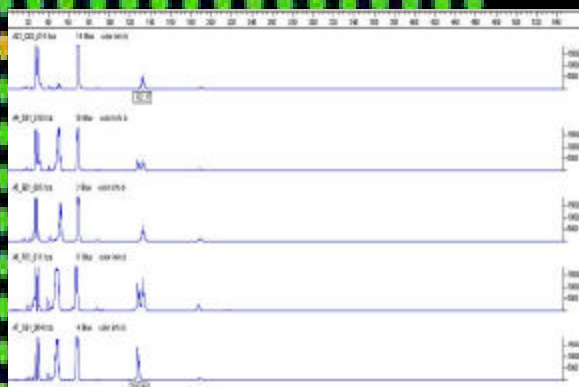


4TH ANNUAL

PLANT MOLECULAR BIOLOGY AND BIOTECHNOLOGY

RESEARCH SYMPOSIUM

THE OHIO STATE UNIVERSITY



**4TH ANNUAL
PLANT MOLECULAR BIOLOGY AND BIOTECHNOLOGY
RESEARCH SYMPOSIUM**

SCHEDULE

- 8:00-8:30 Registration and Reception, Coffee and Pastries
- 8:30-8:35 Welcome, Dr. Richard Sayre
- 8:35-8:55 Activities and New Acquisitions at *Arabidopsis* Biological Resource Center in 2001-2002, Dr. Randy Scholl
- 8:55-9:15 Plant-Microbe Genomics Facility, Michael Zianni, PMGF Research Associate

RESEARCH PRESENTATIONS

Session Chair: Dr. Eric Stockinger

- 9:15-9:35 Dr. Erich Grotewold: "Subcellular Trafficking of Phytochemicals"
- 9:35-9:55 Dr. Wolfgang (Dietz) Bauer: "Plants Secrete Compounds That Affect Quorum Sensing-Regulated Behaviors in Associated Bacteria"
- 9:55-10:15 Dr. Greg Armstrong: "Why Angiosperms Need Light to Green"
- 10:15-10:35 *Break: Refreshments, Posters*

Session Chair: Dr. Guo-Liang Wang

10:35-10:55 Dr. Esther van der Knapp: "Genetic and Molecular Dissection of Variation in Tomato Fruit Morphology; Tools to Unravel Molecular Mechanisms of Fruit Development"

10:55-11:15 Dr. Jyan-Chyun Jang: "Molecular and Genetic Dissection of Sugar Signal Transduction Pathways"

11:15-11:35 Dr. Morris G. Cline: "Regulation of Syllaptic Outgrowth in Lateral Buds of Hybrid Poplar "

11:35-11:55 Dr. Iris Meier: "Plant-Specific Targeting of RAN Signaling to the Nuclear Envelope"

Noon-2:00 *Lunch and Posters*

Session Chair: Dr. Iris Meier

2:00-2:20 Dr. Guo-Liang Wang: "Dissection of Disease Resistance Pathway in Rice Using Genetic and Genomic Approaches"

2:20-2:40 Dr. Biao Ding: "Cell Biology and Genetics of Intercellular Protein and RNA Traffic"

2:40-3:00 Dr. David E. Somers: "Research into the *Arabidopsis* Circadian Clock"

3:00-3:20 Dr. Eric J. Stockinger: "The CBF Transcriptional Activators and Their Role in Plant Cold Acclimation"

3:20-3:40 *Break*

3:40-4:00 Dr. Terry Graham: "Local, Distal and Systemic Defense Potentiation in Soybean"

- 4:00-4:20 Dr. Fred Sack: "Too Many Mouths Modulates Stem Cell Behavior in Developing Leaves"
- 4:20-4:40 Dr. Richard T. Sayre: "Metabolic Engineering of Algae and Plants; Applications for Addressing Basic Issues in Biology and Biotechnology"

OPTIONAL TOURS

Arabidopsis Biological Resource Center, guided by Dr. Randy Scholl

Plant-Microbe Genomics Facility, guided by Michael Zianni

TABLE OF CONTENTS

ABSTRACT

NUMBER

TITLE

1. Subcellular Trafficking of Phytochemicals, Erich Grotewold and Yakang Lin
2. Plants Secrete Compounds That Affect Quorum Sensing-Regulated Behaviors in Associated Bacteria, Wolfgang (Dietz) Bauer, et al
3. Why Angiosperms Need Light to Green, Greg Armstrong
4. Genetic and Molecular Dissection of Variation in Tomato Fruit Morphology; Tools to Unravel Molecular Mechanisms of Fruit Development, Esther van der Knapp
5. Molecular and Genetic Dissection of Sugar Signal Transduction Pathways, Jyan-Chyun Jang, et al
6. Regulation of Syllaptic Outgrowth in Lateral Buds of Hybrid Poplar, Morris G. Cline
7. Plant-Specific Targeting of RAN Signaling to the Nuclear Envelope, Iris Meier
8. Dissection of Disease Resistance Pathway in Rice Using Genetic and Genomic Approaches, Guo-Liang Wang
9. Cell Biology and Genetics of Intercellular Protein and RNA Traffic, Biao Ding, et al
10. Research into the *Arabidopsis* Circadian Clock, David E. Somers
11. The CBF Transcriptional Activators and Their Role in Plant Cold Acclimation, Eric J. Stockinger

12. Local, Distal and Systemic Defense Potentiation in Soybean, Terry Graham and Madge Graham
13. Too Many Mouths Modulates Stem Cell Behavior in Developing Leaves, Fred Sack and Jeanette Nadeau
14. Metabolic Engineering of Algae and Plants; Applications for Addressing Basic Issues in Biology and Biotechnology, Richard T. Sayre
15. A Genome Sequence Survey of the Mollicute Corn Stunt Spiroplasma, *Spiroplasma Kunkelii*, Xiaodong Bai and Saskia Hogenhout
16. The Identification of Novel Proteins of the *Arabidopsis Thaliana* Nuclear Matrix - A Proteomic Approach, Tomasz Calikowski, Tea Meulia, and Iris Meier
17. Two-Hybrid Screening for Partners of Four Lips, A Regulator of Stomatal Patterning, Jeremy Carpenter, et al
18. Utilizing SAG₁₂-IPT Petunias to Investigate the Role of Cytokinin in Flower Senescence, Hsiang Chang, David G. Clark, and Michelle L. Jones
19. Histidine-Tagging and Purification of Maize Chlorotic Dwarf Virus Functional Proteins, Rym Chaouch, et al
20. Metabolite Profiling as a Functional Genomics Tool, Anusha P. Dias and Erich Grotewold
21. Creation of a Heavy Metal "Chameleon" Biosensor Protein, Jaime D. Ewalt and Richard T. Sayre
22. Identification of Nonhost Resistance Genes from *Nicotiana* to *Phytophthora Infestans*, Walid Hamada, Guo-Liang Wang, and Sophien Kamoun

23. The Regulatory Roles of Sterols in the Development of *Arabidopsis Thaliana*, Jun Xian He, et al
24. Coactivator Dependent and Independent Transcription of Flavonoid Genes, J. Marcela Hernandez, Niloufer G. Irani, and Erich Grotewold
25. Callose Synthases at the Forming Cell Plate During Cytokinesis, Zonglie Hong, Xiaoyun Dong, and Desh Pal S. Verma
26. Nonhost Resistance of Arabidopsis to the Oomycete Pathogen *Phytophthora Infestans*, Edgar Huitema, Vivianne G. A. A. Vleeshouwers, and Sophien Kamoun
27. Modification of African Cassava for Faster Starch Production, Uzoma E. Ihemere, Diana I. Arias-Garzon, and Richard T. Sayre
28. Chalcone Isomerase: An Enzyme by Mistake? Niloufer G. Irani, Xiaoyun Dong, and Erich Grotewold
29. Identification and Function Study of Defense Responsive Gene in Rice, Chatchawan Jantasuriyarat
30. MFP1, a DNA-Binding Protein Localized in the Thylakoid Membranes, is Associated with Photomorphogenesis and Chloroplast Development, Sun Yong Jeong, Annkatrin Rose, and Iris Meier
31. Molecular and Genetic Dissection of an ABA-Independent Sugar Signal Transduction Pathway, Shin Gene Kang and Jyan-Chyun Jang
32. Post Pollination Signaling and Senescence in Ethylene-Insensitive Petunias, Brennick Jay Langston and Michelle L. Jones
33. Distinct Sugar Signalling Pathways that Control Seed Germination and Hypocotyl Elongation, Dongmei Li and Jyan-Chyun Jang

34. The Sterol Biosynthetic Gene *Fackel* is Feedback Repressed by Sterols and Activated by Auxin, Tsai-Chi Li, Jun-Xian He, and Jyan-Chyun Jang
35. *Zeitlupe* Associates with *Arabidopsis* SKP1-Like Proteins (ASKS) Via the F-Box, Mary Mason, et al
36. Influence of Explant Source, and Light on Efficiency of Agrobacterium-Mediated Transformation of Cassava, W. Msikita, et al
37. The Statement of MAPKKK and MAPK in Rice by the Treatment of Autoclaved Conidia of *M. Grisea* and Lipid Extract from *M. Grisea*, Sunjin Oh and Guo-Liang Wang
38. A Complete Electron Microscopic Investigation of Corn Stunt Spiroplasma Accumulation and Replication in its Leafhopper Vector *Dalbulus Maidis*, Elvan Özbek, Bill Styer, and Saskia A. Hogenhout
39. Interaction of AtRanGAP1 with Filament like Protein 2 (FLIP2), Shalaka Patel and Iris Meier
40. Mechanisms of Glucose Signaling During Germination of *Arabidopsis Thaliana*, John Price, et al
41. Inducible *AC/DS* Elements for Activation Tagging in Rice, Shaohong Qu, et al
42. Identification and Fractionation of Algal Exudates that Affect Bacterial Quorum Sensing, Sathish Rajamani, et al
43. Identification and Isolation of Genes from Soybean (*Glycine Max*) Orthologous to Cold-Regulated Genes Found in *Arabidopsis Thaliana*, Stephanie M. Roberts and Eric J. Stockinger

44. Targeting of Plant RanGAP to the Nuclear Envelope, Annkatrin Rose, Shalaka Patel, and Iris Meier
45. Proline Action on Heavy Metal Detoxification in Microalgae, Surasak Siripornadulsil, et al
46. Reduction in the Cyanogenic Potential of Cassava Roots; Transgenic Plants Expressing Hydroxynitrile Lyase in the Roots, Dimuth Siritunga, Diana Arias-Garzon, and Richard T. Sayre
47. Domestication of Cassava: Generation of Cyanogen-Free Cassava, Dimuth Siritunga and Richard T. Sayre
48. Identification of Proteins that Interact with *Arabidopsis* Ran GTPase Activating Protein 1, Kelly A. Threm, (Annkatrin Rose, and Iris Meier)
49. Evidence for Heterogeneity of Ribosomes in *Arabidopsis*, Michael Tilley and Randy Scholl
50. Characterizing *Arabidopsis* Callose Synthase and Callose Synthase Complex, Chunbo Wang and Desh Pal S. Verma
51. Molecular Characterization of AN11/TTG-Like WD40 Genes, George Wang, J. Marcela Hernandez, and Erich Grotewold
52. Functional Asymmetry of Photosystem II D1 and D2 Peripheral Chlorophyll Mutants of *Chlamydomonas Reinhardtii*, Jun Wang and Richard T. Sayre
53. Modulation of the Directionality of PSII Electron Transfer by Mutagenesis of the D1-E130 in *Chlamydomonas*, L. Xiong, et al
54. Towards Molecular Cloning and Functional Analysis of *SPL11*, a Gene Involved in Programmed Cell Death and Broad-Spectrum Disease Resistance in Rice, LiRong Zeng, et al

55. Cloning and Characterization of the Broad-Spectrum Blast Resistance Gene *Pi2*,
Bo Zhou, et al

ACTIVITIES AND NEW ACQUISITIONS AT ARABIDOPSIS BIOLOGICAL RESOURCE CENTER IN 2001-2002.

Randy Scholl*, Doreen Ware, Deborah Crist, Emma Knee, Luz Rivero, Jeff Cotrill, Staci Putney. Dept. of Plant Biology, Ohio State Univ. and Dept. of Computer Science and Engineering, Michigan State Univ.

The Arabidopsis Biological Resource Center (ABRC) cooperates with the Nottingham Arabidopsis Stock Centre (NASC) to collect, preserve and distribute seed and DNA stocks of Arabidopsis.

Diverse, new stocks have been added to our collections in the past year, including: A) many new mutant lines, B) pools of T-DNA lines, so that the total lines available is near 230,000, C) characterized insertion lines including GFP and sequence-tagged lines, C) a recombinant inbred population, D) ecotypes from new locations, E) clone accessions, D) libraries, and E) full length cDNA clones. Two large stock collections currently being received will be highlighted. These are: 1) the 140,000 sequence-indexed T-DNA insertion lines currently being received from Joe Ecker's laboratory, and 2) the 8,000+ full length cDNA clones being developed by the SSP consortium.

ABRC is striving to fulfill as completely as possible a number of objectives: a) Enhance the collection of characterized mutants and clones to reflect to the maximum extent the published mutants and clones; b) Offer insertion populations that represent as nearly as possible a genome-saturating set of insertions and comprise diverse types of functional insertions; c) Make available various advanced seed resources arising from genome projects, including sequence-tagged insertion mutants; d) Incorporate a complete set of full-length cDNA clones; e) Organize and confirm the identity of the genomic clones related to the published genome sequence data; f) Incorporate enhanced-function clone collections from genome and other projects such as transformable BACs, and incorporate additional genome-related seed and DNA resources as they are developed. Beginning in summer, 2001 informatics activities of the Resource Center were assumed by The Arabidopsis

Information Resource (TAIR, <http://www.arabidopsis.org>) with informatics support from the National Center for Genome Resources (NCGR). During the past year, ABRC distributed approximately 54,000 seed and 25,000 DNA stocks to researchers. ABRC is supported by the National Science Foundation.

RESEARCH PRESENTATIONS

SUBCELLULAR TRAFFICKING OF PHYTOCHEMICALS

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We have used flavonoid biosynthesis as a model system to investigate plant metabolic pathways and the mechanisms by which plants control gene expression. In maize, two branches of flavonoid biosynthesis are independently regulated by the Myb-domain proteins P and C1, providing one of the best understood regulatory networks in plants. The ectopic expression of the P and C1 regulators in cultured maize Black Mexican Sweet (BMS) cells provides an attractive alternative to engineering plant secondary metabolite production. The analysis of the compounds induced by the ectopic expression of these Myb factors indicated that they play novel regulatory functions, not obvious from previous studies. Autofluorescent compounds induced by P provide unique markers to investigate trafficking and subcellular localization of secondary metabolites. P induces the accumulation of two fluorescent compounds targeted to distinct subcellular structures. A yellow fluorescent compound accumulates in discrete bodies within the vacuolar compartment. Bodies filled with a green fluorescent compound accumulate, upon P induction, in the cytoplasm of BMS cells. These bodies move to the cell surface, fuse with the cell membrane, and release the green fluorescent compound to the cell wall. Golgi-disturbing agents don't inhibit the transport of these bodies to the cell membrane. Rather, the number of cells with green fluorescent bodies and green fluorescence in the cell wall increases. In contrast, the transport of the yellow fluorescent compound to the vacuole is not affected by these treatments. Together, our findings suggest that the transport of plant secondary metabolites involves novel trafficking pathways. Unique tools to dissect these pathways have now become available.

PLANTS SECRETE COMPOUNDS THAT AFFECT QUORUM SENSING-REGULATED BEHAVIORS IN ASSOCIATED BACTERIA

WD BAUER¹, M Teplitski¹, M Gao¹, J Robinson², HC Chen³ and B Rolfe³.
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Department of Biology, U. of Dayton², and Plant-Microbe Genomics
Group, Australian National University, Canberra³.

Many plant-associated bacterial species use the exchange of small, intercellular signal molecules, typically *N*-acyl homoserine lactones (AHLs), to regulate the expression of many of their genes in relation to local population density. Such regulation is known as "quorum-sensing" (QS), and allows nearby individual bacterial cells to act in a coordinated manner. We have discovered that a diversity of higher plants secrete unknown substances that can either stimulate or inhibit QS-regulated behaviors in bacteria (Teplitski et al. 2000. *Molecular Plant-Microbe Interactions* 13:637-648). The ability of plants to manipulate QS-regulated behaviors in bacteria by production of compounds that mimic QS signals could be of broad consequence to agriculture and medicine in preventing disease and enhancing symbioses. We are currently focusing on the QS mimic/effector compounds produced by the model legume, *Medicago truncatula*. We have evidence that *M. truncatula* produces compounds that affect the AI2-luxS dependent QS system of enteric bacteria as well as the AHL-dependent QS system of many other Gram negative species. The secretion of some of the QS-active compounds by *M. truncatula* seems to be subject to developmental regulation and to induction by the prior exposure of seedlings to bacteria, thus indicating a dynamic relationship between the plant and bacteria in terms of the nature and amount of QS mimics secreted. We are also characterizing QS regulation in *Sinorhizobium meliloti*, the N-fixing bacterial symbiont of *M. truncatula*. The long range goal of these studies is to determine how QS mimic compounds from the host plant affect QS-regulated behaviors in the bacterial symbiont and thus affect establishment of an effective symbiosis. Proteomic analysis has revealed that over 80 proteins are differentially accumulated in *S. meliloti* in response to addition of AHLs purified from culture filtrates of the bacterium. We are currently working

to identify the AHLs produced by *S. meliloti*, the genes required for their synthesis and perception, and the responses of the bacterium to mimic compounds from the host plant.

WHY ANGIOSPERMS NEED LIGHT TO GREEN

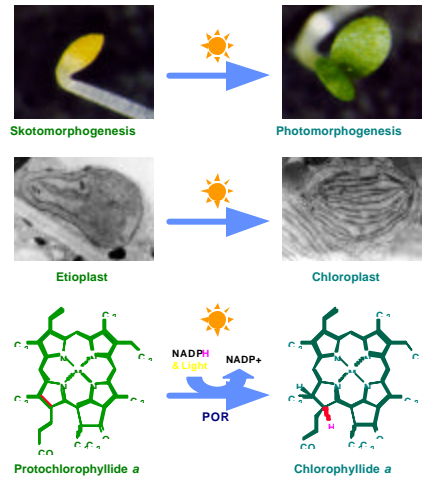
Gregory Armstrong: Department of Plant Biology, Ohio State University, Columbus, 43210; phone (614) 292-4817; email, armstrong.275@osu.edu

Light is one of the most important environmental factors governing plant growth and development. My research focuses on understanding how plants perceive, respond to and protect themselves from light at different developmental stages, using a spectrum of molecular, genetic, biochemical, physiological and genomic approaches. Visible greening is a central light-dependent event in angiosperm seedlings. Greening results because the Mg-tetrapyrroles chlorophylls *a* and *b*, together with carotenoids, are produced in large amounts in plastids and are incorporated into the membrane-bound light-harvesting antenna and reaction center complexes of photosystems I and II. Although essential for photosynthesis, this process also exposes the developing seedling to the risk of photodynamic damage sensitized by chlorophyll precursors. Tetrapyrrole biosynthesis is therefore tightly regulated to prevent the accumulation of free intermediates.

Dark-grown angiosperm seedlings contain no chlorophyll because the reduction of protochlorophyllide (Pchl_{id}) to chlorophyllide requires the activity of a highly unusual, strictly light-dependent enzyme, the NADPH:Pchl_{id} oxidoreductase (POR). POR is not only the most abundant protein in etioplast inner membranes, but also an active determinant of plastid inner membrane architecture. In etioplasts POR exists in a dark-stable ternary complex together with NADPH and Pchl_{id}. This complex is enzymatically active upon illumination at temperatures as low as -70°C. POR thus couples light-dependent greening and chloroplast differentiation to photomorphogenesis, while simultaneously protecting seedlings from Pchl_{id}-induced photodynamic damage.

We are currently applying transgenic and reverse genetic approaches to obtain information about the functions, regulation and *in situ* expression patterns of the three differentially light- and developmentally-regulated POR enzymes of Arabidopsis, PORA, PORB, and PORC. The goals of this research area are to establish the roles of these proteins throughout

angiosperm ontogeny in plastid development, light-dependent chlorophyll biosynthesis and protection against photooxidative damage.



GENETIC AND MOLECULAR DISSECTION OF VARIATION IN TOMATO FRUIT MORPHOLOGY; TOOLS TO UNRAVEL MOLECULAR MECHANISMS OF FRUIT DEVELOPMENT

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The objectives of my research program are to identify molecular mechanisms underlying variation in tomato fruit morphology, with the ultimate goal to gain insight into fruit developmental processes. Years of domestication and selection have resulted in a large number of fruit morphological variants present in the tomato germplasm collection. As a first step to identify molecular processes controlling fruit morphology, we are taking a genetic approach by crossing cultivated tomatoes carrying extremely shaped fruit to one of its undomesticated wild relatives. Progeny of these crosses are scored for segregation of fruit morphological characters, which are nearly always quantitatively inherited. Major quantitative trait loci identified in these experiments can be subjected to further study such as developmental analyses into understanding the function of the locus *in planta* to map-based cloning approaches allowing identification of the gene(s) underlying variation. Currently, we are chromosome-walking to a locus controlling fruit elongation: the cultivated allele causes fruit to be elongated, creating a Roma-type tomato, while the wild and undomesticated allele causes the fruit to be round. The identification of the gene present at this locus will allow us to address questions such as (1) how during domestication changes at the molecular level resulted in changes in fruit morphology, (2) what are the growth processes affected by allelic substitution at this locus, (3) what is the role of the gene product in the cell, tissue and/or *in planta*.

MOLECULAR AND GENETIC DISSECTION OF SUGAR SIGNAL TRANSDUCTION PATHWAYS

Jyan-Chyun Jang, Vijaya Bobba, Shin Gene Kang, Dongmei Li, Tsai-Chi Li, Jong-Kuk Na, John Price, and Marie Prutell. Department of Horticulture and Crop Science, The Ohio State University, Columbus, OH 43210; phone: 614-292-8496; email: jang.40@osu.edu

The research in my lab is focused on the roles of metabolites in the regulation of plant growth and development. In order to control specific processes in plant growth and development, sugars need to be sensed and transmitted by precise mechanisms. However, our understanding of sugar sensing and signaling in plants is very limited. To dissect sugar-signaling process in plants, we and several other labs have isolated many sugar insensitive mutants. Surprisingly, most of them turn out to be ABA mutants. These results raise many interesting questions. Does sugar cause stress? Is ABA indeed a second messenger of sugar signaling? How does sugar signaling crosstalk with ABA signaling and how does ABA regulate sugar-dependent processes? And finally, do ABA-independent sugar signaling pathways exist? To address some of the outstanding questions, my laboratory is pursuing the following objectives.

1) Determine the role of ABA in sugar signalling: Our recent data indicate that exogenous glucose as low as 0.5% can cause significant delay of seed germination. Remarkably, this effect cannot be mimicked by mannitol and ABA is still involved. Because the level of glucose used was low in the experiment, this is the first demonstration that ABA-mediated sugar signaling is not a simple consequence of a stress response. In addition, we have found that although high sugar concentrations cause ABA accumulation, the cellular ABA concentration does not determine whether germination will occur. Rather, germination appears to be controlled by the duration of ABA accumulation.

2) Understand interaction between sugar and ABA signalling pathways: As both sugar and ABA have broad effects on plant growth, we use two strategies to dissect their relationship in signal transduction. We first used DNA microarrays to determine gene expression profiling in response to glucose and ABA. Another effort is to use mutational analysis to dissect crosstalk between sugar and ABA signalling and discover unique sugar

signalling pathways. We are using a novel method to identify sugar signalling component by isolating the enhancer and suppresser of an ABA-deficient mutant *aba2*.

3) Dissect an ABA-independent sugar signalling pathway: To discover a unique sugar signalling pathway that does not involve ABA, we select *trans* mutations that can affect the expression of a marker gene. We have found that *Asparagine Synthetase 1 (ASN1)* is ideal for this work. We have generated transgenic plants containing the promoter of *ASN1* fused to the *LUC* or *GFP* marker genes. These lines have been characterized and we are ready to conduct mutagenesis.

REGULATION OF SYLLEPTIC OUTGROWTH IN LATERAL BUDS OF HYBRID POPLAR

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To elucidate the control mechanisms of shoot branching in herbaceous and woody species various workers have employed a combination of physiological, genetic and molecular approaches (Shimizu-Sato and Mori, 2002). In the past decade a number of branching mutants have been identified and have provided bases for initiating fruitful investigations of these control mechanisms. In our study of sylleptic branching in the economically important hybrid poplar wherein shoots grow out from lateral buds in the same season in which they are formed instead of overwintering and sprouting in the following spring, we have been utilizing three clones of this hybrid exhibiting contrasting degrees of sylleptic branching. The three clones of hybrid poplar, *Populus trichocarpa* (black cottonwood) x *P. deltoides* (eastern cottonwood), are 11-11, 47-174, 49-177 with high, low and intermediate levels of sylleptic branching, respectively. Some evidence for differential sensitivities to auxin (a known repressor of axillary bud outgrowth) and cytokinin (a known promoter of bud outgrowth) treatments has been found among the three clones. Investigations are also under way to analyze for differential branching sensitivity to different forms of nitrogen (NH_4 , NO_3 , NH_4NO_3 , glutamic acid, asparagine and glutamine) among the three clones. Significant enhancement of sylleptic branch outgrowth was observed in the high sylleptic clone (11-11) in the absence of any other fertilization with 5 mM NH_4 , NO_3 or NH_4NO_3 treatments with a somewhat greater effect in the latter treatment. However, there was absolutely no effect observed with any of these three treatments in the low sylleptic clone (47-174). Preliminary results from experiments involving the grafting of high sylleptic scions to low sylleptic rootstocks suggest the existence of a transmissible branching repressor which moves acropetally from the root to the shoot where branching is reduced.

PLANT-SPECIFIC TARGETING OF RAN SIGNALING TO THE NUCLEAR ENVELOPE

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Ran is a small signaling GTPase, which is involved in nucleocytoplasmic transport. Two additional functions of animal Ran in the formation of spindle asters and the reassembly of the nuclear envelope in mitotic cells have been recently reported. In contrast to Ras or Rho, Ran is not associated with membranes. Instead, the spatial sequestering of its accessory proteins, the Ran GTPase-activating protein RanGAP and the nucleotide exchange factor RCC1, appear to define the local concentration of RanGTP versus RanGDP involved in signaling. Mammalian RanGAP is bound to the nuclear pore by a mechanism involving the attachment of SUMO to its C-terminus and the subsequent binding of the SUMOylated domain to the nucleoporin Nup358. We have shown that plant RanGAP utilizes a different mechanism for nuclear envelope-association, involving a novel targeting domain, which appears to be unique to plants. The N-terminal WPP domain is highly conserved among plant RanGAPs and the small, plant-specific nuclear envelope-associated protein MAF1, but not present in yeast or animal RanGAP. Confocal laser scanning microscopy of GFP fusion proteins showed that it is necessary for RanGAP targeting and sufficient to target the heterologous protein GFP to the plant nuclear rim. The highly conserved tryptophan and proline residues of the WPP motif are necessary for its function. The 110 amino acid WPP domain is the first nuclear-envelope targeting domain identified in plants. Its fundamental difference to its mammalian counterpart implies that different mechanisms have evolved in plants and animals to anchor RanGAP at the nuclear surface.

DISSECTION OF DISEASE RESISTANCE PATHWAY IN RICE USING GENETIC AND GENOMIC APPROACHES

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Our research focus is to understand the mechanisms of plant-pathogen interaction, and the signal transduction pathways leading to the induction of disease resistance responses. We use rice as the model plant to clone disease resistance genes and genes involved in resistance response to rice fungal and bacterial pathogens. Both genetic and genomic approaches are being used. We are currently working on the following four projects. Details of each project will be presented in separate posters at the meeting.

1. Map-based cloning of the two broad-spectrum resistance genes, *Pi2* and *Pi9*
2. Identification of defense response genes using subtractive suppression hybridization and microarray methods
3. Map-based cloning of a lesion mimic gene and identification of genes involved in cell-death and disease resistance using Genechip hybridization method
4. Generation of rice mutant populations using a novel Ac/Ds activation tagging system.

CELL BIOLOGY AND GENETICS OF INTERCELLULAR PROTEIN AND RNA TRAFFIC

Biao Ding¹, Asuka Itaya¹, Fengshan Ma¹, Yijun Qi¹, Yali Zhu¹, Neela Kumari¹, Yoshie Matsuda¹, Stephen Raj² and Venkat Gopalan², Department of Plant Biology and Plant Biotechnology Ctr¹ and Department of Biochemistry², The Ohio State University, Columbus, OH 43210, ding.35@osu.edu

Gene products require correct cellular localization for function. In plants, increasing evidence indicates that many proteins and RNAs produced in one cell can traffic into neighboring cells and even distant organs to perform specific functions. We are interested in understanding how such intercellular protein and RNA traffic is regulated. Using a variety of experimental systems, we have obtained results showing that traffic of a protein between different cellular boundaries can be regulated differently. We have developed an *Arabidopsis thaliana* system to genetically investigate the cellular factors that regulate protein traffic. A number of *Arabidopsis* mutants defective in supporting protein targeting to plasmodesmata have been isolated. We have also obtained data showing that the polarity of an RNA is a factor for intranuclear traffic, and that specific structural motifs of an RNA mediate its traffic between the vascular tissue and neighboring cells.

RESEARCH INTO THE ARABIDOPSIS CIRCADIAN CLOCK

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The circadian clock controls a wide range of physiological and developmental processes in plants and animals. *ZEITLUPE (ZTL)* is a novel protein comprised of an amino-terminal PAS/LOV domain, an F-box and six carboxy-terminal kelch repeats. Mutations in the kelch domains lengthen free-running period, implicating this protein in the control of the circadian clock. The LOV domain bears a high degree of similarity to domains of the blue-light receptor involved in phototropism (*NPH1*), as well as the *Neurospora* circadian associated protein *white collar 1 (WC-1)*. The fluence rate-dependent effects of the *ztl* mutations suggests that ZTL defines a new class of photoreceptor dedicated to the control of circadian period in higher plants. Since other F-box proteins have been implicated in ubiquitin-dependent proteolysis, ZTL may participate in a novel light-regulated proteolytic system involved in the degradation of components of the circadian clock.

We have taken a variety of biochemical, mutational and molecular genetic approaches to test this hypothesis and will present the most recent data.

THE CBF TRANSCRIPTIONAL ACTIVATORS AND THEIR ROLE IN PLANT COLD ACCLIMATION

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Many plants have the ability to increase in freezing tolerance after exposure to low nonfreezing temperatures, a process known as cold acclimation. Using *Arabidopsis thaliana* to study this biological phenomenon revealed that the CBF proteins play a key role in the cold acclimation process. In response to cold temperatures, the CBFs activate the expression of downstream structural-protein encoding genes, the CBF regulon, whose polypeptides ameliorate the effects of the cold stress on the plant. The CBFs perform this function by binding to the CRT/DRE, a *cis*-acting element present in the promoters of the *Arabidopsis* cold-regulated (*COR*) genes and then activating *COR* gene expression. While many plants are not capable of cold-acclimation, it is apparent through data base searches that most plants harbor genes encoding proteins highly similar to those encoded by the *CBF* and *COR* genes. Similarly the *cis*-acting CRT/DRE appears to be conserved across species. Thus diverse plant taxonomic groups may share many aspects of the *Arabidopsis* CBF regulon. A central objective of my lab is to determine whether the *Arabidopsis* CBF responsive pathway is highly conserved in plants and whether differences in plant cold tolerance can be traced to differences in CBF cold-response pathways. To do this we are undertaking the molecular cloning and analysis of the CBF genes and regulons from numerous related crop plant species exhibiting a range of cold tolerance levels. In addition we will also determine the low temperature gene expression profiles of these same species in order to create a low-temperature EST (expressed sequence tag) database for each plant. It is anticipated that these comparative and functional genomic analyses will greatly enhance our understanding of the gene networks that play critical roles in determining cold tolerance levels exhibited in different plants.

Complimentary to these analyses, another long-term goal of my lab is to determine the mechanisms by which the CBF proteins activate transcription. To do this we began by the identification and functional characterization of *Arabidopsis* proteins predicted to facilitate the structural modification of chromatin towards an architecture more conducive for gene expression. We have extended these analyses to site directed mutagenesis studies in which the

amino acids comprising the CBF1 trans-activation domain have been systematically altered in order to identify key residues for trans-activation. We next plan to biochemically copurify nuclear factors complexed with CBF1 such that we may construct models depicting CBF, the *COR* gene promoter, and associated proteins as the plant responds to low temperature. It is anticipated that these analysis will not only be of great value in the quest to enhance the freezing tolerance of plants but will also contribute to a general overall understanding of the mechanisms plants use to activation gene expression.

LOCAL, DISTAL AND SYSTEMIC DEFENSE POTENTIATION IN SOYBEAN

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The chemical or genetic enhancement of induced disease resistance responses in plants is an attractive strategy for disease control in that it involves activation of naturally occurring defense responses. A particularly attractive form of induced resistance is defense potentiation. In this form of resistance, defense responses are not permanently induced, but cells are "primed" to respond more rapidly to the pathogen upon attack. Recently we have uncovered three different levels of defense potentiation in soybean. These responses are proximal, distal and truly systemic from the point of the inducing signal.

Proximal defense potentiation is programmed in cells immediately adjacent to the hypersensitive response in *Phytophthora sojae*-infected soybean by factors associated with HR dying or wounded cells. We have termed this potentiation "elicitation competency" in that it programs the capacity of proximal cells to enable their response to the cell wall glucan elicitor from *P. sojae*, which subsequently induces a complex set of phenylpropanoid responses in these cells, including phenolic polymer deposition and phytoalexin (glyceollin) accumulation. The multiplicity and complexity of these various responses were well delineated using metabolic profiling approaches.

Recently, we have shown that glucan elicitor treatment of competent proximal cells also raises the defense potential of cells distal (200-300 cells distant) to the point of treatment. Two different types of defense potentiation were found to be involved. The first, which leads to lesion-limiting resistance, appears to involve the distal induction of proximal elicitation competence. Thus, this is simply a potentiation of the normal proximal or local phenylpropanoid defense responses. The second leads to complete containment of the pathogen and involves a non-phenylpropanoid response, which we are currently studying.

Finally, we have discovered that treatment of soybean leaves with the herbicide lactofen leads to a truly systemic form of defense potentiation to both *P. sojae* and *Pseudomonas syringae* pv. *glycinea*. While lactofen induces a massive local

accumulation of isoflavone conjugates, we do not yet know how this relates to the systemic defense potentiation.

As a complement to metabolic profiling, we have also begun to mine the extensive soybean EST database to facilitate expression analysis of the genes involved in local, distal and systemic defense potentiation. In addition to genes for the phenylpropanoid pathways, we are examining genes for various classes of pathogenesis-related (PR) proteins and those involved in regulation of response (e.g., genes involved in signaling, the establishment of elicitation competency, transcription factors, etc.).

TOO MANY MOUTHS MODULATES STEM CELL BEHAVIOR IN DEVELOPING LEAVES

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Although stomata are key to the survival of land plants, the genes controlling stomatal development are poorly understood. Stomata are formed after a series of asymmetric divisions of transiently self-renewing precursors termed meristemoids. Stomata are continually produced during the mosaic development of the leaf, and many of these form by division of cells next to pre-existing stomata. Patterning of stomata requires the plane of formative asymmetric divisions to be oriented so that the new precursor, the satellite meristemoid, does not contact the pre-existing stoma or precursor. Intercellular signaling provides spatial cues that regulate the orientation of these divisions and may also block asymmetric divisions in cells adjacent to two stomata or precursors. The recessive *too many mouths* mutation disrupts both these processes by randomizing the division plane in cells next to a single stoma or precursor, thus producing clusters of stomata in contact, and by permitting asymmetric divisions in cells next to two stomata or precursors. Also, *tmm* meristemoids divide fewer times before assuming the determinate guard mother cell fate. These phenotypes suggest that TMM is required for cells to respond appropriately to their position during stomatal development, and that TMM participates in intercellular signaling. *TMM* is an apparent receptor expressed in dispersed stem cells in the developing leaf. These results support a role for TMM in a position-dependent signaling pathway that functions at several hierarchical levels including control of the plane of patterning divisions as well as the balance between stem cell divisions and differentiation in stomatal and epidermal development.

METABOLIC ENGINEERING OF ALGAE AND PLANTS; APPLICATIONS FOR ADDRESSING BASIC ISSUES IN BIOLOGY AND BIOTECHNOLOGY

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Research projects in our lab range from addressing questions in fundamental process in bioenergetics to engineering improved crop plants for subsistence farmers in Africa. Specifically our programs include, 1) characterization of the structural and functional asymmetries in electron and energy transfer processes in photosystem II (PSII), 2) the functional genomics of heavy metal stress in *Chlamydomonas* and generation of algae with enhanced heavy metal binding properties, 3) production of oral vaccines using transgenic algae, and 4) the domestication of cassava for subsistence farmers in Africa through transgenic approaches. I will discuss recent advances in the lab in each of these four research areas including, progress towards elucidation of redox active cofactors in PSII, the identification of novel gene products involved in cadmium tolerance in algae, transgenic approaches to enhance the heavy metal binding properties of *Chlamydomonas*, the development of a novel fluorescent heavy metal biosensor molecules (chameleon-like protein), the development microalgal-based oral vaccines (patents pending), and the generation of the first acyanogenic cassava as well as cassava having altered starch metabolism.

POSTER ABSTRACTS

A GENOME SEQUENCE SURVEY OF THE MOLLICUTE CORN STUNT SPIROPLASMA, *SPIROPLASMA KUNKELII*

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The mollicute corn stunt spiroplasma (*Spiroplasma kunkelii*) is a leafhopper-transmitted pathogen of maize. Sequencing of the ~ 1.6-Mb genome of *S. kunkelii* was initiated to aid understanding the genetic basis of spiroplasma interactions with their plant and leafhopper hosts. In total, 144,712 nt of non-redundant, high-quality *S. kunkelii* genome sequence was obtained. Sequence tags were searched against the Mycoplasmataceae and *Bacillus/Clostridium* databases. Results showed that, in addition to spiroplasma phage SpV1 DNA insertions, spiroplasma genomes harbor more purine and amino acid biosynthesis, transcription regulation, cell envelope and DNA transport/binding genes than Mycoplasmataceae genomes. This investigation demonstrates that survey sequencing is an efficient procedure for gene discovery and genome characterization.

The results of the *S. kunkelii* sequencing project are available at the Spiroplasma WebPage: www.oardc.ohio-state.edu/spiroplasma/genome.htm.

THE IDENTIFICATION OF NOVEL PROTEINS OF THE *ARABIDOPSIS THALIANA* NUCLEAR MATRIX – A PROTEOMIC APPROACH

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The nuclear matrix (NM) can be defined as the proteinaceous, insoluble material left after removal of chromatin and the majority of proteins from the interphase cell, by subsequent treatment with detergents, nucleases and high salt buffers. It is thought to consist of the three-dimensional meshwork of protein filaments, forming multiple DNA-binding loops, and playing an important role in diverse intra-nuclear processes. They are likely to include transcription, splicing and replication. However, the precise spatial structure and molecular composition of NM remains largely elusive. Studies in animal systems have revealed a few filament-like nuclear matrix proteins, most notably lamins, NuMA and LUMA. Plants do not appear to contain lamins, but a small number of filamentous nuclear proteins has recently been described. The emerging field of proteomic studies has the potential to yield a comprehensive and complete set of NM proteins.

The aim of the current research is to describe novel components of plant nuclear matrix, by the isolation of the NMs from the tobacco *BY-2* and *Arabidopsis thaliana* suspension cell cultures, their two-dimensional electrophoretic resolution, subsequent analysis by Western immunoblotting and identification by mass spectroscopy. The antibody specific for the NM protein 1 (NMP1) has been used as a positive control of the nuclear matrix preparations, both with the traditional 1-D SDS-PAGE analysis, and with 2-D Westerns. A considerable attention has been devoted to developing an efficient sample solubilization system, a necessary prerequisite for the 2-D electrophoresis. Electron microscopy has been employed for the visualization of the three-dimensional meshwork of tobacco cell NM filaments, whereas the immunogold staining has shown the discrete location of the NMP1 protein within the

branched core filaments. The ongoing research focuses on improving the resolution of *A. thaliana* NM 2-D electrophoresis to allow the mass spectroscopic analysis of selected proteins. The comparison of resulting oligopeptide sequences against a database should result in identification of novel proteins of the plant nuclear matrix, thus allowing for the further studies of their functions and reciprocal interactions.

TWO-HYBRID SCREENING FOR PARTNERS OF FOUR LIPS, A REGULATOR OF STOMATAL PATTERNING

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Wild type stomata are patterned in that they do not contact each other. The *FLP* (*FOUR LIPS*) gene is required for this spacing pattern in *Arabidopsis*. Mutations in *FLP* result in adjacent stomata, as well as an abnormal number of guard cells. Stomatal clusters in *flp* result from extra cell divisions due to the persistence of the guard mother cell (GMC) program. *FLP* may help terminate the GMC program and restrict the number of GMC divisions to one. *FLP* encodes a protein with a conserved DNA-binding domain, a nuclear localization signal, and a coiled-coiled region that may participate in specific protein-protein interactions. The structure of this protein suggests that *FLP* may control stomatal patterning by regulating gene expression. To identify possible interacting proteins, a yeast two-hybrid screen was initiated using the full-length *FLP* protein as bait. Initial results from this screen will be discussed.

UTILIZING SAG₁₂-IPT PETUNIAS TO INVESTIGATE THE ROLE OF CYTOKININ IN FLOWER SENESCENCE

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Cytokinins are closely associated with senescence and increased cytokinin levels can delay senescence in plants. Previous research has shown that the introduction of the *ipt* gene into plants is associated with an increase in cytokinin content, and leaves of plants transformed with SAG12-*ipt* exhibit delayed senescence. *Petunia hybrida* 'V26' has been transformed with SAG12-*ipt* in order to investigate the role of cytokinins in flower and leaf senescence. Transgenic lines of petunia have been identified based on increased flower longevity. The presence of the *ipt* construct has been confirmed by PCR. The unpollinated SAG12-*ipt* flowers last approximately two weeks longer than wild type controls. *Ipt* transcripts are detected in corollas by RT-PCR at 24 hours after pollination. Ethylene production rates and senescence-related gene expression will be compared between these SAG12-*ipt* and wild type flowers to further investigate the role of cytokinins in flower senescence.

HISTIDINE-TAGGING AND PURIFICATION OF MAIZE CHLOROTIC DWARF VIRUS FUNCTIONAL PROTEINS

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Maize chlorotic dwarf virus (MCDV; genus *Waikavirus*) is a phloem-limited, single-stranded RNA virus that causes vein banding and stunting in maize. Waikaviruses include economically important insect-borne maize and rice pathogens. MCDV is transmitted to maize (*Zea mays* L.) by the deltocephaline leafhopper, *Graminella nigrifons*, in a semi-persistent manner in which virus particles bind to the insect's foregut with help of a viral-encoded helper component.

The genome of the severe Ohio MCDV isolate (MCDV-S) is 11.8 kb long and encodes a single ~ 400 kDa polyprotein that is post-translationally cleaved into several smaller functional proteins. While regions of the genome encoding the three MCDV capsid proteins, a cysteine protease and RNA-dependent RNA polymerase are identified, functions have not been assigned to proteins encoded by three separate regions of the virus genome. We cloned these three regions into the pTrcHis plasmid. The His-tagged fusion proteins were produced in *Escherichia coli*, and affinity purified for raising antibodies. Here we report on the identification and localization of proteins in Western blot assays and microscopy of infected plant material and leafhoppers.

Keywords: MCDV, *Graminella nigrifons*, helper component, expression, *Escherichia coli*

METABOLITE PROFILING AS A FUNCTIONAL GENOMICS TOOL

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Recent developments in metabolite profiling has made it an exciting tool for plant functional genomics providing numerous advantages over the existing mRNA profiling, phenotypic profiling, and proteomic techniques for elucidating the function of genes that participate in metabolic pathways. Very little is known on how plant metabolic pathways are regulated despite the enormous economic significance of phytochemicals. Metabolomics of biochemical pathways regulated by transcription factors provide a unique opportunity to study transcription factor function in the regulation of phytochemical accumulation. By experimental analysis of cells in culture or by comparing mutants and wild type plant tissues, we are investigating which metabolic pathways are regulated by plant Myb transcription factor. This is achieved by profiling metabolites (small molecules <1000 Da) by a combination of HPLC, GC-MS and LC-MS. We have used wild type and mutant maize silk tissues expressing the transcription factor P and maize Black Mexican Sweet (BMS) cells over-expressing the ZmMyb-IF35 transcription factor. An array of metabolites was identified in both the over-expressed cell lines and silk tissue. Our studies provide the first application of metabolite profiling to establish the functions of plant transcription factors.

CREATION OF A HEAVY METAL "CHAMELEON" BIOSENSOR PROTEIN

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Heavy metals are pervasive environmental contaminants and remediation relies on monitoring levels in waters, soils and point sources. The current technologies for measuring trace metals are atomic or mass spectroscopy. These approaches require invasion of the natural system and pre-treatment of the environmental sample prior to analysis. We are interested in creating a biomonitoring system using *Chlamydomonas* to determine bioavailable trace and heavy metals. Our approach is modeled from the successful construction of a Ca²⁺ indicator of Miyawaki, et al, (1) based on calmodulin/calmodulin binding protein sandwiched between two green fluorescent protein (GFP) variants. The "sensing" is based on Fluorescence Resonance Energy Transfer (FRET); upon a binding-induced conformational change the two fluorescent proteins are brought within 100Å, the minimal distance for energy transfer from the donor fluorophore (cyan fluorescent protein, CFP) to the acceptor (yellow fluorescent protein, YFP). Metallothionein is a small (63aa) protein that has seven metal binding sites contained in two preferential binding domains and in its metal bound conformation the N- and C- termini are 61Å apart. We have successfully constructed a heavy metal chameleon by mutating a *Chlamydomonas* codon-biased GFP to create CFP and YFP and fusing these proteins to the N- and C-termini of metallothionein via threonine-alanine and (alanine)³ linkers, respectively. Preliminary data suggests that our synthetic protein is indeed binding metal and the expected FRET is resulting. Current work includes protein isolation and the determination of metal-specific dissociation constants and metal binding competition studies. Future work includes expression in *Chlamydomonas*, including the potential targeting to organelles and the cell membrane.

1. Miyawaki A, Llopis J, Heim R, McCaffery JM, Adams J, Ikura M and Tsein R, (1997) "Fluorescent indicators for Ca²⁺ based on green fluorescent proteins and calmodulin", Nature, 388, 882-887

IDENTIFICATION OF NONHOST RESISTANCE GENES FROM *NICOTIANA* TO *PHYTOPHTHORA INFESTANS*

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Phytophthora infestans, the causal agent of late blight of potato and tomato is considered one of the most devastating plant pathogens worldwide. The association of the hypersensitive response (HR) with all types of resistance suggests that disease resistance (R) genes could mediate durable and nonspecific resistance to *P. infestans*. For example, HR to *P. infestans* was observed in nonhost species of the genus *Nicotiana* suggesting that resistance could be transferred from these species to economically important host plants. These *Nicotiana* species recognize a family of elicitor proteins, known as elicitins. Among *Nicotiana*, different responses to INF1, the major elicitin of *P. infestans*, were observed. While some species, such as *Nicotiana otophora*, exhibited a strong HR response to INF1, others, such as *Nicotiana sylvestris*, did not. To identify the genetic determinants of INF1 recognition, we selected a complementation approach based on *Agrobacterium* transient assays. We constructed a transformation and complementation-ready genomic library of *N. otophora* in a binary vector containing an *inf1* expression cassette in the T-DNA. We will describe our progress in characterizing the genomic library, identifying candidate clones, and developing high throughput complementation assays.

THE REGULATORY ROLES OF STEROLS IN THE DEVELOPMENT OF *ARABIDOPSIS THALIANA*

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Sitosterol and campesterol are the two major sterols produced in plants. To date, only campesterol and brassinosteroids (BR) derived from campesterol have been shown to act as hormones in post-embryonic growth. Other sterols have been considered mainly as membrane structural components. However, our recent studies of the *fackel* (*fk*) mutant in *Arabidopsis* have suggested that besides BR, other sterols are critically involved in the control of not only post-embryonic but also embryonic development. We have cloned the *FK* gene and demonstrated that it encodes a sterol C-14 reductase. Besides having reduced levels of brassinosteroids (BR) and sterols, *fk* mutants accumulate the substrate of sterol C-14 reductase plus several uncommon sterols. Sterols were thought to act as regulators of plant development because *fk* mutant could not be complemented by the application of exogenous BR.

While these discoveries are important, there is no direct evidence to clearly demonstrate that non-BR sterols have a regulatory role in plant development. Here we show several new lines of direct evidence that both common sterols and "*fk* sterols" (i.e. uncommon sterols accumulated in *fk* mutants) are biologically active in affecting development and in changing the expression of specific genes. The sterols resemble hormones in their actions. Nano to micro molar concentrations of these sterols can affect cell expansion, cell division, and the expression of specific genes. The regulatory function of sterols in plant development is further demonstrated by the results that fenpropimorph can phenocopy the *fk* mutant at both morphological and gene expression levels. Using GC-MS, we have analyzed 19 sterol intermediates in WT plants treated with 0, 10, or 100 ppm of fenpropimorph. On the basis of the results of this chemical analysis, we

conclude that fenpropimorph at 10 ppm can specifically block FK (sterol C-14 reductase). Together these results indicate that fenpropimorph can be a powerful reagent in the mutant screen for the components involved in sterol metabolism or response.

COACTIVATOR DEPENDENT AND INDEPENDENT TRANSCRIPTION OF FLAVONOID GENES

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The Myb-domain proteins C1 and P control the transcription of the genes involved in the flavonoid biosynthetic pathway in maize (Mol et al. 1998. *Trends Plant Sci* 3:212). In spite of the fact that these two proteins have Myb domains over 70% identical, they control distinct branches of the pathway and have different mechanisms of activation. C1 activates the branch of the pathway that leads to the accumulation of anthocyanins, but only if the helix-loop-helix coactivator protein R is present. P does not require R and only activates the genes involved in the production of phlobaphenes. We have identified the amino acid residues in C1 responsible for the specificity of the interaction with R. When those residues are mutated in P to match the C1 sequence, we obtained a protein that interacts with R, and through this interaction it is able to turn on the anthocyanin branch of the pathway. This novel P protein, P*, has retained the ability to activate *a1* in the absence of R. Thus we have shown that the specificity of these proteins is defined by their ability to interact with the cofactor R (Grotewold et al. 2000. *PNAS* 97:13579). We are now taking advantage of the dual role of P* to understand how C1 and P activate *a1* in co-activator dependent and independent fashions. We are using mutants in each of the three *cis*-regulatory elements identified in the promoter of the *a1* gene to understand how R contributes to the regulatory specificity of C1.

CALLOSE SYNTHASES AT THE FORMING CELL PLATE DURING CYTOKINESIS

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Callose is synthesized on the cell plate and several other locations in the plant. The synthesis of callose also can be induced by wounding, pathogen infection, and physiological stresses. The deposition of callose at the cell plate precedes the synthesis of cellulose and may provide a spreading force that converts the tubulo vesicular network into a fenestrated sheet. We cloned an *Arabidopsis* cDNA encoding a callose synthase (CaIS1) catalytic subunit. The deduced peptide contains 16 predicted transmembrane helices with the N-terminal region and a large hydrophilic central loop facing the cytoplasm. CaIS1 interacted with a novel UDP-glucose transferase (UGT1) that copurified with the CaIS complex. Following fusion with GFP, both CaIS1 and UGT1 were colocalized at the growing cell plate. Expression of CaIS1 in transgenic tobacco cells resulted in higher levels of CaIS activity and enhanced callose synthesis on the forming cell plate. UGT1 also interacted with phragmoplastin and Rop1 that may regulate activity of the CaIS enzyme. These data suggest that plant CaIS may form a complex with UDP-glucose transferase to facilitate the transfer of substrate for callose synthesis. In addition, other proteins such as annexin and Rop1 may provide further regulatory function to control the activity of different CaIS complexes in response to biotic and abiotic stresses as well as under tissue-specific developmental control.

NONHOST RESISTANCE OF ARABIDOPSIS TO THE OOMYCETE PATHOGEN *PHYTOPHTHORA INFESTANS*

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Phytophthora infestans, a host-specific oomycete plant pathogen, causes the late blight disease on some solanaceous plants causing severe economic losses. Control of late blight has proven difficult partly due to a lack of sustainable sources of genetic resistance. A number of plant species however, including Arabidopsis, are fully resistant to all known strains of *P. infestans* or also called nonhosts, and could reveal novel defense genes and pathways. We initiated a number of studies aimed at exploiting Arabidopsis nonhost resistance to *Phytophthora* and establish a key model for understanding nonhost resistance to oomycete pathogens. Following inoculation of Arabidopsis rosette leaves with zoospores of *P. infestans*, penetration of epidermal cells occurred, followed by active defense responses, including a hypersensitive response (HR)-like cell death. Although, induction of *BGL2* gene expression, a marker for the salicylic acid (SA) mediated defense pathway, was recorded, Arabidopsis mutant genotypes, compromised in their SA mediated defense response, did not display a susceptible phenotype. To further investigate Arabidopsis response to *P. infestans*, we used DNA microarray analyses to determine expression profiles for ca. 8,000-10,000 genes during nonhost resistance. Gene expression data generated an overview of activated and repressed disease response pathways between water and zoospores inoculated rosette stage Arabidopsis (Col-0) plants. Results presented here provides a rationale for functional assays of the identified pathways using knockouts and mutant analyses. These studies should lead to the prospect of utilizing Arabidopsis as a source of resistance genes to important pathogens.

MODIFICATION OF AFRICAN CASSAVA FOR FASTER STARCH PRODUCTION

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Cassava's high photosynthetic rate, ability to grow on poor soils and its protection from many pests and herbivores due to the presence of cyanogens make it a good crop to study starch biosynthesis. The objective of our research has been to increase root starch production potential of cassava by introducing a modified *E. coli glgC* gene driven by the tuber-specific patatin promoter (which is also expressed in cassava roots) into cassava. Our choice of the *E. coli glgC* gene, which encodes ADP-glucose pyrophosphorylase (AGPase), over the plant genes is because the *glgC* gene encodes a homodimeric AGPase while the plant AGPase is a heterodimeric enzyme rendering it difficult for cloning. Though the *E. coli* gene has been modified, it still encodes an enzyme with higher activity than the plant enzymes. We have obtained 23 putative transformed plants of which 7 has been proven to be carrying the transgene by PCR and RT-PCR analysis. AGPase enzyme assay indicates that the transformed plants have increase in enzyme activity of more than 22%. Southern blotting analysis to further confirm the transformation of the plants is underway. We propose that enhanced conversion of sugars to substrates for starch synthesis in roots will generate plants with higher starch yields and faster root maturation.

CHALCONE ISOMERASE: AN ENZYME BY MISTAKE?

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The phenylpropanoid pathway, a genetically and biochemically well worked out system serves as a good model to investigate regulation, multienzyme complexes and metabolic flux in plants.

Chalcone isomerase (CHI), a central enzyme of the flavonoid/isoflavonoid pathway, cyclises chalcones into flavanones, a reaction known to occur spontaneously *in vitro*. Contrasting roles of CHI in *Zea mays* BMS cell lines and *Arabidopsis* CHI mutants, where no need for the enzyme activity in the former versus total dependence of the enzyme in the latter in the production of anthocyanins, prompted investigation of the role of CHI in this pathway.

Similar to the other steps in the pathway, maize CHI complements the *Arabidopsis* tt5 mutant. To establish whether the catalytic activity of CHI is required for this complementation, we mutated a highly conserved, catalytically important tyrosine to phenylalanine (*ZmCHI*^{Y104F}). This mutant form of the enzyme also complements the *Arabidopsis* tt5 mutant, with no obvious reduction in the levels of anthocyanin production. Further investigation is on to characterize the activity of *ZmCHI*^{Y104F} *in vitro* and *in vivo*.

If evidence for reduction in the mutated enzyme activity is shown, with a fully functional pathway, it would promote this provocative idea that the role of CHI not only being one of catalysis, but a structural protein involved in holding together various branches of the pathway to efficiently partition metabolites, depending on the need of the environmentally challenged plant.

IDENTIFICATION AND FUNCTION STUDY OF DEFENSE RESPONSIVE GENE IN RICE

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Many genes were induced in plant while attacked by the pathogen. To identify defense genes in rice by incompatible and compatible blast pathogen induced, two subtractive cDNA libraries were constructed using the PCR-select cDNA subtraction kit of Clontech Company. In the first library, which was named Forward subtractive cDNA library, we use 75-1-127, which contains Pi-9 resistance gene to blast disease as "tester", and the reference IR31917, which have not resistance gene as "driver". In another library, which was name Reverse subtractive cDNA library, we use IR31917 as "tester", and 75-1-127 as "driver". Therefore, incompatible pathogen induced genes will be screened from the first library, and compatible pathogen induced genes be screened from the second library. Following the subtraction, amplification products were directly cloned into the pGEM-T vector and approximately 4000 clones were gained in each library. After differential screening, 188 positive clones from Forward part and 285 clones from the Reverse part were selected to do the further analysis. 30 clones were randomly picked up to sequence. Database searches revealed that these genes encode putative proteins have high homology with alcohol dehydrogenase gene (ADH) of some organisms including *Arabidopsis thaliana*, probable cytochrome P450 monooxygenase of maize, homeobox gene of rice, unknown function gene of *A. thaliana*, ALG-2 (apoptosis-linked gene) interacting protein 1 of rat. Also some genes cannot find homology with database. Northern blot analysis showed that the ADH gene was highly induced both in resistance plant (75-1-127) and susceptible plant (IR31917) by blast pathogen, but stronger much in resistance plant. More screening and sequencing are done in our lab now.

MFP1, A DNA-BINDING PROTEIN LOCALIZED IN THE THYLAKOID MEMBRANES, IS ASSOCIATED WITH PHOTOMORPHOGENESIS AND CHLOROPLAST DEVELOPMENT

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Plastid development is related to photomorphogenesis, or vice versa. MFP1 is a nuclear encoded DNA-binding, coiled-coil protein, which is localized in the thylakoid membranes of mature chloroplasts. *AtMFP1*-GFP fusion protein was targeted to plastids in transgenic *Arabidopsis* and BY2 cells. Chloroplast fractionation showed that MFP1 is localized in the thylakoid membrane. This is consistent with the fact that MFP1 has a chloroplast transit peptide and thylakoid signal peptide. Light-grown *Arabidopsis* seedlings express higher level of MFP1 than dark-grown seedlings, which indicates that MFP1 expression is correlated to green tissues. This is consistent with the data that MFP1 expression level is increased 9 hours after 7-day-old etiolated seedlings are moved to light, correlating with greening of cotyledons. MFP1 expression level abruptly decreases during tomato fruit ripening, when chloroplasts develop into chromoplasts. To further correlate MFP1 expression and photomorphogenesis, we investigated MFP1 level in *det1*, *det2*, *det3*, and *cop1* mutants. These mutants develop a light-grown phenotype in the dark, including short hypocotyl, open cotyledons, pigmentation, and the production of true leaves. Dark-grown 7-day-old *det1*, *det2*, *det3*, and *cop1* mutants seedlings express higher level of MFP1 than do dark-grown wild-type seedlings. Together, these data indicate that MFP1 expression is tightly correlated to photomorphogenesis. Its function in photomorphogenesis and/or chloroplast development will be investigated by reverse genetic approaches.

MOLECULAR AND GENETIC DISSECTION OF AN ABA-INDEPENDENT SUGAR SIGNAL TRANSDUCTION PATHWAY

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Recent studies have shown that abscisic acid (ABA) and ethylene interact closely with glucose signaling pathway in the control of *Arabidopsis* seedling development. For example, glucose insensitive mutant *gin6* is allelic to ABA insensitive mutant *abi4*, *gin1* is allelic to ABA-deficient mutant *aba2*, and *gin5* is allelic to *aba3*. Although these results indicate that ABA may act as a second messenger for sugar signal transduction, they might not reflect a physiological role of sugar signaling for normal plant growth and development. Therefore, it is important to use a more defined mutant selection scheme to identify mutants not directly related to ABA.

To identify a unique sugar signaling pathway that does not involve ABA, we attempt to select *trans* mutations that can affect the expression of a marker gene. We have found that *Asparagine Synthetase1* (*ASN1*) is completely repressed by low levels of exogenous glucose (0.1%) in 2 h but is de-repressed upon the removal of glucose. The repression does not require *de novo* protein synthesis (CHX insensitive) but is affected by protein phosphorylation (K-252a sensitive). Interestingly, hexokinase appears to be required for the repression. Critically, we have confirmed that this glucose repression is independent of ABA. We have generated transgenic plants containing the promoter of *ASN1* fused to the *LUC* or *GFP* marker genes. These lines have been obtained and characterized and we are ready to conduct mutagenesis. Unexpectedly, we have already found two mutants prior to the mutagenesis. One transgenic line displays constitutive repression and the other displays constitutive de-repression of the marker gene in response to glucose. It has been confirmed that the phenotype is caused by a *trans*-acting factor and may be specific to sugar. A strategy for the systematic mutagenesis, mutant characterization, and cloning will be discussed.

POST POLLINATION SIGNALING AND SENESCENCE IN ETHYLENE- INSENSITIVE PETUNIAS

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Utilizing ethylene-insensitive petunias, we have investigated the role of ethylene in post pollination signaling and flower senescence. *Petunia hybrida* cv. Mitchell plants expressing the mutated ethylene receptor (35S:*etr1-1*) from *Arabidopsis* are insensitive to ethylene and show extended flower longevity of at least 3 times that of wild type flowers. Senescence of wild type petunia flowers is accelerated by compatible pollination. This pollination-induced senescence is not observed in *etr1-1* flowers, indicating that components of post-pollination signaling are dependent on ethylene. Following pollination, a climacteric peak of ethylene production is detected in wild type corollas at 36 hours after pollination, which initiates senescence within the corolla. In *etr1-1* flowers, pollination is not found to induce a climacteric peak of ethylene production up to 72 hours after pollination. While *etr1-1* flowers eventually die, the symptoms of corolla senescence are visually distinct from the characteristic symptoms observed in wild type flowers. Senescence of wild type flowers begins with wilting at the petal margins followed by complete wilting of the corolla. *Etr1-1* flower senescence is characterized by drying of the petal margins, and subsequent drying of the entire corolla without wilting. To determine if senescence progresses similarly in *etr1-1* and wild type flowers, a typical morphological characteristic of programmed cell death was investigated with an enzymatic *in situ* assay known as TUNEL (TdT-mediated X-dUTP nick end labeling). TUNEL revealed that DNA fragmentation by endonuclease activity coincides with the climacteric peak of ethylene production detected at 36 hours after pollination

DISTINCT SUGAR SIGNALLING PATHWAYS THAT CONTROL SEED GERMINATION AND HYPOCOTYL ELONGATION

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Because of metabolic and structural needs for proper amount of carbon source, different organisms are able to sense levels of sugar and accordingly adjust cellular and metabolic activities. These regulatory mechanisms are particularly important for plants, because sugar production, consumption, and storage occur in the same organism. On one hand, plants have developed sophisticated programs in managing sugar production in source tissue and sugar storage in sink tissue, on the other hand, a complex regulatory circuit controlling gene expression has been evolved to accommodate constant changes of sugar-dependent cellular activities.

Besides gene regulation, sugars are known to affect developmental processes including seed germination, hypocotyls elongation, cotyledon expansion and greening, and root growth. As these processes are also regulated by various plant hormones, we began to dissect the interaction between sugar and hormone signalling pathways in the control of seed germination and hypocotyls elongation. We have found that both glucose and ABA repressed while brassinosteroids (BR) enhanced seed germination. BR-deficient mutants displayed hypersensitivity to the inhibitory effects of both glucose and ABA on seed germination, suggesting an interaction between these response pathways. Interestingly, exogenous BR could correct the hypersensitive response to ABA but not to glucose, suggesting BR and ABA regulate seed germination through a common pathway, which is independent of glucose signalling pathway. Furthermore, exogenous BR could not rescue glucose-induced reduction of hypocotyl elongation, again confirming that sugar controls certain developmental processes through unique signalling pathways. The role of glucose in the control of the expression of genes critical for cell expansion will be further discussed.

THE STEROL BIOSYNTHETIC GENE *FACKEL* IS FEEDBACK REPRESSED BY STEROLS AND ACTIVATED BY AUXIN

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Major plant sterols such as sitosterol and stigmasterol are produced via sterol-specific biosynthetic pathway and are similar in overall chemical structure to ergosterol, the major sterol in fungi, and cholesterol, a major sterol in animals. A regulatory role for sterols in plant development has recently been proposed based on the identification of the *FACKEL* (*FK*) gene encoding a sterol C-14 reductase and the *fk* mutants defective in development and sterol biosynthesis (Jang et al., 2000; Schrick et al., 2000). The sterol C-14 reductase catalyzes a reaction that is upstream of the bifurcation of brassinosteroid-specific pathway and sterol-specific pathway. The block of FK in *fk* mutants has lead to the severe reduction of both brassinosteroids (BR) and sterols.

Because sterol intermediates can regulate the expression of sterol biosynthesis genes in yeast, we examined the effects of various sterols on *FK* expression. In general, *FK* expression was enhanced by both sterols and BL in the short term but was repressed at longer times, indicating a possible negative feedback regulation by these end products. These results indicate that *FK* is subject to potentially important and specific regulation at the transcriptional level by both sterols and BR. Changes in *FK* expression could subsequently affect the levels of specific sterols and BR and their effects on plant development.

Because many aspects of the *fk* mutant phenotype are similar to the phenotypes caused by altered auxin, cytokinin or ethylene responses, we examined the effects of other plant hormones on the expression of *P_{FK}:GUS* in stable transgenic plants. When treated with IAA, intense GUS staining was found throughout the entire plant, suggesting that IAA enhanced *P_{FK}:GUS* transgene expression. In contrast to auxin, *FK* was repressed by brassinolide, again consistent with a possible end product

feedback regulatory mechanism. Other plant hormones such as gibberellic acid, cytokinin, and ethylene had no detectable effects. ABA appeared to exert a modest repression on *FK* expression. In summary, the auxin induction of *FK* could be potentially important for patterning because auxin is known to be involved in patterning and some of the "auxin effects" may actually achieved by the regulation of sterol synthesis and response.

ZEITLUPE ASSOCIATES WITH ARABIDOPSIS SKP1-LIKE PROTEINS (ASKS) VIA THE F-BOX

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The number of genes associated with circadian clock function in plants has increased rapidly in recent years. We focus on the role of the recently cloned F-box protein, *ZEITLUPE* (*ZTL*) in the *Arabidopsis* circadian system. Lesions at this locus cause a significantly longer free-running period with a difference from wild type that is fluence rate dependent in both red and blue light. The presence of an F-box domain, together with a special sub-class of the PAS domain (LOV domain), strongly suggests a role for this protein in light-dependent ubiquitin-mediated degradation of one or more key period-determining factors. This protein is part of a 3-member class of novel clock-associated components, for which there are no known homologues in any other circadian system. This finding further supports the notion that unique components have been recruited in the evolutionary development of the plant circadian clock, setting it apart from other metazoan timing systems.

Two additional regions within *ZTL* are the F-box domain and the kelch repeat domain. Both are thought to mediate protein-protein interactions with specific partners. We have performed yeast two-hybrid interaction screens using both full length *ZTL* and these specific domains and have identified potential *in planta* *ZTL* interaction partners that have been confirmed *in vitro*. We will present the most recent results of the characterization of these interactions.

INFLUENCE OF EXPLANT SOURCE, AND LIGHT ON EFFICIENCY OF AGROBACTERIUM-MEDIATED TRANSFORMATION OF CASSAVA

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Apical leaves, undifferentiated calli pieces, and somatic embryos for cassava cultivar Mcol 2215 were inoculated with *Agrobacterium*-plasmids coding the genes for phosphomannose isomerase (*pmi*), and neomycin phosphotransferase II (*npt II*). The *pmi* gene confers ability to convert mannose-6-phosphate to fructose-6-phosphate (and hence only transformed cells are capable of utilizing mannose as a carbon source), while the *npt II* gene confers resistance to the antibiotic kanamycin. Following inoculation, explants were incubated in the dark (1, 2, or 3 weeks) or transferred directly to a 12-hour light regime, and maintained on selection media (0.3% mannose for *pmi* gene or 75 mg/l paromomycin for *npt II* gene) for 10-12 weeks. Recovery of putative transgenic plants for both gene types was influenced by the source of explant and duration of dark incubation. Germinating somatic embryos incubated in the dark for one week gave the highest number of putative transgenic plants. Overall, more putative plants carrying the *npt II* gene were recovered than for the *pmi* gene. Putative transgenic plants were rooted on half strength Murashige and Skoog medium supplemented with 10 mg/l μ -naphthlaneacetic acid (NAA). Integration of the genes into the plants was verified by PCR analysis, and ranged between 2% (for apical leaves) and 5% (for germinating somatic embryos). The results demonstrate the importance of choice of explant type, and treatment of explant in transformation efficiency of cassava.

THE STATEMENT OF MAPKKK AND MAPK IN RICE BY THE TREATMENT OF AUTOCLAVED CONIDIA OF *M.GRISEA* AND LIPID EXTRACT FROM *M.GRISEA*.

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Plants defend themselves against infectious diseases by launching both preformed and induced defense mechanisms such as the generation of reactive oxygen species, the accumulation of phytoalexin, and a form of programmed cell death called the hypersensitive resistance response (HR). When a pathogen penetrates plant tissue, plants perceive signals brought about by a pathogen. These signals, known as elicitors, can be oligosaccharides, proteins, lipids, and glycoproteins released by the hydrolysis of fungal and plant cell walls. These elicitors can turn on the defense responses by way of rapid changes of transcription factor activity. One of the major routes by which elicitors can trigger plant response is the MAP kinase signaling cascade. This cascade is known to be conserved throughout eukaryotes and to be composed of three protein kinases that act in series: a MAP kinase kinase kinase (MAPKKK), a MAP kinase kinase (MAPKK), and a MAP kinase (MAPK). Previous works have shown that specific MAP kinases become activated during plant responses to invasion by pathogen. Among them, EDR in Arabidopsis, SIPK/WIPK in tobacco, ERMK in parsley suspension cells, BWMK1 in rice and OsMEK1 in rice suspension-cultured cells were reported to be involved in the response to microbial pathogens. Recently, we identified a rice cDNA, named *OsMK3*, which is highly homologous to known plant MAPKKKs and induced by rice blast. We are investigating whether *OsMK3* is induced in rice by the treatment of autoclaved conidia of *M.grisea* or lipid extract from *M. grisea* through Northern blot analysis.

A COMPLETE ELECTRON MICROSCOPIC INVESTIGATION OF CORN STUNT SPIROPLASMA ACCUMULATION AND REPLICATION IN ITS LEAFHOPPER VECTOR *DALBULUS MAIDIS*

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Spiroplasmas belong to the Class Mollicutes, which lack an outer cell wall and have diverged from low G/C Gram-positive bacteria. Three plant pathogenic spiroplasma species are transmitted by phloem-feeding leafhoppers to plants in a propagative persistent manner. This implies that spiroplasmas replicate in various insect organs and move from the gut lumen to the hemolymph and, from there, across the cell layers of the salivary glands into the saliva. With the insect saliva, spiroplasmas are introduced into the plant phloem when the leafhopper feeds. The entry and traversal pathway of spiroplasmas in their insect vectors are not clearly understood. We undertook an electron microscopic investigation of corn stunt spiroplasma (CSS; *Spiroplasma kunkelii*) accumulation and replication in its leafhopper vector *Dalbulus maidis*. Leafhoppers fed from *S. kunkelii*-infected corn plants for two weeks were collected. The head, thorax and abdomen were fixed promptly and embedded in Spurr's resin. Thin sections were stained with uranyl acetate and lead citrate, and examined with a Hitachi-7500 transmission electron microscope (TEM). Thin sections prepared from abdomen contained many spiroplasmas attached to midgut cell microvilli that protrude into the gut lumen. Individual spiroplasma cells were observed in the cytoplasm. High numbers of spiroplasmas were found in the spaces between the plasmalemma and basal lamina of gut cells. Interestingly, numerous spiroplasmas accumulated in between the lamina rara and lamina densa of the basal lamina. Spiroplasmas seemed to detach the layers of the basal lamina from each other and had diverse morphologies, including round, elongated, and flask-like shapes, suggestive of replication, and spiroplasmas showed long fimbriae-like extensions on the cell surface. In addition, at several locations, spiroplasmas appeared to degrade the lamina densa and move into the hemolymph. Thin sections prepared from the leafhopper head revealed many spiroplasmas around and

in between several cell types of the salivary gland. Of the 10 types of salivary gland cells, spiroplasmas were only seen in the cytoplasm and accumulate in the canaliculi of type III cells. In other salivary gland cells, spiroplasmas were observed in between the basal lamina and plasmalemma but were never found in the cytoplasm or canaliculi. Therefore, it is likely that spiroplasmas accumulate in the canaliculi of type III gland cells and move with the saliva into the main ducts and, from there, spiroplasmas are likely to be introduced into the sieve tubes of the plant host. Apart from the gut and salivary gland, spiroplasmas were found in nervous and muscle tissue, Malpighian tubules, tracheal cells and macrophages. This TEM investigation is the first complete analysis of spiroplasma movement in a leafhopper host.

INTERACTION OF AtRanGAP1 WITH FILAMENT LIKE PROTEIN 2 (FLIP2)

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The small GTPase Ran has been implicated in nucleocytoplasmic transport during interphase. The directionality of this transport is determined by a gradient of RanGTP and RanGDP across the nuclear envelope. RanGAP (GTPase activating protein) is involved in establishing this gradient. Animal RanGAP is shown to localize to the nuclear rim by binding to NUP358. No NUP358 ortholog could be identified in the annotated *Arabidopsis* genome, indicating that the anchoring of plant RanGAP to the nuclear rim utilizes different interaction partners. A novel protein domain (WPP domain) was identified that is shared between plant RanGAPs and the plant protein MAF1 (MFP1 associated protein 1), but is not present in RanGAPs from animals. The WPP domain is present at the N-terminal region of RanGAP. Transient MAF1-GFP expression analysis shows that MAF1 localizes to the nuclear rim. Deletion analysis shows that the WPP domain of RanGAP is responsible for its nuclear localization (1).

My experiments have identified a novel plant protein, Filament Like Protein 2 (FLIP2), which has a coil-coiled C terminus. I have shown that it binds to both MAF1 and RanGAP. Co-localization studies will indicate whether FLIP2 is their interaction partner at the nuclear rim.

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MECHANISMS OF GLUCOSE SIGNALING DURING GERMINATION OF *ARABIDOPSIS THALIANA*

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Glucose signaling, along with ABA signaling, has been implicated in regulating early plant development in *Arabidopsis*. It is generally believed that high levels of exogenous glucose cause ABA accumulation, which results in a delay of germination and an inhibition of seedling development- a typical stress response. In order to decipher the complex interactions that occur in the signaling pathways, we determine the effects of sugar and ABA on one developmental event, germination. We show that exogenous glucose as low as 0.5% can cause significant delay of seed germination. Remarkably, this effect cannot be mimicked by an osmotic effect, and ABA is still involved. Because the level of glucose used was low in the experiment, this is the first demonstration that ABA-mediated sugar signaling is not a simple consequence of a stress response. We've found that moderate levels of glucose can relieve repression of germination caused by exogenous ABA, and the kinetics of this relief are remarkably similar in both wild types and glucose- or ABA-insensitive mutants. In addition, we have found that although glucose at different levels can cause transient ABA accumulation, the cellular ABA concentration does not determine whether germination will occur. On the basis of our findings, we propose that germination is controlled by the duration of ABA accumulation and by the temporal expression of genes involved in ABA perception, both processes are likely to be regulated by sugar signaling.

INDUCIBLE *Ac/Ds* ELEMENTS FOR ACTIVATION TAGGING IN RICE

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Rice genome sequencing projects are defining thousands of new rice genes. However, the critical tools required to determine the functions of these genes are not yet available to public-sector researchers. To remedy this deficiency, we are working on rice functional genomics by creating activation-tagged rice mutant collections using the chemical-inducible *Ac/Ds* system.

Previous studies reported the use of an *Ac/Ds* transposon tagging system for generation of plant activation tagged mutants. Transposition of the *Ds* element was achieved by crossing the *Ds* plants with plants carrying an *Ac* transposase gene. Two disadvantages became apparent with this approach. Firstly, the transposition of the *Ac* element was constitutively expressed under the control of 35S promoter and, therefore, both somatic and germinal transposition could occur. Mutations caused by secondary transposition of the *Ac* element confound gene cloning efforts. Secondly, the *Ds* insertions in the genome were not random. To overcome these problems, we have developed vectors using chemical inducible expression, and are using these vectors we will develop a set of mapped starter lines to generate *Ac/Ds* activation tagged lines that are expected to carry insertions throughout the genome.

Using *Agrobacterium*-mediated transformation, rice cultivar Nipponbare was transformed with vectors where the *Ac* transposase is controlled by a GVG-DEX inducible promoter, and the *Ds* transposition can be detected by GUS activity. 30 independently transformed rice calli were transferred to media with DEX to induce expression of the *Ac* transposase. After 20 hrs, the calli were stained for GUS activity. DEX treated calli exhibited stronger GUS staining than untreated controls.

Alternatively, we have developed and are testing a vector that employs the Cre-Lox recombination system to reduce *Ds* transposition. In vector Ds-AcGR/Cre-Lox, the *Ac* transposase is fused with the GR (glucocorticoid receptor). Localization of the AcGR protein in the plant cell depends on DEX treatment. In the presence of DEX, the AcGR is bound with DEX, and transferred into the nucleus where it mediates *Ds* transposition. In the absence of DEX, the AcGR protein remains in the cytosol, and *Ds* transposition is not activated. This system will facilitate gene tagging by removal of *Ac* transposase after *Ds* transposition.

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IDENTIFICATION AND FRACTIONATION OF ALGAL EXUDATES THAT AFFECT BACTERIAL QUORUM SENSING

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For bacteria to be successful pathogens they need to present in sufficient numbers to overcome the host defense systems. Bacteria use a mechanism called quorum sensing to self monitor their population densities. Signaling molecules including Autoinducer1 (acylated homoserine lactone involved in gram negative bacteria signaling) and Autoinducer2 (a furanosyl borate diester derivative for Inter-species bacterial communication), as well as others signaling molecules (gram positive bacteria) activate a number of genes including genes related to virulence, symbiosis, light production, biofilm formation, etc¹. Plants have been shown to produce mimic compounds that interfere with these signals². None of the plant mimic compound structures have been solved to date. Our interest is to identify, purify, and characterize bacterial quorum sensing signal mimic compounds from the algae *Chlamydomonas reinhardtii*. This organism is a single celled eukaryote with fast reproductive cycle and is a good model for genetic analysis. Preliminary analysis with solvent partitioning of algae cell filtrate and HPLC fractionations indicated the presence of Autoinducer1 and Autoinducer2 mimic compounds. Interestingly, there were both stimulatory and inhibitory (nontoxic) activities. Further aim of this project is to purify each fraction for ESI-Mass Spectrometry analysis and NMR analysis. Our long-term objective is to carry out genetic analysis of the genes involved in the mimic production. This will help us to manipulate algae for several useful pharmaceutical applications.

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IDENTIFICATION AND ISOLATION OF GENES FROM SOYBEAN (*GLYCINE MAX*) ORTHOLOGOUS TO COLD-REGULATED GENES FOUND IN *ARABIDOPSIS THALIANA*

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Many plants have the ability to increase in freezing tolerance after exposure to low nonfreezing temperatures, a process known as cold acclimation. Numerous cold regulated (*COR*) genes, which contribute to this cold acclimation process, have been identified in *Arabidopsis thaliana*. The *COR* genes are a battery of genes whose coordinate low temperature expression is controlled by the CBF family of transcriptional activator proteins, themselves induced by low temperatures and together make up the CBF regulon. While many plants are not capable of cold-acclimation, it is apparent through data base searches that most plants harbor genes encoding proteins highly similar to those encoded by the *CBF* and *COR* genes. One hypothesis is that one or more of these *CBF* orthologous genes no longer responds to low temperatures making the entire regulon non responsive to low temperature. One objective of this research is to identify genes in soybean, which are potential orthologs of those making up the Arabidopsis CBF regulon, and to characterize their expression after subjecting soybean plants to low temperature. To accomplish this, the TIGR Soybean EST database was searched using the Arabidopsis sequences as queries. Numerous candidates to both the CBF regulators and the downstream genes they might control were identified. These genes have been isolated from soybean genomic DNA and mRNA using PCR and RT-PCR respectively and the identity of the resulting clones confirmed by DNA sequence analysis. This is an early step in which the long-term goal is to understand the molecular mechanisms by which different plants tolerate extremes in cold temperatures.

TARGETING OF PLANT RANGAP TO THE NUCLEAR ENVELOPE

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Ran is a small GTP-binding protein that is required for the trafficking of proteins and RNA in and out of the nucleus. The directionality of transport is determined by a gradient of RanGTP and RanGDP across the nuclear envelope. In mammalian cells, this gradient is formed by the spatial separation of RanGAP (Ran GTPase activating protein) outside and RCC1 (Ran nucleotide exchange factor) inside the nucleus. RanGAP is associated with the outer basket of the nuclear pore complex and contains a C-terminal domain required for binding to the nuclear pore protein NUP358. This domain is not present in yeast and plant RanGAP proteins, therefore suggesting a different mechanism or no targeting of RanGAP to the nuclear envelope in those organisms. We have determined that a GFP fusion of Arabidopsis RanGAP1 (AtRanGAP1) is targeted to the nuclear envelope in tobacco BY-2 cells. A novel protein domain was identified that is shared between plant RanGAP and a plant protein (MAF1) previously shown to be associated with the nuclear envelope. The analysis of AtRanGAP1 deletion clones showed that this domain is necessary and sufficient for nuclear envelope targeting. It is not present in RanGAPs from animals and yeast, indicating a plant specific mechanism of RanGAP targeting. A protein that interacts with both AtRanGAP1 and MAF1 was identified in a yeast two-hybrid screen and was shown to bind to the nuclear envelope-targeting domain of AtRanGAP1. Its potential function in anchoring RanGAP to the nuclear envelope in plants is under investigation.

PROLINE ACTION ON HEAVY METAL DETOXIFICATION IN MICROALGAE

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Free proline has been shown to play an important role in ameliorating environmental stress in plants and microorganisms, including heavy metal stress. Here we describe the expression of a plant pyrroline-5-carboxylate synthase (*P5CS*) gene in the green microalga, *Chlamydomonas reinhardtii*. We show that transgenic algae expressing the *P5CS* gene have 80% higher free-proline levels than wild-type cells, tolerate toxic cadmium concentrations (100 μ M), and bind four-fold more cadmium than wild-type cells when grown in the presence of 50 μ M Cd. Extended X-ray absorption fine structure (EXAFS) studies indicated that the cadmium does not directly bind to free proline. Analyses of the fatty acid products of free-radical damage, malondialdehyde (MDA), and determinations of redox state of the cell (oxidized glutathione (GSSG) levels) indicated that free proline reduces free radical damage and maintains a more reducing environment in the cells are exposed to toxic heavy metals (50 μ M Cd). These results suggest that the free proline likely acts as an antioxidant in cadmium stressed cells. The resulting elevated glutathione (GSH) levels would presumably allow for increased phytochelatin synthesis since GSH-heavy metal adducts are the substrate for phytochelatin synthetase. Increased phytochelatin synthesis would in turn lead to increased cadmium accumulation and detoxification.

**REDUCTION IN THE CYANOGENIC POTENTIAL OF CASSAVA ROOTS;
TRANSGENIC PLANTS EXPRESSING HYDROXYNITRILE LYASE IN THE ROOTS**

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Unlike leaves, cassava roots lack the enzyme hydroxynitrile lyase (HNL) which converts acetone cyanohydrin (from linamarin de-glycosylation) into cyanide plus acetone. As a result poorly processed cassava roots may contain sufficient quantities of acetone cyanohydrins to cause cyanide-associated disorders in consumers. We report here the stable genetic transformation of cassava with a cassava HNL gene whose expression is regulated by the 2X35S constitutive promoter.

Hydroxynitrile lyase activity from leaves and stems of transformed plants was up to 2.3 fold higher than wild-type. Higher HNL levels were associated with a 61% decrease in total acetone cyanohydrins in roots following processing for 120 minutes.

Cassava transformants expressing HNL in roots are expected to accelerate the cyanogen detoxification process and in turn provide a safer food product for human consumption. Significantly, these plants will maintain normal levels of cyanogenic glycosides prior to cell rupture, thus providing the same herbivore deterrent capabilities as wild-type.

DOMESTICATION OF CASSAVA: GENERATION OF CYANOGEN-FREE CASSAVA

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Cassava (*Manihot esculenta*) is one of the most important food crops in tropical Africa, Asia and South America. Cassava, however, contains the cyanogenic glucoside linamarin which when hydrolyzed yields cyanide. Consumption of poorly processed cassava having residual cyanogens can cause goiter, tropical atoxic neuropathy, permanent paralysis and, in rare cases, death.

The first dedicated step in linamarin synthesis is the conversion of valine to its oxime catalyzed by a cytochrome P450. In 2000, Anderson et al. isolated two full-length cDNA clones (CYP79D1 and CYP79D2) that encode the cytochrome P450s that catalyze this reaction.

Our objective has been to produce a safer cassava food product having reduced linamarin content. In addition, we are interested in analyzing the potential movement of cyanogenic glucosides between plant organs. Our strategy was to produce acyanogenic cassava plants by down regulating the expression of the CYP79 genes using anti-sense technology. To accomplish this goal a recombinant DNA plasmid was constructed using the *Agrobacterium* binary vector pBI121. The modified vector has the 5' ends of the CYP79D1 and CYP79D2 genes cloned in anti-sense orientation. Each of the CYP79 genes is expressed under the control of a leaf specific Cab1 promoter. The modified binary vector was transformed into *Agrobacterium* strain LBA4404, which was then used to transform cassava germinated somatic embryos.

We have successfully generated transgenic cassava plants in which the steady-state levels of the CYP79D1 and CYP79D2 transcripts have been inhibited or substantially reduced in leaves. Using GC-MS, the linamarin content of leaves from six independent transformants lacking or having reduced CYP79 transcript levels was shown to be reduced by as much as 94%. The same transformants also exhibited a substantial reduction in root linamarin content, down to 1% of wild-type levels. This is the first cassava plant that has essentially no linamarin in its roots. Interestingly, this reduction of linamarin content in the roots of the Cab1 transformants was not associated with a reduction in the root CYP79D1 or D2 transcript steady-state

levels. This observation suggests that linamarin made in the leaves may be transported down to the roots.

In summary, we have generated acyanogenic cassava providing a safer alternative to cultivars currently grown by subsistence farmers in Africa. In addition, acyanogenic cassava plants provide a more marketable food product, potentially providing additional sources of income generation for subsistence farmers. Finally, acyanogenic cassava also would help alleviate the release of cyanide waste from commercial cassava processing facilities.

Anderson M, Busk P, Svendsen I, and Moller B. 2000. Cytochromes P-450 from Cassava (*Manihot esculenta* Crantz) catalyzing the first steps in the biosynthesis of the cyanogenic glucoside linamarin and lotaustralin. *J. Biological Chemistry* 275:1966-1975.

IDENTIFICATION OF PROTEINS THAT INTERACT WITH *ARABIDOPSIS* RAN GTPASE ACTIVATING PROTEIN 1

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Ran GTPase activating protein, RanGAP, is a protein that is involved in nucleocytoplasmic transport. Animal RanGAP is associated with the outside of the nuclear pore complex by the binding of its C-terminal domain to the nucleoporin Nup358. Plant RanGAP has been identified, but its function in nuclear import/export has not yet been characterized. It has recently been shown that *Arabidopsis* RanGAP 1 (AtRanGAP1) uses a different N-terminal domain, called the WPP domain, for binding to the nuclear envelope. It is now important to find the interaction partner for this domain at the plant nuclear envelope. The yeast two-hybrid system has been employed in identifying six proteins that bind to AtRanGAP1. Their cDNAs have been sequenced and their interaction with AtRanGAP1 deletion clones, a WPP mutant, and other WPP domain proteins has been tested. Five proteins interact with the AtRanGAP1 WPP domain, varying in their specificity for other WPP domain proteins. All have putative nuclear localization signals. One protein contains a kelch domain and a transmembrane domain, and another has homology to centromere proteins. The goal of the present research is to further characterize these novel proteins with respect to their subcellular location.

EVIDENCE FOR HETEROGENEITY OF RIBOSOMES IN ARABIDOPSIS

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Ribosomes are essential organelles composed of ribosomal RNA (rRNA) and ribosomal proteins (rProteins). The main function is to synthesize a protein molecule based on information contained in a messenger RNA (mRNA). Ribosomes are composed of three to four rRNA molecules and a large number of proteins (up to 80 or more). Because stoichiometric amounts of each rProtein must be present for efficient assembly and the number of genes involved, rProtein genes have been the subjects of studies in coordinated gene regulation in bacteria and animals.

In *E. coli* each rProtein is encoded by a single gene, in *Saccharomyces* a single gene or a two-member gene family encodes most proteins. In mammals there are small gene families for each rProtein, however, it appears that only one member is functional and the rest are pseudogenes. In plants a small gene family with two or three members encodes most proteins. Most of these appear to be functional and represented in the (expressed sequence tag) EST collection. The deduced amino acid sequence of each member in plants varies from identical to 74% identity. The high degree of similarity among different family members has made it difficult to determine if products from all of the gene families are incorporated into plant ribosomes and if some of the family members have specialized roles.

We have characterized two ribosomal protein genes (L3A and L3B) from *Arabidopsis* that encode a homolog of the *E. coli* L3 protein. These two genes are relatively divergent, sharing approximately 75% nucleotide identity and 85% amino acid identity. L3A appears to be constitutively transcribed at high levels in all tissues. Its mRNA is polyadenylated and can be isolated from the polyribosome fraction. The protein can be isolated from shoots and roots and it is abundant in polyribosomes.

L3B, appears to be transcribed in all tissues, it is more abundant in root than shoot. Its mRNA has several unusual features. First it has a very short 5' UTR. Despite its short length it does have a CT rich region, typical of the 5' UTR of ribosomal protein mRNAs. Second, its mRNA is found in the poly A- fraction after separation on an oligo-dT column. Sequence analysis and RT-PCR using an oligo-dT primer for first strand synthesis has located a polyadenylation site. At this site a short polyA tail appears to be present on at least a majority of the messages and there is evidence that mRNA processing is a method of regulation for this gene. L3B mRNA can also

be isolated from polyribosomes; Western analysis confirms the presence of L3B protein in shoot and root polyribosomes, with it being more prevalent in root tissue.

TDNA insertion mutants from the SALK collection have been identified for both L3A and L3B. The TDNA is inserted in the second exon of L3A and in the 3' UTR for L3B. The L3A mutation is lethal for the homozygote and the hemizygote shows reduced viability. The L3B mutation also appears to be lethal for the homozygote and the hemizygote shows reduced viability.

CHARACTERIZING ARABIDOPSIS CALLOSE SYNTHASE AND CALLOSE SYNTHASE COMPLEX

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Callose is β -1,3 glucan with β -1,6 branches. It is found in plant cell plate, pollen tube, phloem cells and plasmadesmata. Callose synthesis is location specific and highly regulated. Arabidopsis membrane protein was extracted from, and the properties of callose synthase was studied. The pH optima for callose synthase is 7.1, and within the range of pH 6.9 to 7.3, the activity is not significantly changed. The Ca^{2+} optima is 0.3 to 0.6 mM, with higher concentration inhibiting the activity. The enzyme is salt sensitive. Callose synthesis can continue linearly for up to at least 60 minutes when 6 to 12 μ g total protein was used. The K_m is 5.0 mM for callose synthase. Product entrapment procedure was used to purify callose synthase enzyme, and 4 protein bands were detected. Further sucrose gradient fractionation of the enzyme was carried out to further separate the proteins, and one peak callose synthase activity was detected, which contained 3 major protein bands. Further work will be done to identify the proteins of the callose synthase complex.

MOLECULAR CHARACTERIZATION OF AN11/TTG-LIKE WD40 GENES

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Proteins containing the WD40 domain are widely distributed in all major kingdoms and participate, largely through protein-protein interactions, in a wide range of processes including signal transduction, control of gene expression, and RNA splicing. One group of these WD40 proteins include TTG in *Arabidopsis* and AN11 in petunia. These proteins regulate trichome and/or anthocyanin accumulation, probably by modulating the activity of corresponding Myb and HLH transcriptional activators. The cloning of the maize *mp1* gene, originally thought to correspond to the maize ortholog of *an11*, indicated that within the *an11* group of WD40 genes, there are two distinct classes. One class is represented by *ttg*, *an11* and the maize *pac1* gene (a regulator of anthocyanin accumulation) and a second class contains *mp1* and two *an11*-related genes from *Arabidopsis*, *AtAn11a* and *AtAn11b*. The *AtAn11a* and *AtAn11b* genes have been identified and cloned and their functions are currently being investigated. Loss-of-function mutants are being constructed using double-stranded RNA interference (dsRNAi).

FUNCTIONAL ASYMMETRY OF PHOTOSYSTEM II D1 AND D2 PERIPHERAL CHLOROPHYLL MUTANTS OF CHLAMYDOMONAS REINHARDTII

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The peripheral accessory chlorophylls of the photosystem II reaction center are coordinated by a pair of symmetry related histidine residues (D1-H118 and D2-H117). These chlorophylls participate in energy transfer from the proximal antennae complexes (CP43 and CP47) to the reaction center core chromophores. In addition, one or both of the peripheral chlorophylls is redox active and participates in a low quantum yield electron transfer cycle around photosystem II. We demonstrate that conservative mutations of the D2-H117 residue result in decreased chlorophyll fluorescence quenching efficiency attributed to altered formation of the peripheral accessory chlorophyll cation, Chl_z^+ . In contrast, identical symmetry related mutations at residue D1-H118 had no effect on chlorophyll fluorescence yield or quenching kinetics. Mutagenesis of the D2-H117 residue also altered the line width of the Chl_z^+ EPR signal but the lineshape of the D1-H118Q mutant remained unchanged. The D1-H118 and D2-H117 mutations also altered energy transfer properties in photosystem II reaction centers. Unlike wild type or the D1-H118Q mutant, D2-H117N reaction centers exhibited a reduced circular dichroism doublet in the red-region of chlorophyll absorbance band, indicative of reduced energetic coupling between P680 and the peripheral accessory chlorophyll. In addition, transient absorption measurements of D2-H117N reaction centers, excited on the blue side of the chlorophyll absorbance band, exhibited a new (~400 fs) pheophytin Q_x band bleach lifetime component not seen in wild-type or D1-H118Q reaction centers. The origin of this component may be related to delayed fast energy equilibration of the excited state between the core pigments of this mutant.

MODULATION OF THE DIRECTIONALITY OF PSII ELECTRON TRANSFER BY MUTAGENESIS OF THE D1-E130 IN CHLAMYDOMONAS

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Two potential parallel pathways for the reduction of pheophytins (Pheo) by the primary electron donor, P680, are present in the photosystem II reaction center complex. However, only one of them is physiologically active. We have perturbed the protein environment of the putative active Pheo by site-directed mutagenesis of the glutamate residue at position 130 of D1 (D1-E130) to determine its influence on the electron transfer. It was observed that the D1-E130 residue hydrogen bonds (H bond) to the active Pheo with longer wavelength absorbance, similar to the glutamate residue at position 104 on the L subunit (L-E104) in the bacterial photosynthetic RC. The D1-E130L mutant neither shows substantial oxygen evolution activity, nor forms high fluorescence state P680Q_A⁻ in nanosecond timescale. Spectroscopic analyses show that D1-E130L mutation induces a distinct blue shift in both the ground state and transiently reduced Pheo Q_x absorption band, indicating that the H bond strength changes, and/or inactive Pheo is partially reduced. In contrast, a D1-E130H mutant had near wild type (WT) rates of oxygen evolution and had an almost undistinguishable effect on the ground state or the reduced Pheo Q_x spectrum, consistent with the observation that only a minor perturbation of the hydrogen bonding interaction as found by high field EPR studies. Significantly, the D1-E130Q mutation caused a distinct blue shift only on the reduced spectrum. The rate constant for charge separation measured by both femtosecond transient absorption and time-resolved chlorophyll fluorescence kinetics, S₂-multiline signal, as well as the steady-state accumulation of the Q_A-Fe²⁺ EPR signal at cryogenic temperatures are essentially the same in all the D1-E130 mutants as that in WT. Therefore, the decreased efficiency of electron transfer around PSII of D1-E130L mutant evidenced by oxygen evolution rate and microsecond fluorescence decay kinetics is indicative of

structural changes of Pheo or Q_A. Furthermore, the directionality of the ultrafast electron transfer may be partially modified by D1-E130L mutation.

**TOWARDS MOLECULAR CLONING AND FUNCTIONAL ANALYSIS OF *SPL11*,
A GENE INVOLVED IN PROGRAMMED CELL DEATH AND BROAD-SPECTRUM
DISEASE RESISTANCE IN RICE**

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Rice lesion mimic mutant *sp11* was previously shown to confer non-race specific resistance to both rice blast and bacterial blight diseases. To gain an in-depth insight into the molecular basis underlying the cell death and broad-spectrum disease resistance in rice, the *Sp11* gene is being cloned using a positional strategy. Three RAPD markers linked to *sp11* were developed and the gene was mapped on rice chromosome 12. Based on genetic mapping and physical mapping analysis, a BAC contig that spanned the *Sp11* region was constructed. Fine mapping indicated that *Sp11* is located in a 150 kb region on BAC78. This BAC clone was partially sequenced and candidate genes were uncovered. One of the candidates, mitochondrial inner membrane translocase, showed differential expression between *sp11* and wild type plant IR68. High-resolution fine mapping with a large F2 population (1,800 individuals) is being carried out to pinpoint *sp11* on BAC78. Complementation of *sp11* using 50-100 kb insert in a transformation competent artificial chromosome (TAC) vector is in progress. To identify components that may involve in *sp11*-mediated cell death and defense signaling pathway, *sp11* seeds were treated with the chemical mutagen diepoxybutane (DEB). Twenty-nine lines that showed differential phenotypes in the suppression of the lesion mimics were identified in the M2 population. Genetic and molecular analysis of these suppressors is undergoing.

CLONING AND CHARACTERIZATION OF THE BROAD-SPECTRUM BLAST RESISTANCE GENE *PI2*

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Rice blast is the most devastating disease in rice production worldwide. The yield loss due to the epidemic of the disease is estimated to be about US\$ 5 billions per year. *Pi2* is one of the major genes with broad-spectrum resistance to the rice blast. To fine-map the gene on chromosome 6, DNA was extracted from over 500 susceptible F2 and F3 plants. Five primers pairs (RG64, NBS1, NBS2, NBS4, NBS6), based on the genomic sequence at the *Pi9* region, were used in the screen. No recombinant between NBS2 and NBS4 was found in all screened plants. Together with our previously RFLP mapping results, we confirmed that NBS2 has no recombination with *Pi2* in over 3000 F2 plants. Both BAC and TAC library of the *Pi2* resistant line A51 were constructed with the average size about 40kb. About 400,000 BAC and TAC clones were stored, which is over 40 equivalents of the rice genome. Three primer pairs (NIP, NBS2, NBS4) were used to screen the two libraries. Four clones (BAC70, BAC23, TAC40, BAC6) spanning the *Pi2* region were identified. Using primer pairs designed based on the end sequence of BAC6, three recombinants were found in the same mapping population, confirming that *Pi2* is within the BAC contig. Three shotgun libraries were constructed from BAC70, TAC40 and BAC6. Over 1600 subclones containing only the insertion were picked and are being sequenced. Transformation of the *Pi2* candidate genes is in process.

COVER IMAGES

POSITION	IMAGE	SOURCE
BACKGROUND	MICROARRAY	PLANT-MICROBE GENOMICS FACILITY
TOP UPPER	PLANT CELLS	DEPARTMENT OF PLANT BIOLOGY
TOP LOWER	INDUCTION OF SOMATIC EMBRYOS IN APICAL LEAVES OF CASSAVA	RICHARD T. SAYRE
BOTTOM UPPER	CATTELYA INTERMEDIA 'JOHN FINER'	ED MERKLE/JOHN FINER
BOTTOM LOWER	GENOTYPING ANALYSIS DATA*	XIAO YANG/TARA VAN TOAI/ PLANT-MICROBE GENOMICS FACILITY

*A SIMPLE SEQUENCE REPEAT (SSR) ANALYSIS WAS DONE WITH CORN GENOMIC DNA TO IDENTIFY THE PRESENCE OF MARKERS FOR A WILDTYPE OR LOW-FATTY ACID PHENOTYPE IN THE KERNEL. THE TOP AND MIDDLE TRACES HAVE A SINGLE FRAGMENT OF 132 BASES INDICATING IT IS HOMOZYGOUS FOR THE MUTANT MARKER WHEREAS THE BOTTOM TRACE HAS A BAND 128 BASES LONG INDICATING IT IS HOMOZYGOUS FOR THE WILDTYPE MARKER. THE TRACES SECOND FROM THE TOP AND SECOND FROM THE BOTTOM HAVE TWO PEAKS INDICATING THESE INDIVIDUALS ARE HETEROZYGOUS FOR THE MARKER.