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MAS-ASPB Annual Spring Meeting
American Society for Plant Biologists (ASPB)

12th Annual Minisymposium on Plant Biology
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MAS-ASPB Annual Spring Meeting
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12th Annual Minisymposium on Plant Biology
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Mid Atlantic Section ASPB Annual Spring Meeting
Friday, April 8, 2011
Crist Board Room, Riggs Alumni Center
University of Maryland, College Park, MD

8:30 Registration, coffee and pastry

8:55 Welcome, **Gary Coleman**, ASPB Mid-Atlantic section chair

9:00 Platform presentations

Session 1 (**Gerry Deitzer**, session chair)

9:00 **Thomas Boothby** (Cell Biol. & Mol. Genetics, Univ. of Maryland)
" Subnuclear storage of masked mRNA in the microspore of *Marsilea vestita* "

9:20 **Sijacic Paja** (Cell Biol. & Mol. Genetics, Univ. Maryland)
" A recessive antimorphic allele overcomes genetic redundancy and reveals TSO1 function in flower and meristem development "

9:40 **Salil Chanroj** (Cell Biol. & Mol. Genetics, Univ. Maryland)
" Arabidopsis CHX16-20 are endomembrane cation/proton transporters with distinct activities and emerging roles in protein sorting"

10:00 **Qingmei Guan** (Plant Science and Landscape Architecture, Univ. Maryland)
" Identification of a Salt Tolerance Determinant, SRS2"

10:20 Coffee Break

Session 2 (**Mark Holland**, session chair)

10:35 **Cheng Dai** (Univ. Maryland)
" Effects and functional mechanism of rice EL1, a casein kinase I, in gibberellin acid signaling"

10:55 **Nabanita Kundu** (Biology, Howard University, Washington, DC)
" Tyrosine Phosphorylation and Sumoylation based homo-dimerization of Arabidopsis RACK1A protein regulates Distinct Oxidative Stress Signaling Pathways"

11:15 **Ross Sager** (Delaware Biotechnology Inst, Dept. Plant and Soil Sciences, Univ. Delaware)
" PDLP5, a dual regulator of the SA defense pathway and PD-mediated cell-to-cell communication"

11:45 Move to 1103 Bioscience Research Building for noon talk

12:00 **Featured Speaker**: **Richard Jorgensen** (1103 Bioscience Research Bldg)
(Centro de Investigación y Estudios Avanzados (CINVESTAV), Irapuato, Guanajuato, Mexico)
"Evolutionary and Functional Diversification of the Epigenome: a 'Paragenetic' Perspective on the Role of RNA Silencing in the Biology of Plants"

1:00 **Lunch on your own**

Session 3 (**Gary Coleman**, session chair)

2:00 **Arianne Tremblay**
(USDA-ARS, Soybean Genomics & Improvement Laboratory, Beltsville, MD)
" Using Illumina mRNA-Sequencing to understand *Glycine max-Phakopsora pachyhrizi* interaction"

2:20 **Mandy Kendrick**
(USDA-ARS, Foreign Disease-Weed Science Research Unit, Fort Detrick, MD)
" Identification of soybean genes that confer resistance to Asian soybean rust "

2:40 **Robert Berkey** (Center for Biosystems Research, Institute for Bioscience and Biotechnology Research, Rockville, MD)
" PI3P, a landmark for trafficking at the host-pathogen interface?"

3:00 **Keynote speaker: Manuel Lerdau,**
Dept. of Environmental Sciences, University of Virginia
"Bi-Directional Scaling of Isoprene Physiology: From Membranes to the Atmosphere"

3:45 Coffee Break

Session 4 (**Zhongchi Liu**, session chair)

4:00 **Zhongchi Liu** (MAS-ASPB, section representative)
"Updates from the Am. Soc. Plant Biologists: ambassador program & beyond"

4:10 **Invited speaker: Dong Wang** (Department of Biology, Stanford University)
"Herding bacteria: host factors required for nitrogen-fixing symbiosis"

4:40 **Invited speaker: Ludmila Tyler**
(University of California, Berkeley and Plant Gene Expression Center)
"*Brachypodium distachyon* as a model for bioenergy grasses: Natural and induced variation in cell walls"

5:10 Mid-Atlantic section meeting and announcements

April 9 (Sat)-12th Annual Plant Biology Minisymposium
University of Maryland, College Park, MD
1103 Bioscience Research Building

8:30 **Coffee and pastry** (outside of room Rm 1103)
poster installation

9:00 **Opening remarks** (Hua Lu, organizer)

9:05 **Session I** (Hua Lu, session chair)

9:05 **Charles Delwiche**
(University of Maryland, Dept. of Cell Biology and Molecular Genetics)
"Charophyte transcriptomics and the evolutionary origin of land plants"

9:25 **Stephen Miller**
(Univ. Maryland, Baltimore County, Dept. of Biological Sciences)
"Chlamydomonas, Volvox, and the evolution of multicellularity"

9:45 **Janet Slovin** (USDA)
"Genomic resources for the diploid woodland strawberry: a reference plant for the Rosaceae"

10:05 **Coffee break** (outside of Room 1103) and poster installation

10:40 **Session II** (Ganesh Sriram, session chair)

10:40 **Priscila Chaverri**
(University of Maryland, Dept. of Plant Science and Landscape Architecture)
"The fungal genus /Trichoderma: evolution of ecological roles and nutritional modes and its relationships with plants"

11:00 **Basil Nikolau**
(Iowa State University, Dept. of Biochemistry, Biophysics and Molecular Biology)
"The importance of surfaces in biological processes. "

11:20 **Sharlene Weatherwax**
(Department of Energy)
"Biological and Environmental Research at the Department of Energy"

11:40 **Bruce McClure**
(Univ. Missouri & National Science Foundation)
" S-RNase-based Self-incompatibility: following S-RNase in pollen tubes"

12:00 **Lunch and poster viewing** (BRB ground floor)

1:40 **Session III** (Shunyuan Xiao, session chair)

1:40 **John M. McDowell**
(Virginia Tech., Dept. of Plant Pathology, Physiology and Weed Science)
" How do biotrophic pathogens survive inside hostile hosts?"

2:00 **Harsh Bais**
(University of Delaware, Dept. of Plant and Soil Sciences and the Delaware Biotechnology Institute)
"Probiotics for plants: Impact of belowground beneficials on aboveground pathogens"

2:20 **Yinong Yang**
(Penn State University, Dept. of Plant Pathology and Huck Institute for the Life Sciences)
"Role of ethylene, abscisic acid and MAP kinase pathways in rice disease resistance"

2:40 **Break and poster viewing**

3:10 **Session IV** (Hemayet Ullah, session chair)

3:10 **Rich Jorgensen**
(Centro de Investigación y Estudios Avanzados (CINVESTAV), Irapuato, Guanajuato, Mexico)
" Conserved Peptide Upstream Open Reading Frames in Plant mRNAs are Associated with Regulatory Genes "

3:30 **Steve Wolniak**
(Dept. of Cell Biology and Molecular Genetics, Univ. Maryland)
"Regulatory Control over Rapid Development in Marsilea"

3:50 **Taishi Umezawa**
(Visiting scholar, Univ. Maryland & RIKEN Plant Science Center, Tsukuba, Japan)
"Protein phosphorylation network in abscisic acid signaling"

4:10 **Wine and beer reception** (outside BRB 1103)
Posters (final chance to see any posters that are still up)

5:00 **End of meeting. Take down all posters**

7:39 **Sunset** (You have over two hours of daylight to enjoy the cherry blossoms!)

ORAL PRESENTATION

1. Subnuclear storage of masked mRNA in the microspore of *Marsilea vestita*.

Thomas Boothby and Stephen M. Wolniak
Dept. of Cell Biology and Molecular Genetics, Univ. of Maryland, College Park, MD 20742

Like many rapidly developing systems, the microspore of *Marsilea vestita* relies on the regulated translation of stored transcripts for the formation of new proteins essential for morphogenesis. Here, we present evidence that the microspore of *Marsilea vestita* stores translationally-inactive, or "masked," mRNAs in a large aggregate within its nucleus. Desiccation is essential for the maturation of microspores from *M. vestita*. Using a newly developed differential DNA/RNA staining assay, we have found that dehydration triggers the accumulation of RNA within several nuclear particles, which share some protein constituents with nuclear speckles. These particles coalesce during spore desiccation to form a single large aggregate in the interchromosomal space of the nucleus of the dried spore. Rehydration of the microspore triggers rapid gametophyte development and spermatogenesis. During rehydration, masked subnuclear mRNAs are inaccessible to traditional FISH probes. We have developed a modified in situ hybridization assay that allows us to distinguish between masked and unmasked mRNAs. Masked transcripts are initially observed exclusively within the nucleus of the gametophyte and immunolabeling reveals that these mRNAs are stored in association with pre-mRNA processing proteins. The proteins and transcripts apparently aggregated in the microspore nucleus during desiccation. The large nuclear aggregate is displaced to the cytoplasm during nuclear envelope breakdown of the first mitotic division in the gametophyte. As successive division cycles occur in the developing gametophyte, the RNA/protein aggregate undergoes fragmentation, and is passed asymmetrically to spermatogenous, but not sterile cells. This asymmetric distribution of masked mRNA and protein to the spermatogenous cells requires EJC core component Mv-Mago. Supported by NSF grants MCB-0720486 and DBI-0842525 to SMW.

2. A recessive antimorphic allele overcomes genetic redundancy and reveals TSO1 function in flower and meristem development

Sijacic Paja and Zhongchi Liu
Dept. of Cell Biology and Molecular Genetics, Univ. of Maryland, College Park, MD 20742

Arabidopsis TSO1 encodes a putative transcription factor that belongs to an eight-member gene family. TSO1 contains two CXC motifs, a cysteine-rich regions that can bind zinc ions *in vitro* and serve as DNA anchoring domains. *tso1-1*, which results from a missense mutation, develops inflorescence meristems that are often enlarged and fasciated. *tso1-1* floral organs of inner three whorls are missing and instead are replaced by a callus-like tissue rendering *tso1-1* plants sterile.

In contrast, *tso1-3*, a nonsense mutation leading to premature protein termination, showed very mild phenotype, affecting only male and female gamete development and dramatically reduced fertility.

To answer the question why *tso1-1* missense mutation causes much stronger developmental defects than *tso1-3* nonsense mutation, we used several different approaches. First, we knock-downed *TSO1* expression using artificial microRNA as an attempt to identify loss-of-function or null phenotypes. Second, we identified and characterized T-DNA insertional mutations in *TSO1*. Third, we performed double mutant analyses between *TSO1* and two closest *TSO1* homologs, *SOL1* and *SOL2*, to test for genetic redundancy. These results strongly support that *tso1-1* is a recessive antimorphic allele that not only knocks down TSO1 function but also reduces functions of other family members.

To further understand TSO1 function, a microarray was performed to identify potential target pathways that TSO1 and its family members regulate. Further verification of TSO1 targets is in progress.

3. Arabidopsis CHX16-CHX20 are endomembrane cation/proton transporters with distinct activities and emerging roles in protein sorting

Salil Chanroj[a], Yongxian Lu[a], S Padmanaban[a], K Nanatani[c], N Uozumi[c], Rajini Rao[b] & Heven Sze[a]
[a]Dept. Cell Biology & Molecular Genetics, Maryland Agr Exp Sta, Univ. Maryland, College Park, MD
[b]Dept. Physiology, Johns Hopkins University School of Medicine, Baltimore, MD, USA
[c]Dept. Biomolecular Engineering, Tohoku University, Sendai, Japan

The dynamic endomembrane system is emerging as a critical coordinator of plant growth, development and adaptation to stress, yet mechanisms regulating the environment of the intracellular compartments in plants are not well understood. A large family of cation/H^+ exchangers, represented by 28 *CHX* genes in *Arabidopsis thaliana*, is associated with diverse endomembrane compartments and tissues in plants. We expressed a phylogenetically-related cluster of CHX proteins, encoded by CHX15-20, in yeast and bacterial cells engineered to lack multiple cation handling mechanisms. Of these, CHX16-20 were implicated in pH homeostasis as their expression rescued alkaline pH-sensitive growth phenotype of the host yeast strain. A smaller subset, CHX17-19, also conferred tolerance to the cationic drug hygromycin B. Further differences were observed in K^+- and low pH- dependent growth phenotypes. Although expression of CHX17 did not alter cytoplasmic or vacuolar pH in yeast, CHX20 elicited acidification and alkalization of the cytosol and vacuole, respectively. Using heterologous expression in *E. coli* strains lacking K^+ uptake systems, we provided evidence for K^+ (^{86}Rb) transport mediated by CHX17 and CHX20, and estimated cation selectivity and affinity. Finally, we obtained preliminary evidence that CHX17 and CHX20 affected protein sorting as measured by carboxypeptidase Y secretion in yeast mutants grown at alkaline pH. In plant cells, CHX20-GFP was localized to the ER; whereas GFP-tagged CHX17-CHX19 co-localized with prevacuolar compartment and endosome markers. Together, these results suggest that multiple CHX transporters differentially modulate K^+ and pH homeostasis of distinct intracellular compartments that could influence protein sorting and membrane trafficking events in different cell-types. (Supported by DOE BES to HS)

4. Identification of a Salt Tolerance Determinant, SRS2

Qingmei Guan, Sanghyun Han, and Jianhua Zhu
Dept. of Plant Science and landscape Architecture, Univ. of Maryland, College Park, MD 20742

Excessive level of salts in soil solution causes inhibition of plant growth and development and has become an increasing threat to agriculture worldwide. To identify salt tolerance determinants, we used EMS mutagenized *Arabidopsis thaliana* to isolate a recessive mutant, *short root in salt medium* (*srs2-1*), with hypersensitivity to NaCl and KCl. SRS2 encodes a chitinase-like protein related to abiotic stress. Our results show that SRS2 is a positive regulator which is essential for plant salt stress tolerance.

5. Effects and functional mechanism of rice EL1, a casein kinase I, in gibberellin acid signaling

Cheng Dai, Hong-Wei Xue*

Plant hormone GA is crucial for multiple aspects of plant growth and development. To study the relevant regulatory mechanisms, a rice T-DNA insertion mutant *earlier flowering1, el1*, which is deficient in a casein kinase I that plays critical roles in both plants and animals, was identified. *el1* had an enhanced GA response including the elongated second leaf sheaths and α-amylase activities. The transcriptions of GA biosynthesis-related genes were reduced in *el1*, which is consistent with the stimulated GA signaling. Biochemical characterization showed that EL1 specifically phosphorylated the rice DELLA protein SLR1, proving a direct evidence for SLR1 phosphorylation. The severe dwarf phenotype and suppressed GA signaling by overexpression of SLR1 was significantly reduced by *EL1* deficiency, indicating the negative effect of SLR1 on GA signaling requires the EL1 function. This study demonstrates EL1, a novel and key negative regulator of the GA response, and provides important clues on casein kinase I activities in regulation of GA signaling and plant development.

Key words: rice, flowering time, Gibberellic acid response, EL1, casein kinase I

6. Tyrosine Phosphorylation and Sumoylation based homo-dimerization of Arabidopsis RACK1A protein regulates Distinct Oxidative Stress Signaling Pathways

Nabanita Kundu and Hemayet Ullah, Department of Biology, Howard University, Washington, DC 20059

Scaffold proteins are known as important cellular regulators that can interact with multiple proteins to modulate diverse signal transduction pathways. RACK1 (Receptor for Activated C Kinase 1) is a WD-40 type scaffold protein, conserved in eukaryotes, from *Chlamydymonas* to plants and humans, expresses ubiquitously and plays regulatory roles in diverse signal transduction and stress response pathways. Model plant *Arabidopsis thaliana* genome maintains three different *RACK1* genes termed *RACK1A, RACK1B*, and *RACK1C* with a very high (85-93%) sequence identity among themselves. Loss of function mutant in Arabidopsis indicates that RACK1 proteins regulate diverse environmental stress signaling pathways including drought stress resistance pathway. Recently deduced crystal structure of Arabidopsis RACK1A- very first among all the RACK1 proteins, indicates that it can potentially be regulated by post-translational modifications, like tyrosine phosphorylations and sumoylation at key residues. Here we show evidence that RACK1A proteins, depending on diverse environmental stresses, are tyrosine phosphorylated and sumoylated. Utilizing site-directed mutagenesis of key tyrosine and lysine residues, it is found that tyrosine phosphorylation and sumoylation of key residues can potentially dictate the homo-dimerization of RACK1A proteins. The homo-dimerization status of RACK1A proteins is found to regulate diverse cellular stress signaling pathways including drought, UV-B, and oxidative stress pathways. The novel and attractive hypothesis of tyrosine phosphorylation based sumoylation of RACK1 proteins is being investigated as the leading mode of RACK1 protein mediated oxidative stress signaling pathways.

7. PDLP5, a dual regulator of the SA defense pathway and PD-mediated cell-to-cell communication

Ross Sager, Weier Cui, Xu Wang, and Jung-Youn Lee
Delaware Biotechnology Institute, Dept. Plant and Soil Sciences, Univ Delaware, Newark, DE 19711

Programmed cell death that takes place during the hypersensitive response (HR) in plant defense has been speculated to require coordinated cell-to-cell communication. However, the role of plasmodesmata (PD) in this process has not been explored. Here we present experimental evidence that a novel member of a PD-localized protein family, PDLP5, provides a mechanism that links PD regulation and salicylic acid (SA)-mediated defense signaling. Overexpression of PDLP5 (35S::PDLP5) reduced plant growth, induced necrotic lesions, and accumulated a marker gene for SA upregulation, *PR1*. We introduced the salicylate hydroxylase construct NahG into PDLP5-overexpressing plants to confirm that the above phenotypes were due to SA overaccumulation, and found that indeed the phenotype reverted back to that of wild-type plants. SA treatment upregulated PDLP5 expression in wild-type plants, and molecular profiling of knockouts of critical SA defense pathway genes revealed that *eds1*, *ics1*, and *npr1* had lower basal and SA-induced expression of PDLP5, suggesting that PDLP5 is part of a positive feedback loop through the SA defense pathway. We have confirmed that PDLP5 is essential for proper PD gating; 35S::PDLP5 showed a stark reduction in PD permeability, while a severe knockdown line (*pdlp5*) had the opposite effect. Furthermore, callose accumulation at PD is also positively correlated with the level of PDLP5 expression. Based upon our findings, we have developed a model whereby PDLP5 is induced via the SA defense pathway during a defense response, limiting the intercellular movement of harmful pathogenic toxins or endogenous cell death signals, while simultaneously adding to local defense via positive SA feedback.

(This research is supported by NSF grant (IOB-0954931) awarded to J.-Y. L.)

Featured Seminar: **Evolutionary Diversification of the Epigenome: A 'Paragenetic' Perspective on the Role of RNA Silencing in the Biology of Plants**

Richard Jorgensen, Carolyn Napoli and Karla Gendler

RNA silencing plays a key role in establishing and maintaining chromatin states in the plant epigenome. Our work on epigenetic states of RNA silencing in transgenic petunias is based on transgenes designed for protein overexpression that instead trigger RNA silencing. Duplication of such a transgene results in heritable, but reversible epigenetic changes in morphology-based changes in patterns of silencing in flowers. These epigenetic events are 'paramutations' because they persist after segregation of the 2nd gene copy and are referred to as 'paragenetic' because they are chromosomally based (R.A. Brink, 1960). Changes in phloem and plasmodesmal trafficking of RNA silencing signals appear to interact with particular epialleles of the transgene. Building on R. Alexander Brink's concept of 'paragenetic' chromosome functions, we argue that interactions between RNA silencing signals and paragenetic states, which may occur at many loci throughout the genome and could involve systemic trafficking of RNA and protein molecules that can influence these states, create the possibility of a highly sensitive, dynamic regulatory system that transmits

(via plasmodesmata) and stores (via chromatin) information that is potentially useful in plant growth, development, and adaptation to the environment.

Chromatin (i.e., the “epigenome”) is comprised of dozens of protein families with diverse molecular functions ranging from histone and DNA modifying enzymes to nucleosome remodeling complexes and RNAi machinery components, which collectively comprise the "epigenome" and which control and integrate patterns of gene expression and mediate chromosomal processes such as genetic recombination, DNA repair and chromosome condensation. Through molecular phylogenetic analysis of dozens of protein families we have estimated the number of clades of chromatin proteins that existed prior to divergence of plants and animals, of monocots and dicots, of fission and budding yeasts, and of insects and vertebrates, and identified new clades representing a variety of ancient gene duplications that arose prior to each divergence event. New proteins have arisen at similar rates in plants and in animals (roughly, 1 new clade per 10My), whereas yeasts have dispensed with more types of chromatin proteins than have been gained. In plants histone methyltransferases have diversified at several times the rate of duplication of histone acetylases/deacetylases, whereas both classes have expanded at similar rates in animals, and yeasts show a pattern opposite to that of plants. Most strikingly, factors responsible for RNA-directed chromatin modification have expanded dramatically in plants as compared to animals, consistent with a much greater role for RNAi-mediated control in plants, possibly involving systemic RNA signals that act on chromatin and transmit epigenetic information throughout the plant. The evolution of new RNAi-mediated functions was often associated with positive selection on branches leading to new clades and seems to have paralleled the evolution in land plants of increasing developmental complexity, including complexity of the vasculature.

8. **Using Illumina mRNA-Sequencing to understand *Glycine max-Phakopsora pachyhrizi* interaction**

Arianne Tremblay[1], Parsa Hosseini[1,2], Nadim W. Alkharouf[2], Shuxian Li[3], Benjamin F. Matthews[1]
[1]United States Department of Agriculture-Agricultural Research Service (USDA-ARS), Soybean Genomics & Improvement Laboratory, Beltsville, MD 20705, U.S.A., [2]Towson University, 8000 York Road, Towson, MD 21252, [3]USDA-ARS, Crop Genetics Research Unit, Stoneville, MS 38776

Soybean is one of the top five agricultural products in the United States and is highly susceptible to soybean rust (SR), an exotic obligate fungus that arrived in the USA in 2004. We used mRNA-Seq by Illumina to analyze gene expression pattern of the host and pathogen at different time points during infection. Over 6 million sequences were obtained for each time-point. DNA sequences were aligned to the soybean genome and soybean rust sequences. Tag counts were obtained for each genes and the expression of each gene was compared to the uninfected control. Expression levels of genes encoding enzymes were overlaid on biochemical pathway diagrams from the Kyoto Encylcopedia of Genes and Genomes to provide easier analysis of changes in pathways. For example, we found that genes involved in carbohydrate metabolism were generally down-regulated over the infection time-course. There were some exceptions, such as some genes involve in butanoate metabolism that were up-regulated at 7 hai, but decreased at 48 hai. However, by 240 hai many of these genes were up-regulated again. Genes encoding enzymes in the propanoate metabolism followed the same pattern but remained down-regulated at 240 hai. Such information may be use to develop new methods to broaden resistance of soybean to soybean rust.

9. Identification of soybean genes that confer resistance to Asian soybean rust.

Mandy D. Kendrick[1], Bo-Keun Ha[2,3], Michelle Graham[4,5], Chunquan Zhang[6], Steve Whitham[6], John Hill[6],Reid D. Frederick[1], H. Roger Boerma[2], and Kerry F. Pedley[1]
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[5]Department of Agronomy, Iowa State University, Ames, IA, USA 50011
[6]Department of Plant Pathology, Iowa State University, Ames, IA USA 50011

Soybean is the second largest crop produced in the U.S. As a result of selective breeding and genetic engineering the crop has been effectively cultivated for decades with few emerging biotic threats poised to compromise crop yield. However, in 2004 the fungal pathogen *Phakopsora pachyrhizi* was discovered in Louisiana and Alabama. First identified in 1902 in Japan, this obligate biotroph has since spread throughout Asia, Africa, Australia, and South America. Commonly thought to have arrived in the continental U.S. from South America via Hurricane Ivan, the pathogen has since spread to nineteen additional U.S. states. *P. pachyrhizi* is the causal agent of Asian soybean rust (ASR). During severe outbreaks the disease can lead

to defoliation of entire soybean plants, causing a decrease in crop yield by as much as 80%. Due to a lack of resistance in commercially grown soybean cultivars, fungicide application is currently the only option for crop protection against ASR. However, due to costs associated with fungicide application, the negative impact on the environment, and the potential for increasing fungal tolerance to these chemicals, developing ASR-resistant soybean cultivars could be an effective strategy for protecting the global soybean crop. To date five loci that confer resistance to specific isolates of *P. pachyrhizi* have been identified in soybean. *Resistance to P. pachyrhizi-3* (*Rpp3*) was first identified in the soybean cultivar Ankur and mapped to a definded region of chromosome 6. More recently, the soybean cultivar Hyuuga was also found to harbor ASR-resistance, which mapped to the same region of chromosome 6 as *Rpp3*. However, it remained unknown whether resistance in Hyuuga and Ankur were conferred by identical, allelic, or independent genes. In this study we screened both Hyuuga and Ankur with multiple isolates of *P. pachyrhizi* and concluded that Hyuuga carries two resistance genes: *Rpp3* and a second gene on chromosome 3 that maps near *Rpp5*. Now we are using virus-induced gene silencing (VIGS) along with DNA sequencing and gene expression analysis in an effort to identify the resistance genes (R genes) in Hyuuga.

10. PI3P, a landmark for trafficking at the host-pathogen interface?

Robert Berkey, Wenming Wang and Shunyuan Xiao
Center for Biosystems Research, Institute for Bioscience and Biotechnology Reasarch, Rockville, MD 20850

Biotrophic fungal and oomycete pathogens, including the rust and powdery mildews, cause significant damage to a wide range of economically important crop species around the world. Despite the economic importance of these pathogens, not much is known about the molecular basis of host defense mechanisms. In order for these pathogens to survive and proliferate in plants, they penetrate the plant cell wall and produce specialized feeding structures called haustoria to 1) extract nutrients from the host cell and 2) secrete effector proteins to suppress or circumvent host defenses. Our lab research is focused on understanding the molecular basis of the broad-spectrum resistance activated by the Arabidopsis protein RPW8 against powdery mildew pathogens. We recently reported that RPW8.2 is specifically induced and targeted to the extra-haustorial membrane (EHM) that encases the haustorium whereby it activates and enhances host defense against haustorial invasion. The EHM represents a highly specialized interfacial membrane between the host and pathogen, and is presumably modified by both the pathogen and the host for successful invasion and defense, respectively. Despite its importance as a focal point of host-pathogen interactions, the EHM remains largely enigmatic in terms of its origin, and its membrane and protein composition. By using RPW8.2 as the first EHM-specific resident protein, we have been investigating how RPW8.2 is specifically targeted to the EHM and what is the potential trafficking cue from the host-pathogen interface. Our recent results suggest that phosphoinositides may play an important role in regulating membrane/protein trafficking towards the host-pathogen interface, and/or host defense against haustorial invasion. (Supported by NSF grant IOS-0842877 to SX

Poster:

Establishing *Fragaria vesca*, a diploid strawberry, as a model for studying flower and fruit development

Hollender, Courtney A (a) Geretz, Aviva (a) Slovin, Janet (b) Liu, Zhongchi (a)
(a) Dept. of Cell Biology and Molecular Genetics, University of Maryland, College Park (b) Genetic Improvement of Fruit and Vegetables Laboratory, USDA/ARS, Beltsville, MD

The diploid strawberry *Fragaria vesca* is becoming an ideal model for studying flower and fruit development. F. vesca can be easily grown in the lab, has a life cycle of only 3.5 months, can be propagated both sexually and asexually, and is amenable to transformation and genetic analyses. In addition, its ~200Mb genome has recently been sequenced. F. vesca can also serve as a model for other Rosaceae species that possess larger genomes and/or longer juvenile phases. Using a 7th generation diploid inbred line, F. vesca 5AF7, we characterized developmental stages of strawberry flower and fruit development using histological sections and scanning electron microscopy. The description of morphological landmarks during flower and fruit development provides reference points for future molecular, genetic, and genomic studies. Specifically, the shoot and floral meristem architecture of *F. vesca* are being described using morphology and *in situ* hybridization of molecular markers including *FvWUSCHEL.* Second, stage-, tissue-, or floral organ-specific transcriptiome analyses will be produced using next-generation sequencing. The stage and tissue-specific reproductive tissues are being dissected either manually or via laser capture microdissection. Finally, a mutagenized population of 5AF7 is being generated with the ultimate goal of isolating developmental mutants and creating a tilling library for both forward and reverse genetic analyses. (This work is supported by NSF (MCB0923913) to ZL and JS and Hokensen graduate fellowship to CH.)

ORAL PRESENTATION

A. A.M. morning session

1. Charophyte transcriptomics and the evolutionary origin of land plants.
Charles F. Delwiche and Ruth E. Timme

The charophyte green algae are one of two primary lineages of green algae, comprising six orders of freshwater or terrestrial algae. The diversification of green algae probably occurred well over a billion years ago, and the deepest phylogenetic split within them is between the "chlorophyte" and "charophyte" green algae. The land plants (i.e., embryophytes) are the terrestrial lineage that includes seed plants, ferns and other non-seed vascular plants, and bryophytes such as mosses and liverworts; this lineage diversified roughly 450 million years ago and is phylogenetically placed within the charophyte green algae. Consequently, the charophytes are much more closely related to land plants than are green algae for which fully sequenced genomes are available (notably *Chlamydomonas* and *Ostreococcus*), and would be a logical outgroup for comparative analysis. Unfortunately, none of the charophytes is well developed as a model system, and relatively little information is available on genome size and organization. Therefore, to begin genome-scale study of charophytes we undertook an EST/Transcriptomic survey of seven select charophytes representing five of the six recognized orders. Using a combination of Sanger and 454 sequencing, we obtained roughly 2.77 billion nucleotides of data divided among the seven libraries, and identified 12-30,000 unigenes from each organism. These data support the close relationship between charophytes and land plants but call into question some details of this relationship. Several key plant processes appear to trace their origin to the charophytes.

2. *Chlamydomonas*, *Volvox*, and the evolution of multicellularity
Stephen M. Miller

Chlamydomonas reinhardtii, *Volvox carteri*, and their closest relatives—collectively the volvocine green algae—comprise an excellent system for exploring the origins of developmental complexity. Over a relatively short period of time (~200 million years) *Volvox* evolved an impressive suite of developmental traits, including asymmetric cell division, multicellularity with germ-soma division of labor, and embryonic morphogenesis. Both *Volvox* and *Chlamydomonas* are excellent model organisms with sequenced genomes, making it possible to investigate the evolution of new developmental traits at the molecular-genetic level. This talk will focus on what we have learned from comparative analysis of the *Volvox* and *Chlamydomonas* genomes, and from analysis of *regA* and related genes, which encode putative transcription factors that regulate *Volvox* cell-fate determination. Interestingly, the *regA* gene appears to have been generated by a gene duplication event that occurred in the common ancestor of *Chlamydomonas* and *Volvox*, but was lost in the lineage leading to *Chlamydomonas*. Evidence will be presented to support the hypothesis that *regA* function in cell differentiation may have evolved via co-option of components of a stress-response pathway, and might involve indirect regulation of nuclear genes that encode proteins essential for chloroplast function.

3. Genomics resources for the diploid woodland strawberry: a reference plant for the Rosaceae
Janet Slovin, Genetic Improvement of Fruit and Vegetables Laboratory, ARS-USDA, Beltsville, MD

Strawberry is an herbaceous perennial member of the Rosaceae family, which includes several important fruit crops as well as ornamentals. The commercial dessert strawberry, *Fragaria xananssa,* is octoploid and not readily amenable to genetic and genomic research. Genetic and genomic resources are rapidly being developed for the diploid woodland strawberry, *F. vesca*, so that it can be used as a model system for *Fragaria*, as well as for members of the Rosaceae family that are more difficult to transform, have long juvenile stages, or are otherwise difficult to work with in the laboratory. A draft sequence of its small, ~200Mb, genome was recently published. *F. vesca* can be easily grown in the lab, has a seed to seed cycle of only 3.5 months, can be propagated both sexually and asexually, and is amenable to transformation and genetic analyses. Developmental stages of flower and fruit development of a 7th generation diploid inbred line, *F. vesca* 5AF7 are being characterized using histological sections and scanning electron microscopy. A set of about 42,000 ESTs was generated from five separate cDNA libraries representing transcripts from water, temperature and osmotically stressed seedlings and plants.

A mutagenized population of 5AF7 has also been generated for isolating developmental mutants and creating a TILLING resource for both forward and reverse genetic analyses.

4. The fungal genus *Trichoderma*: evolution of ecological roles and nutritional modes and its relationships with plants

Priscila Chaverri, Department of Plant Sciences and Landscape Architecture, University of Maryland

Trichoderma spp. can be found as soil inhabitants, plant decomposers, endophytes, and parasites of other fungi. The fact that many *Trichoderma* species have demonstrated antifungal or plant-growth-stimulating activities has led to their widespread exploitation as biological control agents. Many studies report *Trichoderma* as being both saprophytic and fungicolous, implying that the same species can obtain nutrients from organisms of completely unrelated kingdoms; however, this is unlikely. The objectives of the present study were to (1) infer the evolution of substrate/habitat "preference" (soil, decaying plant material, other fungi, endophyte), and (2) infer the evolution of major host affiliations (e.g. plant vs. fungus). These objectives were addressed through phylogenetic analyses of five genes and ancestral character reconstructions. Results show that a preference for a particular habitat/substrate was gained or lost multiple times in the evolution of the genus. Results also support at least four interkingdom host jumps, two from fungus to plant, and two from plant to fungus. In this study it was possible to infer the role *Trichoderma* endophytes play in their hosts by evaluating their closest relatives and determining the most recent ancestors. For example, many *Trichoderma* species found as endophytes may be fungicolous and thus may protect the host plant from disease. The same above principle applies for species that are "cryptic" mycoparasites living in the soil or in decaying plant material. Findings from the current study may have implications for the discovery and development of novel biological control strategies.

5. Integrating fundamental research into bioengineering solutions: Diversifying fatty acid biosynthesis with biocatalytic functionalities from polyketide biosynthesis for creating a new paradigm in biorenewable chemicals

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The chemical industry is reliant on a historically inexpensive, petroleum-based carbon feedstock that generates a small collection of platform-chemicals, from which highly efficient chemical conversions lead to the manufacture of a large collage of products. Given this paradigm, the Center for Biorenewable Chemicals (CBiRC) is exploring the potential of exploiting the polyketide/fatty acid biosynthetic pathways as a new paradigm for the production of platformbiorenewable chemicals. Polyketide and fatty acid biosynthesis share a common series of biocatalytic mechanisms that reiteratively generate a homologous series of alkyl-chains that range from chain lengths of 4-carbon atoms to greater than 30-carbon atoms. These processes share the common mechanism of carbonylgroup chemistry as intermediates of the biosynthetic origins. By amalgamating biocatalysts from diverse metabolic and phylogenetic origins within an integrated biological system, we are envisioning a single technological platform that will generate a variety of biorenewable molecules. Because the envisioned system is based on the reiteration of a series of simple transformations on a homologous series of substrates, by analogy to combinatorial chemistry, the envisioned platform can be considered an example of combinatorial metabolism.

6. Biological and Environmental Research at the Department of Energy

Sharlene Weatherwax (Department of Energy)

DOE's Office of Biological & Environmental Research (BER) supports basic research programs and scientific user facilities to address DOE's energy, environment, and basic research missions. BER research programs advance fundamental scientific understanding necessary to develop biofuels as a major secure national energy resource, understand relationships between climate change and Earth's ecosystems, predict fate and transport of subsurface contaminants, and develop new tools to explore the interface of biological and physical sciences. BER has spearheaded the development of modern genomics-based systems biology and played a major role in seeding and fostering the contemporary biotechnology revolution, while at the same time supporting forefront research on the impacts of enegy production and use on climate change. DOE offers many opportunities for plant biologists to participate in its research activities and contribute towards its mission.

7. Early steps in S-RNase uptake

Bruce McClure (Univ. Missouri, & National Science Foundation)

S-RNase is secreted in the pistil extracellular matrix. Both compatible and incompatible pollen tubes take up S-RNase, and much of the internalized protein moves to the vacuole lumen. This appears to be a default mechanism since it occurs in both compatible and incompatible pollen tubes in self-incompatible (SI) species like *Nicotiana alata* and in self-compatible (SC) species like *N. tabacum*. The fate of S-RNase, as it moves through the pollen tube, has important implications for S-RNase-based SI. We used immunolocalization and live imaging to follow S-RNase uptake in regions of the pollen tube that are close to the tip in both SC and SI species. Immunolocalization showed S-RNase-containing bodies just behind the tip. In progressively more mature regions of the pollen tube, S-RNase was observed surrounding vacuole-like structures and then in the vacuole lumen. Live-imaging studies using S-RNase:RFP fusions produced similar results.

B. Sat. afternoon p.m. session

8. How do biotrophic pathogens survive inside hostile hosts?

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We investigate the molecular interplay and co-evolution between *Arabidopsis thaliana* and the oomycete pathogen *Hyaloperonospora arabidopsidis* (*Hpa*, downy mildew disease). *Hpa* is an obligate biotroph that extracts nutrients from living plant tissue and cannot exist apart from its host. Recent sequencing of the *Hpa* genome and comparison to genomes of facultative parasites in the related *Phythophthora* genus revealed several genomic signatures of evolution towards an obligate life style. For example, almost every gene family encoding secreted pathogenicity proteins is downsized in *Hpa* compared to *Phytophthora*, presumably to facilitate "stealth" inside the host. We are particularly interested in RXLR effectors, which are exported to the interior of plant cells where they promote host susceptibility or are recognized as signals of invasion. Hpa contains ~130 candidate RXLR genes, compared to ~370-550 in *Phytophthora* genomes. There is little evolutionary conservation between candidate RXLR proteins in *Hpa* and *Phytophthora*, suggesting that oomycetes must continually invent or re-invent RXLR weaponry. However, some RXLR genes are conserved, suggesting that they play general roles in oomycete pathogenicity. We are focusing on these conserved genes from *Hpa* and their homologs in the soybean root/stem rot pathogen *Phytophthora sojae*. Our data indicate that these effectors can suppress plant immune responses across a broad range of host plants.

9. Probiotics for plants: Impact of belowground beneficials on aboveground pathogens.

Harsh P. Bais[1,2], Venkatachalam Lakshmanan[1,2], Amutha Sampath Kumar[1,2], Weier Cui[1,2], Sherry L. Kitto[1], Jung-Youn Lee[1,2], Jeff Caplan[2], Deborah Powell[2], Kirk J. Czymmek[2,3], Delphis F. Levia[4].

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Plants exist in a complex multitrophic environment where they interact with and compete for resources with other plants, microbes and animals. The effects of beneficial microbes on plant performance and disease resistance has been well-studied, however, there has been comparatively much less progress in elucidating the mechanisms for conferring these benefits. Our recent work demonstrated that foliar infection by *Pseudomonas syringae* pv tomato (hereafter PstDC3000) induces recruitment of *Bacillus subtilis* (hereafter FB17), a beneficial rhizobacteria at the root surface. Our data reveals of a molecular mechanism underling the positive feedback response by connecting aboveground and belowground portions. Our results provide the foundations for future investigations into the impact of the root microbiome on stomatal regulation and defense. We believe that these findings will be of interest to wide range scientists who study innate immunity recognition mechanisms in plants and animals.

10. Role of ethylene, abscisic acid and MAP kinase pathways in rice disease resistance

Yinong Yang, Department of Plant Pathology and Huck Institutes of Life Sciences, Pennsylvania State University, University Park, PA 16802

A combination of molecular, biochemical and functional genomic approaches have been used to elucidate the complex network of defense signaling pathways involved in rice resistance to the blast (*Magnaporthe* oryzae, a hemibiotroph) and sheath blight (*Rhizoctonia solani*, a necrotroph) diseases. By generating and characterizing a series of transgenic rice lines defective in hormone biosynthesis or signaling, we have uncovered the distinctive roles of salicylic acid, jasmonic acid, ethylene and abscisic acid in rice defense response against these two most important rice pathogens. For example, ethylene biosynthesis and signaling was shown to play a positive role in rice resistance to *M. oryzae* or *R. solani* infection. By contrast, abscisic acid interacts antagonistically with ethylene and negatively modulates rice blast and sheath blight resistance. In addition, a stress-responsive rice MAP kinase was found to mediate the cross-talk between ethylene and abscisic acid pathways and inversely regulate rice disease resistance and abiotic stress tolerance. To gain insights into the MAP kinase pathway, we recently identified a number of the MAP kinase substrates using an in situ solid-phase phosphorylation screening method. Preliminary analyses demonstrate that these substrates could be phosphorylated by the MAP kinase and play an important role in mediating downstream defense signaling and rice disease resistance.

11. **Conserved Peptide Upstream Open Reading Frames in Plant mRNAs are Associated with Regulatory Genes**

Richard Jorgensen, Carolyn Napoli and Karla Gendler University of Arizona, School of Plant Sciences

The amino acid sequences of upstream open reading frame (uORF) are usually not conserved in evolution, but the small class of uORFS whose encoded peptides are conserved seem likely to play roles in translational control of downstream (major) open reading frames (mORFs). By comparing full-length cDNA sequences from Arabidopsis and rice we identified 30 distinct homology groups of conserved uORFs, only three of which had been reported previously. Pairwise K_a/K_s analysis showed that purifying selection had acted on nearly all uORFs and mORFs. Functions of predicted mORF proteins could be reasonably inferred for 24 homology groups and each of these proteins appears to have a regulatory function, including 6 involved in transcriptional control, 8 involved in signal transduction, 4 involved in small signal molecule pathways, 4 with other known regulatory functions, and two with protein interaction domains. Duplicate copies of genes with a conserved uORF that were created by tetraploidy in an *Arabidopsis* ancestor are much more likely to have been retained in *Arabidopsis* than are duplicates of other genes (39% vs. 14% of ancestral genes, $p=5x10^{-3}$). Two uORF groups were also found in animals, indicating an ancient origin of these uORFs. The inferred ancestral state of one ancient 'uORF' lacks a downstream mORF. It became a uORF in three independent lineages through transcriptional fusions to three different mORFs in plants, arthropods and nematodes, whereas it remained a free ORF in algae, vertebrates, fungi, and slime molds, suggesting that some uORFs may arise by transcriptional fusion of two unrelated ORFs.

12. Regulatory Control over Rapid Development in Marsilea

Steve Wolniak (Univ. Maryland, Dept. of Cell Biology and Molecular Genetics)

We have used the simple, endosporic male gametophyte of the water fern *Marsilea vestita* to study mechanisms that control cell fate determination and cell morphogenesis. The gametophyte develops rapidly after a dry microspore is placed into water and undergoes nine division cycles to produce 7 sterile cells and 32 spermatids. The spermatids then undergo profound changes to become multiciliated, corkscrew-shaped gametes. In the absence of cell movement, cell position within the spore wall defines fate. After the divisions are completed, the spermatids are sites for *de novo* basal body formation, cytoskeletal assembly, nuclear and cell elongation, and ciliogenesis. We found that gametophyte development is controlled post-transcriptionally, and involves several modes of RNA-processing that allow translation of specific mRNAs at distinct stages during gametogenesis. Transcripts stored in the microspore are unmasked and translated at specific times during development. RNA silencing protocols enabled us to block translation of these proteins and thereby establish their necessity and sufficiency for gametogenesis. Distributions of mRNAs and the proteins they encode are not identical in the gametophyte, so transcript processing is important in allowing translation to occur. Transcript polyadenylation in the spermatogenous cells matches the translation of specific mRNAs. The exon junction complex (EJC) plays key roles in transcript regulation and modifications that underlie cell specification in the gametophyte. We have linked the synthesis and redistribution of spermidine in the gametophyte to the control of mRNA release from storage during early development, and later to basal

body formation, cytoskeletal assembly and to nuclear and cell elongation in the differentiating spermatids.

13. Protein Phosphorylation Network in Abscisic Acid Signaling

Taishi Umezawa RIKEN Plant Science Center, Tsukuba, Japan

Abscisic acid (ABA) signaling is initiated by a receptor complex of PYR/PYL/RCAR and a PP2C that strictly regulates the activity of downstream SNF1-related protein kinase 2 (SnRK2). Our current hypothesis is that protein phosphorylation mediated by SnRK2 is a key step of ABA signal transduction. We are using a LC-MS-based phosphoproteomic approach to identify ABA-induced phosphorylation events and candidates for SnRK2 substrates. To date we have obtained multiple phosphoproteome profiles consisting of over 5,000 phospho peptides from Arabidopsis seedlings (WT and SnRK2 mutants) and performed quantitative assessments of ABA- and drought-induced changes in phosphorylation using label-free/18O-labeling approaches. Using this approach we obtained several new candidates for SnRK2 substrates and are currently testing the function of these proteins in ABA signaling.

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