2012 Meeting of the Southern Section of the American Society of Plant Biologists

Abstracts

March 3 - 5, 2012

Ocean Reef Resort

Myrtle Beach, South Carolina

General Session

Soybean Somatic Embryos and Plastids as a Model System for Lipid Metabolism

Salvatore A. Sparace[1], Karen R. Clark[1], Yan He[1], Tammie E. Young[1], Zhigang Li[2], Hong Luo[2] and Kathryn F. Kleppinger-Sparace[1]

[1]Department of Biological Sciences, Clemson University, Clemson, SC, USA.
[2]Department of Genetics and Biochemistry, Clemson University, Clemson, SC, USA.

Soybean (*Glycine max* L.) somatic embryos and their plastids are being used as a model to study the developmental relationships and metabolic interactions of fatty acid and glycerolipid biosynthesis. Batch cultures of somatic embryos exhibit sigmoidal growth kinetics over the course of eight weeks. On a fresh weight basis, embryos accumulate up to 4% protein, 2.5% soluble sugars, 1.9% starch and 1.5% lipid. Plastids with the highest rates of fatty acid biosynthesis from acetate are isolated from embryos early in exponential growth. Although soybean somatic embryos and their plastids are green, the plastids appear to function like heterotrophic plastids. Light has essentially no effect on fatty acid biosynthesis, while ATP, coenzyme A and bicarbonate are all essential. Acetate is the preferred precursor at low ($\leq$ 1 mM) concentrations while pyruvate is greatly preferred at higher concentrations (up to 10 mM). Radioactivity from acetate is recovered in primarily in palmitic and oleic acids, while radioactive fatty acids and ^{14}C-glycerol-3-phosphate are recovered in essentially all the standard chloroplast lipids, but especially in triacylglycerol and phosphatidylcholine (23% and 20%, respectively). Embryos and plastids both express key enzymes of nitrogen assimilation. The activities of these enzymes suggest that nitrogen assimilation has potential to impact lipid biosynthesis in soybean. This research was supported by projects 8233 and 1233 from the United Soybean Board.

Transgenic Soybean Seeds as a Protein Expression System: Separating Production from Purification

Kenneth L. Bost and Kenneth J. Piller. University of North Carolina at Charlotte and SoyMeds, Inc, Charlotte, NC.

Soybean seeds expressing transgenic proteins represent a novel, sustainable platform technology which overcomes some of the current limitations for producing recombinant proteins useful as immunotherapeutics. We have successfully expressed a variety of proteins, including some with immunomodulatory potential, in transgenic soybean seeds, and are evaluating their usefulness as therapeutics. In particular, we have expressed several tolerogenic proteins aimed at limiting the autoimmune response in animal models of Multiple Sclerosis. Expressing multiple autoantigens, which target mucosal sites, and present the autoantigen in a tolerogenic context, is a difficult undertaking. However the ability to express large amounts of these formidable tolerogens in transgenic soybean seeds, and the flexibility of long-term storage of soy in the absence of a cold chain is a significant advantage for this platform technology. Storing transgenic soy powder for extended periods of time, without degradation of the expressed tolerogen, allows production to occur well in advance of purification or formulation. Stated simply, this manufacturing process can separate protein expression from therapeutic administration by years. As protein expression technologies expand and evolve, transgenic soybean seeds represent an emerging technology with some significant advantages.

Hotspots in Viral siRNA Accumulation in Maize

Vicki Vance[1], Sizolwenkosi Mlotshwa[1], Cameron Johnson[2], Amy Wahba Foreman[1], Gail J. Pruss[1], Venkatesan Sundaresan[2], Kay Scheets[2], and Lewis H. Bowman[1]. [1]Department of Biological Sciences, University of South Carolina, Columbia, South Carolina; [2] Section of Plant Biology, College of Biological Sciences, University of California Davis, Davis, California; [3]Department of Botany, Oklahoma State University, Stillwater, Oklahoma

RNA silencing is a sequence-specific RNA degradation mechanism that serves as an antiviral defense pathway in plants. Most plant viruses have single stranded RNA genomes and replicate via double stranded RNA (dsRNA) replication intermediates. The viral dsRNA triggers antiviral silencing in the host. It is processed by Dicer-like ribonucleases to produce short interfering RNAs (siRNAs) that incorporate into a RNA-induced silencing complex (RISC). Within RISC, the viral siRNA acts as a guide to direct the complex to complementary target RNAs, which are then destroyed. In this way, viruses provide the molecular tools (siRNAs) that lead to their own destruction, providing a potent and specific antiviral defense. Here we report an analysis of the population of viral siRNAs that accumulate during infection of maize with three different viruses: maize dwarf mosaic virus (MDMV), maize chlorotic mottle virus (MCMV) and maize necrotic streak virus (MNeSV). In each case, we find that viral siRNAs comprise a large proportion of the total small RNAs in infected cells and that viral siRNAs are generated along both strands of the entire genome. However, the analysis identified a few regions of the viral genome that generated very high levels of siRNAs. The characteristics of these "hotspot" viral siRNAs will be discussed. The data raise the intriguing possibility that these abundant viral siRNAs mediate an additional level of antiviral silencing by targeting host genes that are required for efficient viral replication.

Analysis of Aquaporin Gene Family in Cotton

Wonkeun Park[1,3], P.J. Bauer[1], Brian E. Scheffler[2], and Bruce T. Campbell[1]. [1]USDA-ARS, Florence, SC, [2]USDA-ARS, Stoneville, MS, [3]PDREC, Clemson University, Florence, SC

Aquaporins are intrinsic membrane proteins present across kingdoms. In plants, aquaporins play roles in intercellular and intracellular water movement in response to osmotic and hydraulic potentials resulting from changing environmental conditions. In higher plants, aquaporins consist of five subfamilies that are plasma membrane intrinsic proteins (PIP), tonoplast intrinsic proteins (TIP), NOD26-like intrinsic proteins (NIP), small basic intrinsic proteins (SIP), and previously unrecognized X-intrinsic proteins (XIP). In addition to the role as water channels, aquaporins are functionally versatile and have diverse substrate specificity to specific small, non-polar solutes. We are analyzing aquaporin gene sequences from different *Gossypium* species to determine the sub-genomic makeup of the cotton aquaporin gene family. The recent release of the genome sequence of a D-genome diploid ancestor (*G. raimondii*) will guide the analysis of the complex and large aquaporin gene family in the cultivated, tetraploid cotton genome (AD-genome).

N-Glycosylation Engineering of Tobacco Plants to Produce Asialoerythropoietin

Farooqahmed S. Kittur[1], Mamudou S. Bah[1], Chiu-Yueh Hung[1], Diane E. Darlington[1], David C. Sane[2] and Jiahua Xie[1]. [1]Department of Pharmaceutical Sciences, Biomanufacturing Research Institute & Technology Enterprise, North Carolina Central University, Durham, NC, [2]Carilion Clinic and Virginia Tech Carilion School of Medicine, Roanoke, VA.

Erythropoietin (EPO) is a glycoprotein hormone that displays both hematopoietic and tissue protective functions by binding to two distinct receptors. Recombinant human EPO (rhuEPO) is widely used for the treatment of anemia, but its use for tissue protection is limited because of potentially harmful increases in red blood cell mass when higher doses of rhuEPO are used. Recent studies have shown that asialoerythropoietin (asialo-rhuEPO), a desialylated form of rhuEPO, lacks hematopoietic activity, but retains cytoprotective activity. Currently, a small amount of asialo-rhuEPO is produced by enzymatic desialylation of rhuEPO. The prohibitive cost of rhuEPO, however, is a major limitation of this method. Plants have ability to synthesize complex *N*-glycans but lack enzymatic activities to add sialic acid and β1,4-galactose to *N*-glycan chains. Plants could be genetically engineered to produce asialo-rhuEPO by introducing human β 1,4-galactosyltransferase. The penultimate β1,4-linked galactose residues are important for *in vivo* biological activity. In this proof of concept study, we show that tobacco plants co-expressing human β 1,4-galactosyltransferase and EPO genes accumulate asialo-rhuEPO. Purified asialo-rhuEPO bound to an *Erythrina Cristagalli* lectin column, indicating that its *N*-glycan chains bear terminal β1,4-galactose residues and that the co-expressed GalT is functionally active. Asialo-rhuEPO interacted with the EPO receptor (EPOR) with similar affinity as rhuEPO, implying that it is properly folded. The strategy described here provides a straightforward way to produce asialo-rhuEPO for research and therapeutic purposes.

TLA1, A Novel Gene for the Regulation of the Chlorophyll Antenna Size in the Green Microalga *Chlamydomonas reinhardtii*

Mautusi Mitra[1,2], Henning Kirst[2], David Dewez[2], Sam Ng[2] and Anastasios Melis[2]. [1]Department of Biology, University of West Georgia, Carrollton, Georgia, GA, [2]Department of Plant and Microbial Biology, University of California, Berkeley, CA

TLA1 (Truncated Light-harvesting Antenna 1) is the first gene shown to play a role in a signal transduction pathway determining the chlorophyll (Chl) antenna size of photosynthesis in the model green microalga *Chlamydomonas reinhardtii*. In the *Chlamydomonas* DNA insertional mutant *tla1,* the native *TLA1* gene is transcribed with a new 5' untranslated region sequence, derived from the 3' end of the transforming plasmid. Replacement of the 5' UTR caused reduced translation of the respective *tla1* mRNA, resulting in much lower levels of the TLA1 protein in the cell. The interim conclusion that the Chl antenna size depends on the level of the TLA1 protein in the cell was further investigated. The *TLA1* gene was genetically manipulated for over-expression and RNAi down-regulation to study its effect on the regulation of the Chl antenna size and chloroplast structure. Western blot analyses of the *TLA1* RNAi transformants, showed that reduction of *TLA1* gene expression is paralleled by substantial reduction in photosynthetic proteins such as the Chl *a-b* light-harvesting proteins Lhcb, the D1, D2 protein and VIPP1. Immunolocalization and transmission electron microscopy studies showed that the TLA1 protein is localized in the chloroplast and is involved in the structural organization of the thylakoid membranes. Recent bioinformatics analyses of the TLA1 protein sequence revealed a high degree of identity in the secondary structure organization of the TLA1 with that of the MOV34 domain containing proteins which are involved in regulation of gene expression and protein turnover.
(Supported by grants from the US-DOE).

Wound healing and peculiar underwater polymerization strategies in marine algae

Cliff Ross[1], Matthew Welling[2] and Georg Pohnert[2]. [1]Department of Biology, University of North Florida, Jacksonville, FL; [2]Institute for Inorganic and Analytical Chemistry, Friedrich Schiller University, Jena, Germany

Protection against environmental threats by plants and algae is often mediated by fast wound activated processes in which metabolites that are stored within the tissue are transformed enzymatically. Such reactions can not only be employed to generate efficient chemical defences against herbivore attack or pathogenic invasion, but may also serve to mechanically protect the tissue or cells. Wound sealing is particularly important for siphonous green macroalgae due to their remarkable organization; they can be comprised of a single giant cell. Considering many adhesives do not function very well underwater, it is of interest to determine how marine algae undergo rapid wound healing events (polymerization) in an aqueous environment. In this study we investigated the kinetics and composition of the wound repair process in the tropical green algae Dasycladus vermicularis ([Scropoli] Krasser) using fluorescent probes, chromatography, UV spectroscopy, and histochemistry. The structural components and biochemical mechanisms employed in this underwater wound-sealing process may allow us to establish new insights and strategies regarding the development of novel biopolymer systems.

Soybean Seed Protein, Oil, Sugars, and Boron Altered by Foliar Boron Application Under Water Stress

Nacer Bellaloui[1], Krishna N. Reddy[2] Anne M. Gillen[1], Daniel K. Fisher[2], and Craig A. Abel[3]. [1]Crop Genetics Research Unit, USDA-ARS, Stoneville MS, [2]Crop Production Systems Research Unit, USDA-ARS, Stoneville MS, [3]Corn Insects and Crop Genetics Research Unit, USDA-ARS, Iowa State University, Ames IA

Information on the effect of foliar boron (B) application on soybean (*Glycine max* (L) Merr.) seed composition is limited. The objective of this study was to investigate the effect of foliar B on seed protein, oil, fatty acids, and sugars under water stress. A repeated greenhouse experiment was conducted where one set of soybean plants were subjected to water stress, and the other set was watered. Foliar B was applied at a rate of 0.45 kg/ha as boric acid. Treatments were watered-plants with no foliar B (W), watered-plants with foliar B (WB), water-stress plants with no foliar B (WS), and water-stress plants with foliar B (WSB). The results showed that seed protein and oil percentage were significantly ($P < 0.05$) higher in WB than in other treatments. Oleic acid increased and linolenic acid decreased in WB and WSB. The concentration of B in leaves and seed was significantly ($P < 0.05$) higher in W than in WS, but cell wall B was significantly higher than total B in WS than in W. Application of B resulted in higher seed sucrose in watered and water-stressed plants, but raffinose and stachyose were significantly higher under water-stressed plants than in other treatment plants. Lack of B translocation from leaves to seed under water stress indicates limited B translocation under water stress. These results suggest that foliar B can alter seed composition. This research also suggests that soybean selection for B translocation efficiency is an important trait for soybean B nutrition under drought conditions. Since mechanisms involved in the effect of B on soybean seed composition are not well known, further research is needed.

Genetic Analysis of Resistance to Sudden Death Syndrome (SDS) and Soybean Cyst Nematode (SCN) in Soybean [*Glycine max* (L.) Merr.]

Abdelmajid Kassem[1], Stella K. Kantartzi[2], and Khalid Meksem[2]. [1]Genomics & Biotechnology Lab, Department of Natural Sciences, Fayetteville State University, Fayetteville, NC. [2]Department of Plant, Soil, and Agricultural Systems, Southern Illinois University, Carbondale, IL.

Soybeans [*Glycine max* (L.) Merr.] are susceptible to many diseases including fungal diseases such as soybean sudden death syndrome (SDS) and nematode diseases such as Soybean Cyst Nematode (SCN) which are complex and polygenic. Several studies reported QTL for SDS and SCN resistance on the soybean genome using different populations and low density genetic linkage maps. The objectives of this study were (1) to construct a high density SNP-based genetic linkage map of soybean using the 'PI438489B' by 'Hamilton' (PIxH, *n*=50) recombinant inbred line population, and (2) to map QTL for SDS and SCN resistance using this high-density reliable genetic SNP-based map. The 'PI438489B' by 'Hamilton' SNP-based map was a high density map composed of 31 LGs, 648 SNPs, and covered 1,524.7 cM with an average of 2.35 cM between two adjacent SNP markers. Fourteen significant QTL have identified for SDS resistance using interval mapping (IM) and composite interval mapping (CIM) with LOD scores that ranged between 2.6 and 5.0. Twelve QTL were identified for foliar disease severity (FDS) and three QTL for root rot severity (RRS) of which one QTL underlain both FDS and RRS. The fourteen QTL were mapped onto ten separate chromosomes of the soybean genome. Seven of the intervals encompassing the QTL had been identified previously (on LGs C1, C2, D1b, G, L, N and O) associated with resistance to SDS but seven were novel (LGs A2 (2), B1, C2, D1a, D1b and O). Eight QTL have been identified for SCN resistance to races 3 and 5 on 7 different soybean chromosomes. Four QTL for resistance to SCN race 3 were identified and mapped on LGs M, F, and J. Similarly, four QTL for resistance to SCN race 5 were identified and mapped on LGs A1, A2, and B1. The QTL identified here maybe introduced in breeding programs to develop cultivars with dual resistance to SDS and SCN.

The ANR Pathway of Proanthocyanidin Biosynthesis is Evolutionarally Conserved Across the Vascular Plants

Qing-Zhong Peng[1], Yue Zhu[1], Ci Du[1], Ke-Gang Li1, De-Yu Xie [1,2], [1]College of Biology and Environmental Sciences, Jishou University, Jishou City, Hunan Province, P. R. of China; [2]Department of Plant Biology, North Carolina State University, Raleigh, NC.

Proanthocyanidins (PAs) are oligomers or polymers of plant flavonoids. For plants, PAs provide multiple protective functions, which include UV-protection, anti-herbivore, anti-bacterium, anti-fungus, protection against oxidative stresses, etc. In the late biosynthetic pathway of PAs, there are two branches, the ANR and LAR pathways. However, whether and how the ANR and LAR pathways evolutionally co-exist to contribute to the formation of PAs remains unanswered. Here, we report the development of multiple approaches to demonstrate the ANR pathway in the plant kingdom. These approaches include analysis of ANR from different plants, HPLC-based profiling, phytochemical approaches, RT-PCR, and sequence blast analyses. We have tested 8 plant species. The resulting data show that The ANR pathway is phylogenetically conserved across ferns, gymnosperms and angiosperms. We hypothesize that the ANR pathway globally associates the biosynthesis of PAs in the vascular plants.

Graduate Student Oral Competition Session A.

Physiological and Biochemical Responses of Bald Cypress (*Taxodium distichum*) to Elevated Salinity

Nathan Lauer and Cliff Ross, Department of Biology, University of North Florida, Jacksonville, Florida.

Coastal freshwater wetlands are being lost due to salt water intrusion associated with anthropogenic activities and sea level rise. Slight inputs of elevated salinity are known to convert freshwater swamps into oligohaline marshes. Bald Cypress, an ecologically important species, persists longer than any other species associated with the freshwater wetland plant communities of the Southeastern United States. The salinity tolerance and sub-lethal stress response of this plant was studied by inundating one year old seedlings at salinities of 0, 4, or 8‰ for 10 weeks. Elevated salinity caused a drop in photosynthetic efficiency and an increase in non-photochemical quenching. Hydrogen peroxide content increased in both root and leaf tissue in plants maintained at 8‰. Soluble proline content increased in the leaf tissue of plants exposed to elevated salinity. Furthermore, the specific activity of the antioxidant enzyme superoxide dismutase increased in response to elevated salinity and there was a strong negative correlation between leaf hydrogen peroxide content and photosynthetic efficiency. Interestingly, the specific activity of peroxidase enzymes decreased in the leaf tissue of plants exposed to 8‰, suggesting that Bald Cypress downregulate these enzymes in order to increase hydrogen peroxide content in leaf tissue. Efforts will now be made to see if these trends hold true for Bald Cypress *in situ.*

The Role of *CALMODULIN-LIKE38* in Viral Suppression of RNA Silencing

Amy Wahba Foreman[1], Ansul Lokdarshi[2], Lewis H Bowman[1], Daniel M Roberts[2] and Vicki Vance[1]. [1]Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, USA. [2]Department of Biochemistry and Cellular and Molecular Biology, University of Tennessee, Knoxville, TN.

RNA silencing is a sequence-specific RNA degradation mechanism that serves as an antiviral defense pathway in many eukaryotic organisms. Many components of the silencing machinery have been identified, but the regulation of the process is not well understood. A number of viruses encode proteins that block silencing, often interfering with endogenous small RNA pathways as well. One such viral suppressor of silencing, the helper component proteinase (HC-Pro) of potyviruses, has been shown to interact with a tobacco protein called rgsCaM, which, like HC-Pro itself, blocks silencing when over-expressed in tobacco. Based on computational molecular modeling, the tobacco rgsCaM is a calmodulin-related protein with several unusual features that may be crucial for its function in suppression of silencing. Here we show that *Arabidopsis CALMODULIN-LIKE38* (*CML38*) is a structural homolog of the tobacco suppressor of silencing, *rgsCaM*, based on predicted protein domains, biochemical characteristics, and physical interaction with HC-Pro. In addition our work indicates that *CML38* is also a functional homolog of *rgsCaM* based on its involvement in RNA silencing. We find that *CML38* is required for the phenotypic anomalies associated with HC-Pro transgenic plants as well as for HC-Pro suppression of hairpin transgene-induced RNA silencing. Together these findings point to the importance of endogenous regulatory proteins in viral suppression of RNA silencing.

Coupling of Molecular Modeling with *In Vitro* Enzymatic Analysis to Elucidate how the Toc GTPase Mechanism is Coupled to the Pre-Protein Import Cycle

Kristen Holbrook and Barry Bruce, Department of Biochemistry and Cellular and Molecular Biology, University of Tennessee, Knoxville, TN

Most plastid-localized proteins are nuclear-encoded and post-translationally imported from the cytosol. The mechanism for the selective translocation of preproteins appears to involve the recognition of the transit peptide by the Toc GTPases, which act as gatekeepers. It is hypothesized that the primary targeting specificity is mediated through interactions between the transit peptide and the Toc receptors, Toc34 and Toc159. Although it is known that both Toc34 and Toc159 are GTPases and bind preproteins, it is unclear how their intrinsic GTPase activity is involved in the cycle.

To aid in the elucidation of this complex process, we have developed highly sensitive GTP-binding and hydrolysis assays to characterize the catalytic mechanism of the Toc34 GTPase component. We have extended our analysis of Toc34 to integrate molecular dynamics simulations and QM/MM calculations, which has allowed for the design of site-specific mutations near the active site of Toc34. We plan to enlist in vitro kinetic analyses to test the characteristics of these mutants. Our work is expected to make a major step forward in the understanding of the Toc GTPase catalytic mechanism as well as its importance in preprotein import.

Two conflicting models are available to explain the selective translocation process, and a major unresolved question is the identity of the primary receptor for preproteins. Evidence is available to support either Toc159 or Toc34 as the primary receptor. We have initiated experiments to build a novel in vitro XTP-dependent system for the Toc34 and Toc159 GTPases.

Ferric Reductases and Transporters that Contribute to Mitochondrial Iron Homeostasis

Anshika Jain and Erin Connolly, Department of Biological Sciences, University of South Carolina, Columbia, SC

Iron is an essential nutrient for plants and although the mechanisms controlling iron uptake from the soil are relatively well understood, comparatively little is known about subcellular trafficking of iron in plant cells. Our research focuses on the molecular mechanisms of iron transport between the cytosol and mitochondria. The *Arabidopsis* genome encodes for eight genes that likely encode ferric chelate reductase enzymes (FRO1-8). FRO3 localizes to mitochondria and analysis of *fro3* and *35S-FRO3* lines shows that FRO3 plays an important role in Fe homeostasis. *fro3* mitochondria show a 50% reduction in Fe content as compared to WT. The Fe content of *fro3* shoots and roots is elevated, suggesting that an alteration in mitochondrial Fe homeostasis is associated with changes in Fe homeostasis observed at the level of the whole plant. Currently, we are assessing the effects of loss of FRO3 on the seed production of the plant. Second, we have identified two genes (MFT1 and MFT2) that show considerable homology with mitoferrin Fe transporters of yeast and zebrafish. pMFT1/2-GUS lines show that MFT1/2 are expressed throughout the plant. MFT1 and MFT2 rescue the mutant phenotype of a yeast mitoferrin mutant that is defective in mitochondrial iron import. To precisely define the physiological function of these genes, we are in the process of analyzing the phenotypes of *mft1*, *mft2* and *mft1mft2* lines. These studies are anticipated to contribute to a comprehensive understanding of iron homeostasis in plants which may, in turn, allow the development of iron-rich plant foods that will help to reduce the incidence of iron deficiency anemia.

Mini Spider Silk-Like Protein Production in Transgenic Tobacco Plants

Congyue Peng and William R. Marcotte, Jr., Department of Genetics and Biochemistry, Clemson University, Clemson, SC

Spider silks are protein-based fibers among which the major ampullate silk features superior strength and elasticity that outperforms current available materials. Spider silks are biocompatible which makes them ideal therapeutic materials such as supporters for artificial bones or scaffolds for neuron regeneration. It is envisioned that large scale production of the constituent proteins will accelerate the development of advanced materials. Here, we present the production of spider major ampullate silk-like proteins (mini-spidroins) in transgenic tobacco plants. The assembled *MaSp1* and *MaSp2* genes are driven by the enhanced full-length mirabilis mosaic virus or peanut chlorotic streak caulimovirus promoter with a downstream N-terminus, variable numbers of repeats and C-terminus. The C-terminus is tagged with an intein-chitin binding domain (CBD) for protein purification. A total of 10 constructs had been created and independent transgenic tobacco lines (102) established. Fragments at the N and C-terminus of the assembled mini-spidroin gene were amplified from the genomic DNA extracts. We were able to amplify mini-spidroin intein fragments from the synthesized cDNA strands through reverse transcriptase PCR. Sp1R8 and Sp2R8 proteins were detected in crude leaf extracts and were partially purified by chitin resin binding. We are currently assessing the possibility that mini-spidroin intein will activate after elution from the chitin beads and that non-intein tagged mini-spidroins will be released and fiber can be produced from the proteins.

Employing Functional Genomics to Study the Regulation of Tetrapyrrole Metabolism in the Green Micro-Alga *Chlamydomonas reinhardtii*

Phillip Grovenstein and Mautusi Mitra, Department of Biology, University of West Georgia, Carrollton, Georgia

Chlorophylls (Chl) and Hemes are essential for energy metabolism in photosynthetic organisms. These tetrapyrroles have a common branched pathway of synthesis involving nuclear encoded enzymes. Free Chl and immediate precursors are highly photo-toxic and generate ROSunder aerobic conditions. Hence synthesized Chl and heme are usually bound to apoproteins. Chl and heme biosynthesis in photosynthetic organisms are under complex regulatory control at transcriptional, translational and post-translational levels. This is not well understood.

Unlike higher plants, *Chlamydomonas reinhardtii's* chls and hemes are exclusively synthesized in the chloroplast. As a green micro-alga, *C. reinhardtii* grows heterotrophically (using acetate) and photoautotrophically. A haplontic life cycle, ability to use light-dependent and light-independent pathways for chlorophyll synthesis, and a completely sequenced genome makes it ideal for photosynthetic studies. Furthermore, well-developed molecular tools exist for genetic manipulation. We have generated a random DNA insertional Chl deficient mutant. Mutant #14 cannot grow photoautotrophically, is very sensitive to rapid changes in light intensity, is green in the dark and shows a light intensity dependent photo-bleaching. Preliminary steady state analysis of tetrapyrroles and tetrapyrrole precursors by HPLC indicates #14 is constitutively heme deficient and accumulates tetrapyrrole precursors like protoporphyrin IX, Mg-protoporphyrin IX and Mg-protoporphyrin IX monomethylester in the dark but not in light. We will present our data on this mutant.

Functional Characterization of AtFRO4 and AtFRO5: Two Members of the *Arabidopsis* Ferric Reductase Oxidase Gene Family

Grandon T. Wilson and Erin L. Connolly, Department of Biological Sciences, University of South Carolina, Columbia, SC

Iron and copper are essential nutrients for plants and act as cofactors for enzymes that function in respiration and photosynthesis. Members of the *FRO* (*f*erric *r*eductase *o*xidase) family of ferric chelate reductases are known to reduce ferric chelates to ferrous iron and it is thought that *FROs* may reduce copper as well, like their yeast counterparts. The *Arabidopsis FRO* family contains eight members; the best-characterized member of the family, *AtFRO2*, is responsible for reduction of iron at the root-soil interface. Other FROs localize to internal membranes; AtFRO7 localizes to chloroplasts and plays an essential role in chloroplast Fe homeostasis. Our current work focuses on the role of *AtFRO4* and *AtFRO5*, which reside in tandem on chromosome 5. Expression of AtFRO5-GFP in protoplasts shows that AtFRO5 localizes to the plasma membrane and AtFRO4 likely localizes to the plasma membrane as well. *AtFRO4* is expressed in older leaves and roots, while *AtFRO5* is expressed primarily in the roots. Previously, we showed that expression of *AtFRO5* is induced by deficiencies of iron and copper and recent data suggests that *AtFRO4* also is induced by Cu deficiency. Analysis of *atfro4*, *atfro5* and *atfro4fro5* lines demonstrates that *AtFRO4* and *AtFRO5* function as copper reductases that reduce Cu at the root surface. Taken together, data suggest *AtFRO4* and *AtFRO5* have a role in copper homeostasis, and likely function to reduce Cu(II) to Cu(I) in the rhizosphere for subsequent uptake across the plasma membrane of root cells, while their function in iron homeostasis is still largely unknown.

Physiological Responses of the Seagrass *Thalassia testudinum* Against the Causative Agent of Wasting Disease, *Labyrinthula sp.*

Stacey Trevathan-Tackett and Cliff Ross, Department of Biology, University of North Florida, Jacksonville, Florida.

Seagrass meadows represent a vital component of many coastal ecosystems, but have experienced declines in abundance due to a series of environmental stressors including incidence of wasting disease and changes in salinity. The objective of this study was to investigate the short term physiological responses of *Thalassia testudinum* when exposed to elevated salinity and to determine how this impacts susceptibility to infection by *Labyrinthula*, the causative pathogen of wasting disease. *T. testudinum* was subjected to ambient (30) and elevated (45) salinity treatments for one week under laboratory conditions before being infected by *Labyrinthula*. One week after the onset of infection, a series of physiological and metabolic response variables were measured. Results demonstrated that the occurrence of wasting disease was significantly lower in the hypersalinity treatments. Exposure to elevated salinity caused a reduction in chlorophyll *a* and *b* content, however photosynthetic activity was not affected by either salinity or disease. Interestingly, elevated salinity caused *T. testudinum* to significantly increase *in vivo* H2O2 concentrations in the leaf tissue to approximately three times the values found in specimens maintained under ambient salinity conditions. These H2O2 concentrations were found to be sufficient in inhibiting *Labyrinthula* growth using an *in vitro* bioassay. Our results suggest that even after short term exposure to hypersaline conditions, there were no significant changes to plant physiology that would enhance susceptibility to infection.

Three Arabidopsis AIL/PLT Genes Act in Combination to Regulate Shoot Apical Meristem Function

Janaki Mudunkothge and Beth A. Krizek, Department of Biological Sciences, University of South Carolina, Columbia, South Carolina

The shoot apical meristem, a small dome-shaped structure at the shoot apex, is responsible for the initiation of all post-embryonic shoot organs. Pluripotent stem cells within the meristem replenish themselves and provide daughter cells that become incorporated into lateral organ primordia around the meristem periphery. We have identified three novel regulators of shoot apical meristem activity in *Arabidopsis thaliana* that encode related AIL/PLT transcription factors: *AINTEGUMENTA* (*ANT*), *AINTEGUMENTA-LIKE6* (*AIL6*)/*PLETHORA3* (*PLT3*) and *AINTEGUMENTA-LIKE7* (*AIL7*)/*PLETHORA7* (*PLT7*). Loss of these genes results in plants that initiate only a few leaves prior to termination of shoot apical meristem activity. In seven-day-old *ant ail6 ail7* seedlings, we observed reduced cell division in the meristem region, differentiation of meristematic cells and altered expression of the meristem regulators *WUSCHEL* (*WUS*), *CLAVATA3* (*CLV3*) and *SHOOT MERISTEMLESS* (*STM*). Genetic experiments suggest that these three *AIL* genes do not act specifically in either the *WUS*/*CLV* or *STM* pathway regulating meristem function. Furthermore, these studies indicate that *ANT*, *AIL6* and *AIL7* have distinct functions within the meristem rather than acting in a strictly redundant manner. Our study thus identifies three new genes whose distinct functions are together required for continuous shoot apical meristem function.

The Utilization of a Genome-Scale Model to Explore Mutations that Increase Cellulose in *Arabidopsis thaliana*

Jiun Yen[1], Ryan Senger[1], and Glenda Gillaspy[2], [1]Department of Biological Systems Engineering, [2]Department of Biochemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24060

Plant scientists have pursued the optimization of plant biomass and cellulose production via modulation of single genes and breeding strategies, but to date, this process has not yielded substantial improvements. We are working to develop a computational modeling strategy to predict and modify combinations of genes that will drive biomass and cellulose increases. A metabolic genome scale model of *Arabidopsis* thaliana, called AraGEM, was modified to search for candidate genes that would allow increased cellulose production. AraGEM is constructed from 1601 metabolic reactions using 1737 compartmentalized metabolites. The rate and directionality of each reaction is defined by its flux value, and its magnitude is limited by the upper and lower constraints. The optimization of the fluxes is performed using the COBRA Toolbox and the GNU Linear Programming Kit (GLPK). We constrained the fluxes of the reactions within AraGEM to mimic the physiology of plant mesophyll cells during photosynthesis. We then optimized this system to compute a maximal cellulose accumulation rate at cases of different photon uptake rates. We compared the flux ratios between each case and found reactions with the most significant flux changes. The enzymes that govern these reactions were considered as promising candidates to modify plant biomass and cellulose production. We are examining the genes encoding these enzymes, and are testing the model's predictions by altering these genes. To test our model, six candidate genes are being overexpressed to create gain-of-function transgenic plants. Two other candidate genes are being studied using existing SALK and SAIL T-DNA knock-out lines to obtain loss-of-function plants. We will report on two gene candidates where data support our model's prediction.

Velvetbean: *de novo* transcriptome assembly

Elspeth S. Murday[1] and H.T. Knap[1,2], [1]School of Agricultural, Forest, and Environmental Sciences, Clemson University, Clemson, SC, [2]Department of Genetics and Biochemistry, Clemson University, Clemson, SC, USA

A transcriptome of velvetbean—*Mucuna pruriens*, a tropical legume closely related to soybean—has been assembled de novo from Illumina HiSeq 100 bp paired-end mRNA sequencing. Traditionally used as a forage and cover-crop, velvetbean produces large seeds and displays rapid, vigorous vegetative growth. The plant yields many compounds of medicinal and agronomic interest, such as multiple nematicidal compounds including the growth stimulant triacontanol ("TRIA".) Triacontanol is a primary alcohol produced from a 30-carbon very long chain fatty acid (VLCFA). Nematicidal metabolite production in velvetbean might correlate with known VLCFA pathway genes or endoreduplication, a process which can enhance metabolite production. Endoreduplication is governed at least in part by a group of genes designated CCS52 in plants and could also provide an explanation for the plant's rapid growth. Because velvetbean lacks both a reference genome and published gene sequences, de novo transcriptome assembly of velvetbean was necessary to search for transcripts of putative VLCFA and CCS52 genes. To ensure best assembly of the new transcriptome, raw Illumina reads for plant species with published reference genomes were used to assess de novo transcript-assembly pipelines. Potential bioinformatic processing of velvetbean data—including adapter removal, quality trimming, and sequence assembly—was first tested on the reference species. Reads were then aligned back to their respective reference genomes to compare effects of each treatment. Additionally, assembly programs were compared by detection of verified genes within each set of finished transcripts from reference species. Successful methods for de novo transcript assembly were implemented for velvetbean; CCS52 and VLCFA-pathway gene candidates were identified for comparison to soybean sequence.

Graduate Student Oral Competition Session B.

Ethanol Prolongs Systemin, Flagellin, and Chitosan-Induced Activation of MAP Kinases in *Solanum peruvianum* cells.

Claire Hann and Johannes Stratmann, Department of Biological Sciences, University of South Carolina, Columbia, South Carolina

The perception of elicitors by membrane-bound receptors, followed by the activation of MAP kinases (MAPKs) and an extracellular alkalinization, are components of the signaling pathway that results in defense responses to pathogens and herbivores. In suspension-cultured cells of Solanum peruvianum (a wild tomato species), the durations of MAP kinase activation and extracellular alkalinization post-exposure to the wound-signaling peptide systemin, and the microbe-associated molecular patterns (MAMPs) flagellin and chitosan, have previously been established. The objective of this study was to test whether ethanol affects these two signaling responses. MAPK activation was measured by immunoblot analysis with anti-phospho-ERK antibodies, which recognize phosphorylated, or activated, MAPKs. I found that ethanol concentrations of 2% or more prolonged the duration of MAP kinase activation induced by the elicitors systemin, chitosan, and flagellin. Ethanol alone did not activate MAPKs. In addition, the extracellular alkalinization response to ethanol and elicitors was synergistic, as compared to ethanol or elicitor alone. These results show that ethanol increases the sensitivity of plant cells to elicitors. When ethanol is used as a solvent for test compounds in similar bioassays, appropriate ethanol concentrations should be considered.

Comparative Proteomics of Recalcitrant Seed Death in *Spartina alterniflora*

Yi Wang and Marc Alan Cohn, Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA.

While most seeds can be stored dry for commerce and conservation, some seeds exhibit recalcitrance and die when desiccated. Spartina alterniflora produces recalcitrant seeds, which lose viability when dried below 45% water content. Comparative proteomics between S. alterniflora and orthodox, desiccation tolerant S. pectinata seeds was performed to identify heat-stable (soluble after 40 min at 95oC) proteins that may be associated with desiccation tolerance. The heat-stable proteomes of S. alterniflora and S. pectinata contain 231 and 297 spots, respectively, as resolved by two dimensional gel electrophoresis. Eighty-five unique spots were present in S. pectinata but were missing in S. alterniflora. Some have been sequenced so far, and a number of proteins share homologies to known sequences for group-3 late embryogenesis abundant proteins (LEAs), dehydrin, cystatin, superoxide dismutase, peroxiredoxin, stress-responsive protein, nascent peptide associated complex, abscisic stress ripening protein, glyceraldehyde-3-phosphate dehydrogenase and ubiquitin; these proteins are associated with the desiccation-tolerant state in other organisms.

Gels stained with PRO-Q Diamond revealed phosphorylated cystatins, dehydrin, ubiquitin and abscisic stress ripening protein that were highly expressed in S. pectinata but not in S. alterniflora. These data suggest that a modestly-sized suite of proteins and post-translational modifications confer desiccation tolerance to S. pectinata seeds, and demonstrate the utility of the comparing related species to understand physiological processes.

Examining the Roles of PsToc75 POTRA Domains in Chloroplast Protein Import

Richard Simmerman and Barry Bruce, Department of Biochemistry and Cellular and Molecular Biology, University of Tennessee, Knoxville, TN

During chloroplast evolution, the majority of the enslaved cyanobacterial DNA was appropriated and incorporated by the host eukaryote. Analyses suggest that >95% of the ancestral cyanobacterial proteome is now nuclear encoded, and that now cytosolically-translated proteins required for chloroplast function must be transported into the organelle. The mechanism of translocation is poorly understood, but involves TOC & TIC (translocons at the outer/inner envelope of the chloroplast membranes). A 20-100 residue-long, cleavable, N-terminal extension known as the TP (transit peptide) targets precursor proteins to the chloroplast. Pre-proteins pass through the outer chloroplast membrane via an Omp85 (outer membrane protein of 85 kDa) homologue, Toc75. In addition to a transmembrane beta-barrel, mature Toc75 contains three POTRA (polypeptide transport associated) domains. POTRA domains, numbered increasingly from the N-terminus, have a conserved secondary structure. Despite their inclusion in all members of the PTB (polypeptide-transporting beta-barrel protein) superfamily, homologues of which are present in outer membranes of Gram-negative bacteria, mitochondria, and chloroplasts, the roles of POTRA domains in translocation are unknown. To investigate roles of POTRAs in Toc75, we expressed and purified recombinant P1, P2, P3, and P1-3. Homotypic POTRA interactions are supported by crosslinking and (AUC) analytical ultra centrifugation. Heterotypic POTRA interactions with TP have been studied and supported via: pull-down assays, crosslinking, AUC, BSI (backscatter interferometry).

Analysis of Soybean Stem Characteristics to Maximize Biofuel Yield

Matthew Waalkes and Anne Alerding, Virginia Military Institute, Lexington, VA

Alternative energy sources are being examined to compensate for increased demand. Plant matter (biomass) is one source of biofuels that can help stabilize the world's energy consumption. Leftover crop material, made up mostly of cellulose and lignin, is a potential source of biomass for biofuel. One possible source for biomass is soybeans; however, it is unknown what anatomical characteristics in soybean plants will help produce the best biofuel. Evidence suggests cellulose is more efficient biomass for biofuel than lignin. We investigated morphologies of five soybean cultivars to select the cultivar with ideal characteristics to produce biofuel. Our hypothesis is that cultivars with external and internal characteristics associated with an increased cellulose to lignin ratio will produce the most efficient biomass for biofuel. We conducted four experiments, each with five different cultivars, to determine the highest cellulose to lignin ratio while maintaining seed yield. One experiment in each location was for stem cross-sections to measure internal stem characteristics, while the second is to measure soybean yield. Of the five cultivars we have examined so far, there are significant differences between some of the cultivars' external characteristics, including stem diameters, using ANOVA single factor statistical tests. We are working to determine the cellular basis of these differences and calculate their relationship to the cellulose/lignin ratio. It is our hope to correlate these characteristics with biofuel yield by measuring biofuel through pyrolysis.

Interacting Partners of the SUNN Symbiotic Regulatory Kinase

Ashley Crook and Julia Frugoli, Department of Genetics and Biochemistry, Clemson University, Clemson, SC.

The control of nodule number, or autoregulation of nodulation (AON), exhibited by nodule-forming legume species involves a complex signaling pathway encompassing molecules that act in both the root and the shoot. To date, our lab has focused on two genes known to be involved in the AON of *Medicago truncatula*, *SUNN* and *RDN1*, which have a supernodulation phenotype when mutated and have been shown to act in the shoot and root, respectively. Co-immunoprecipitation of *SUNN* using whole *M. truncatula* transgenic plants in a *SUNN* null background will allow for the identification of novel protein-protein interactions within the AON. Future studies will include a co-immunoprecipitation study of *RDN1* in the same manner. Here we report the generation of whole *M. truncatula* transgenic plants mediated by *Agrobacterium tumefaciens* strain DHA105 using a tissue culture system specially adapted to *M. truncatula*. Our initial transformation is carrying a plasmid containing a YFP/Hemagglutinin-tagged *SUNN* gene driven by the 35S CaMV promoter. Putative transgenic lines were selected using BAR herbicide resistance. A second construct carrying a Strep/His-tagged *SUNN* gene driven by the 35S CaMV promoter was also used to make transgenic plants. Preliminary attempts to identify and precipitate *SUNN* from plants carrying these constructs will be discussed.

Ectopic Expression of AINTEGUMENTA-LIKE 6 Affects Floral Organ Initiation and Identity

Han Han and Beth A. Krizek, Department of Biological Sciences, University of South Carolina, Columbia, SC

A small group of floral stem cells gives rise to a flower with four types of floral organs arranged in a defined pattern in *Arabidopsis thaliana*. Four classes of floral homeotic genes act in different combinations to specify floral organ identity. This process involves cell-fate specification, morphogenesis and tissue patterning, which are precisely controlled but poorly understood. Mutations in *AINTEGUMENTA* (*ANT*), an AP2/ERF gene, result in female sterile and the production of smaller floral organs than wild type. Mutations in the related *AINTEGUMENTA-LIKE 6* (*AIL6*) do not have any visible phenotype, however *ant ail6* double mutants display more severe flower defects than *ant*. These genetic results indicate that *ANT* and *AIL6* have partly overlapping roles in regulating floral organ initiation, identity and growth. To further probe *AIL6* function in flower development, we have ectopically expressed *AIL6* using two different approaches. In plants ectopically expressing *AIL6* under the *ANT* promoter (*ANT:gAIL6-3'*), we observe alterations in floral organ number and the production of mosaic floral organs, in particular the development of petaloid sepals in the first whorl. This phenotype is correlated with misexpression of the class B floral homeotic gene, *APETALA3* (*AP3*), in first whorl organ primordia. We have also used an ethanol inducible system to constitutively express *AIL6* (*35S: AlcR/AlcA:gAIL6-3'*). Our results suggest that *AIL6* is an important regulator of floral homeotic gene expression and that *AIL6* levels must be precisely controlled in early stages of flower development.

Effects of Historical Land Use and Environmental Variation on Plant Community Structure and the Prevalence of Invasion in Weeks Bay, AL

Amanda Ecker, Kelly Major and C. S. Major, Department of Biology, University of South Alabama, Mobile, AL

Weeks Bay is ecologically and economically important, and an excellent case study for how land use and natural variation influence invasive species occurrence in sensitive coastal systems. Specifically, the Swift Tract region of the Weeks Bay reserve is comprised of Southern Flood Plain and mixed hard wood forest cover types that include *Pinus palustris*/*Pinus elliottii*-dominated communities that are notably in decline. Fifteen paired sampling sites were stratified throughout the study area to represent all cover types. Percent cover for all species rooted in each 50 X 20 m nested plot was recorded monthly from Summer 2010 through Fall 2011, along with canopy/subcanopy percent cover, DBH, and seedling/sapling measurements. Lysimeters were placed between paired sites; soil parameters that include salinity, pH, dissolved oxygen (DO), and organics were measured from March-November 2011. To date, 58 taxa from 34 plant families have been recorded. Several genera/species (e.g., *Polygonum* sp., *Rumex* sp., *Boehmeria cylindrica, Toxicodendron radicans*) are distributed across ~75% of the sites. Additionally, two invasive species, *Triadica sebiferum* (popcorn tree) and *Alternanthera philoxeroides* (alligator weed), commonly occur among sites. Data indicate that salinity and DO are low, while organics are generally high, regardless of location. Vegetation, soil, and habitat data (rainfall and temperature) and spatial imagery will be used to develop a management tool to monitor shifts in composition and identify plant communities most susceptible to invasion upon disturbance.

Seagrass Defenses: Elicitation of an Oxidative Burst in the Tropical Marine Angiosperm *Thalassia testudinum*

Kyle Loucks and Cliff Ross, Department of Biology, University of North Florida, Jacksonville, Florida.

Seagrasses are widely distributed marine vascular plants that serve as important species in coastal ecosystems. We have witnessed global declines in seagrass with die-offs attributed to multiple factors that include direct human activities as well as climate change. "Wasting disease", caused by protist pathogens in the genus *Labyrinthula*, has contributed to rapid population declines in both temperate and tropical seagrasses. Unfortunately, we do not have a clear understanding of processes of disease transmission or how seagrass defenses modulate responses to the pathogen.

Although there is an increasing awareness of the impacts of diseases in the marine environment, and considerable effort has been made to understand activated defense responses in terrestrial plants, the biochemical basis of pathogen recognition and host defense responses in seagrasses are virtually unexplored. Reactive oxygen species (ROS), in particular H2O2, serve as antimicrobial agents and are upregulated during the plant defense response cascade. *T. testudinum* can produce H2O2 locally in response to microbial exposure. What is not clear is the functional role of ROS accumulation. By using a combination of fluorogenic probes and a newly developed ROS- chemiluminescence assay we provide insight into the kinetics and abundance of ROS produced in seagrasses during the early stages of infection.

N-Terminal Hsp70 Binding Site is a Major Determinant for Precursor Protein Translocation Into Chloroplasts

Prakitchai Chotewutmontri and Barry Bruce, Department of Biochemistry and Cellular and Molecular Biology, University of Tennessee, Knoxville, TN.

The majority of chloroplast proteins is nuclear-encoded and utilizes N-terminal targeting sequence called transit peptide (TP) to target and translocate into chloroplasts through the general import pathway. Although analysis of plant genomes was fruitful in providing over ten thousand predicted TP primary sequences, it is still poorly understood what constitutes a TP and how these components facilitate TP recognitions. We have shown that the recognition of TP during binding to chloroplast is physicochemical specific. However, the translocation of precursor protein is dictated by the N-terminal sequence of TP. This terminal region has been shown to be highly uncharged and recognized by Hsp70 chaperone. Here we determine the role of these properties by replacing the N-terminus with a series of previously recognized but non-plant based Hsp70 interacting peptides. We confirm the critical role of the N-terminus as an Hsp70 binding peptide and as the dominant determinant for translocation. Analyzing a collection of 208 experimentally confirmed TP sequences from Arabidopsis, we identify the locations of Hsp70 biding sites with respect to the proposed TP motif FGLK. Based on the Hsp70 binding site location, they are classified into 9 subgroups. We observe that a subset of TP harboring the N-terminal Hsp70 has a tendency to carry a FGLK motif around 25 residue away from the N-termini suggesting the important role of their specific placements in TP recognitions. Future work will further address this observation.

Long Distance Signaling: a Mechanism for Nodule Regulation in *Medicago truncatula*

Tessema Kebede Kassaw and Julia Frugoli, Genetics and Biochemistry, Clemson University, Clemson, South Carolina

The most metabolically diverse prokaryotes are the only organisms capable of fixing molecular nitrogen in association with specific group of plants known as legumes. The symbiosis process is energy expensive and plants need to maintain the trade off by limiting the number of nodules they form. Hence, legume plants inherited long distance signaling which is a common phenomenon in plant developmental regulations to control the extent of nodulation. We cloned a *ROOT DETERMINED NODULATOR1 (RDN1)* of *Medicago truncatula*. The *rdn1* mutants have defect in the regulatory circuit and can form 5-10 fold more nodules than the wild type. Grafting experiments and split root systems demonstrated that *RDN1* regulatory function occurs in the roots and prior nodulation events doesn't affect late nodulation events. *RDN1* encodes a protein of unknown function and is a member of a small, uncharacterized, highly conserved gene family unique to green plants. Among the three *RDNs* in Medicago, we found that the ortholog of *RDN2* in Arabidopsis involved in lateral root regulation. The *RDN1* promoter drives expression in the vascular cylinder, suggesting *RDN1* may be involved in initiating, responding to, or transporting vascular signals. Using Inverted-Y grafting we confirmed that *RDN1* is involved in the synthesis or transporting of the root derived signal not responding to the shoot derived signal. We recently found that *RDN1* localized in a large number of tiny moving organelles in the cytoplasm and plasma membrane of the cell suggesting that *RDN1* may engage in vesicle trafficking.

Identification and Characterization of a MAP Kinase-Containing Complex

Carleton Bequette and Johannes Stratmann, Department of Biological Sciences, University of South Carolina, Columbia, SC

Mitogen-activated protein kinase (MAPK) cascades are crucial components of signal transduction pathways, which are involved in transmission of extracellular stimuli to a wide range of cellular responses. These protein phosphorylation cascades consist of three functionally linked kinases: MAPK kinase kinase, MAPK kinase, and MAPK. In plants, MAPK signaling pathways are involved in responses to many environmental stresses and in development. Prior research has identified specific components of MAPK cascades, however, it is largely unknown how MAPK signaling modules are organized in response to a specific stimulus, which induces a specific output response. Using size exclusion chromatography and subsequent immunoblotting, we have identified MAPKs and MAPKKs in high molecular weight (~300-600kDa) fractions of tomato, tobacco, and Arabidopsis leaf tissue. This indicates that MAPKs and MAPKKs associate with other proteins and form a multiprotein complex (MC). After MAPK activation, all active MAPKs are present as free proteins and do not associate with the MC. However, in the presence of a phosphatase inhibitor, active MAPKs associate with the MC. This indicates that the MC plays a role in MAPK dephosphorylation. Our long-term goal is to identify the constituents of the MC.

Poster Session 1. 5:15 P.M – 7:30 P.M.

Undergraduate students – posters

(* - undergraduate poster competition entry)

P1*. Employing Functional Genomics to Identify Novel Genes That Provide Photo-Protection to Plants in High Light Stress

Darryel A. Wilson, Jacqueline M. Smith, Kathryn D. Lankford, Phillip B. Grovenstein, and Mautusi Mitra. Department of Biology, University of West Georgia, Carrollton, GA

The model green micro-alga C*hlamydomonas reinhardtii* can be easily cultured in the laboratory, has a simple haplontic life cycle, grows both photosynthetically and heterotrophically, is amenable to both nuclear and chloroplast transformation and its genome has been sequenced. All of these traits make it an ideal eukaryotic model system to dissect oxygenic photosynthesis. The goal of this research project is to identify novel genes that play a role in photo-protection under high light stress.

Although *Chlamydomonas* mutants that are incapable of photoautotrophic growth can be identified and cultured as heterotrophic mutants in the light, this methodology does not allow for the obtainment of photosynthetic mutants that are light sensitive. Hence to isolate such light sensitive photosynthetic mutants, screening has to be performed in dark. After comparison of the growth of four different wild-type *Chlamydomonas* strains in the dark, the cell-walled strain $4A^+$ was selected as the parental strain for the generation of insertional mutants because of its ability to grow well and stay green in both the light and dark. The linearized bacterial plasmid pBC1, conferring paromomycin resistance, was used to transform the strain $4A^+$ to generate a random DNA insertional mutant library. To date we have identified 90 high light stress sensitive mutants which vary in their degree of light sensitivity. We will be presenting our research about some of these light sensitive mutants.

P2*. Analyzing the Mechanism of Antibiotic Resistance with Atwbc19 through the Analysis of Protein-Protein Interaction

La'Mayah Hodges, Raven Hardy, and Mentewab Ayalew, Department of Biology, Spelman College, Atlanta Georgia

ABC (ATP-binding cassette) transporter systems couple the transport of the solutes through the cell membrane to ATP hydrolysis. Atwbc19 is an ABC transporter that confers antibiotic resistance; however, in doing so it does not behave as a typical ABC transporter. In an attempt to understand the mechanism of antibiotic resistance associated with Atwbc19, we sought to identify proteins that interact with Atwbc19. CK2 (Casein Kinase 2) was found to interact extensively with the N-terminal region in a yeast two hybrid experiment. To confirm this interaction, a Bimolecular Fluorescense Complementation (BiFC) was used. BiFC allows us to determine whether the two proteins interact as well as their subcellular localization *in planta*. To do this, cDNA was synthesized and the gene was cloned into an expression vector that tagged it with nYFP or cYFP. For the seedling transformation, the 'Fast Agrobacterium Seedling Transformation' (F.A.S.T.) method was used as a quick and efficient assay tool. Confirming the interaction between Atwbc19 and CK2 suggests that Atwbc19 is phosphorylated by the kinase, the significance of which has yet to be determined.

P3*. The Effects of NIP on Cellular Growth in *Arabidopsis thaliana* Leaves

Bailey Wattron, Mohammad Salehin, Rebecca Dickstein, Department of Biological Sciences, University of North Texas, Denton, TX.

In the legume *Medicago truncatula*, the *NIP* gene encodes a protein (NIP) necessary for the development of nitrogen-fixing nodules. *NIP* has been transformed into the genome of Col-0, a variety of the model plant *Arabidopsis thaliana*. Col-0 mutants with the transformed *NIP* gene yield larger leaves than wild-type Col-0 specimens. It has been hypothesized that the gene affects leaf size by causing increased cell expansion in the leaves. To test this hypothesis, epidermal leaf cells from multiple wild-type Col-0 and transgenic Col-0 specimens (Col-0 + *NIP*) are observed with a scanning electron microscope. The observable areas of all sampled cells are calculated, resulting in an average area of Col-0 cells and an average area of Col-0+*NIP* cells. Comparison of these averages is used to determine the effects of *NIP* on cell expansion. Measurements gathered thus far show only a meager difference in the sizes of Col-0 and Col-0+*NIP* cells. These results do not verify the hypothesis that *NIP* enhances leaf-size in *Arabidopsis thaliana* primarily through the increase of cell expansion. Although the hypothesis may be correct, it is possible that *NIP* affects alternative or additional mechanisms of leaf-growth, such as cell proliferation. In order to yield more accurate size measurements, additional leaf cell samples will be analyzed, including samples from cotyledons. Cotyledons have a small and highly observable surface area and the average cell size in these leaves can be calculated more accurately than in larger leaves. We acknowledge funding by NSF #IOS-0923756.

P4*. Selenate (SeO4) Induced the Up-Regulation of miRNA395 in *Arabidopsis thaliana*

E. Patrick Vallentine and Doug Van Hoewyk, Department of Biology, Coastal Carolina University, Conway, SC.

MicroRNA's are a class of noncoding RNA molecules about 20bp long. Their function is to regulate proteins on a post-transcriptional level. MiRNA395 is one of the identified miRNAs and is conserved across many plants. During times of sulfate starvation, a plant must have a mechanism that makes certain that the limited sulfate is used in the shoots for photosynthesis. *Arabidopsis thaliana* has accomplished this through miRNA395 which binds to the transcripts of less important proteins and destroys them before translation. The experiment I performed was to determine if selenate induced miRNA395 expression. It has been observed that excess selenium induces sulfur starvation-like symptoms in some plants. We expected to find that miRNA395 would be up-regulated in selenate fed plants as is the case for sulfur starved plants. *Arabidopsis thaliana* plants were grown in one of three treated agar growth plates. The treatments were a control, -Sulfate, and +Selenate. After a period of growth, the samples were processed by grinding, miRNA395 and 167(control) was extracted, and reverse transcription was performed. The miRNA was then run through a real time PCR machine to quantify the amount of miRNAs present. The preliminary results show that there is an increase in miRNA395 within the leaves of plants grown in selenate. The finding that selenate may also be involved in the regulation miRNA395 could change the way we understand miRNA395. The results presented here are still preliminary and the research is ongoing.

P5*. The Effect of the Arabidopsis ABC Transporter Atwbc19 on Transcript Levels of Iron-Regulated Genes

Morayo Gloria Adebiyi and Mentewab Ayalew, Department of Biology, Spelman College, Atlanta, GA

Atwbc19 encodes for the ATP Binding Cassette (ABC) transporter from Arabidopsis thaliana.This gene was discovered to confer antibiotic resistance. Atwbc19 is expressed throughout the plant's vascular system and is coexpressed with iron transport genes. Because iron transporters might serve as a gatweway to kanamycin, we hypothesize that the antibiotic resistance associated with Atwbc19 relies on the downregulation of iron transport genes.To quantify transcript levels of iron transport genes, real time-PCRs were performed using cDNA generated from plants grown in the presence or absence of kanamycin. When plants were exposed to kanamycin, iron transport genes were downregulated. However, when Atwbc19 is knocked out, the transcripts of iron transport genes remained high. These experiments provide support for the hypothesis that when Atwbc19 is functional iron transport genes are downregulated.

P6*. Genetic Mapping of a Novel MicroRNA Translational Regulation Mutant in Plants

Kala Peek, Caleb Kirkpatrick, Cody Mullins and Charlotte Song, Department of Biology, Charleston Southern University, North Charleston, SC.

Recently, small RNAs have been found to be significant in the regulation in eukaryotes. MicroRNAs (miRNA) are a class of small RNAs. In plants, cleavage is believed to be the main mode of regulation by miRNAs, but recently translational regulation has been found to also be playing a role. We have generated transgenic plants with an artificial miRNA targeted to *chalcone synthase* (*CHS*) in *Arabidopsis thaliana*. *Chalcone synthase* is an ideal gene to study because it is involved in the synthesis of anthocyanins, which are easily observable under certain experimental conditions. We introduced a nucleotide loop out in the target to prevent cleavage and shifted the regulation to translational repression. Then, we generated EMS mutants to find signaling components involved in translational repression of miRNA targets. The EMS pools were screened for suppression mutants. The mutants were collected and crossed to a different ecotype of *Arabidopsis thaliana* to generate a mapping population. In the laboratory, I have collected seeds, planted mapping populations, extracted DNA samples for one of the mutants. We are currently mapping the mutant to find the gene. The mutant I am studying does not have any distinguishable phenotypes, and it does not have any overlapping phenotypes with known miRNA mutants. It is likely to be novel mutant.

P7*. Confirmation of Mini-spidroin Expression in Transgenic Tobacco

Charlene Gravgaard and William R. Marcotte, Department of Genetics and Biochemistry, Clemson University, Clemson, SC

Spider silks are proteinaceous fibers that display a variety of attractive physicomechanical properties. The major ampullate or dragline silk of spiders is composed of two proteins (spidroin 1 and spidroin 2) and is of interest for the development of new materials because it provides great strength along with elastic properties. Silk-like materials have been shown to be promising for biomedical applications due to their biocompatibility and biodegradability and are likely to be useful as scaffolds for tissue engineering. This study involved the introduction of recombinant silk-like genes into tobacco with the intent of producing large quantities of the constituent proteins for new materials development. The constructs contained one of two plant viral promoters to drive expression on mini-spidroin coding regions. We have verified the identity of transgenic plants harboring both spidroin 1 and spidroin 2 constructs, demonstrated expression at the RNA level by RT-PCR and are now characterizing protein expression levels. In addition, we are exploring a variety of scalable protein purification strategies.

P8*. Evaluation of Natural Pathogens of *Salvinia molesta* as Potential Agents of Biological Control.

Danielle B. Handrop, Stephen W. Banks, and Dalton R..Gossett. Louisiana State University at Shreveport, Shreveport, LA.

The floating water fern *Salvinia molesta* ,now classified as the world's number one aquatic pest has infested lakes in the southern United States. This fern reproduces asexually, and can cover large expanses of water very rapidly which leads to habitat destruction. The experiments described in this presentation were carried out in an effort to investigate the efficacy of bacterial and fungal pathogens of *Salvinia* being used as agents of biological control. Our approach was to investigate the effects of natural pathogens on *Salvinia* when combined with physical injury to the plant structure. Field isolates of fungi and bacteria were applied in various ways to determine which combinations caused the most tissue damage. Our results revealed that an, as yet unidentified fungus and an unidentified bacteria in certain combinations did in fact hinder new growth and killed portions of *Salvinia*. Our future work will be to identify these pathogens and further investigate the possibility of them as agents of biological control.

P9*. Creation and Characterization of Transgenic Tobacco Plants Expressing Human Recombinant Erythropoietin

Mamudou S. Bah, Farooqahmed S. Kittur, Chiu-Yueh Hung and Jiahua Xie. Department of Pharmaceutical Sciences, Biomanufacturing Research Institute & Technology Enterprise, North Carolina Central University, Durham, NC.

Erythropoietin (EPO) is a glycoprotein hormone produced by the adult kidney. It is a cytokine and has two basic functions: the production of red blood cells and protecting tissues from diverse injuries. Currently, recombinant human EPO (rhuEPO) is being used for the treatment of anemia, and is the most commercially valuable biopharmaceutical on the market. The mammalian cell culture currently being used to produce rhuEPO is however, very expensive. There is a need for an alternate expression platform to produce rhuEPO inexpensively for use as cytoprotective agent. Plants offer many advantages such as inexpensive to grow and maintain, free of pathogens and most importantly they can synthesize complex *N*-glycans. The objective of the present study was to create transgenic tobacco plants for the production of rhuEPO for cytoprotective function. To achieve that, a genetic cassette containing human β1, 4-galactosyltransferase gene *(GalT)* driven by GapC promoter and erythropoietin gene (*EPO*) driven by CaMV35S promoter was created. *Agrobacterium*-mediated transformation approach was employed to transfer an *EPO + GalT* genetic cassette into tobacco plants. Transgenic plants were confirmed for the presence of *EPO* and *GalT* genes by PCR. Gene expression levels were measured by RT-PCR and qRT-PCR. T0 transgenic plants with high expression levels were propagated by tissue culture. Created transgenic plants are being used for the purification of rhuEPO for downstream work, including peptide mapping, glycan mapping and functional studies.

Poster Session 2. 6:30 P.M. – 7:30 P.M.

P10. Positive Effects of the Gall Forming Midge, *Asphondylia borrichiae* (*Diptera*: *Cecidomyiideae*), on Its Host Plant *Borrichia frutescens* (*Asteraceae*)

Anthony Rossi and Cliff Ross. Department of Biology, University of North Florida, Jacksonville, FL

In the current study, we assessed the effects of herbivory caused by the stem tip gall midge, *Asphondylia borrichiae* (Diptera: Cecidomyiidae), on the survival and performance of its composite host plant, sea oxeye daisy (*Borrichia frutescens*). Rates of galling, which differ greatly between populations of *Borrichia*, are significantly affected by environmental and genetic variation. *Borrichia* is a highly clonal species and some genotypes are resistant to attack by *Asphondylia*, while other populations inhabit poor-quality environments. However, for a population of *Borrichia* that was "susceptible" to *Asphondylia*, mean number of stems was positively correlated with the number of galls on a plant. Moreover, this susceptible population of *Borrichia* had significantly more stems than a population that was "resistant" to galling and marked stems in the susceptible population that were galled had higher rates of survival and lived longer than non-galled stems. Our data suggest that if attack by stem-tip specific herbivores such as *Asphondylia* positively affects the survival and/or performance of its potential host(s) then selection against host range expansion may be relaxed.

P11. Cotton Line Development for Physiology, Genetics and Biochemistry Research

Rick B.Turley, USDA-ARS, Jamie Whitten Delta States Research Center, Stoneville, MS

Plant line development provides an incredible resource for scientists, especially in identifying mutants and producing near isogenic lines (NIL's). This work is first, an introduction to some of the known cotton mutations and then a description of development of cotton NILs. For the majority of the NIL production in this report, the single seed descent model was used. This production technique began by developing NIL parental lines (wild-type) by selecting a single plant from each generation for nine generations (F9). These plants were then crossed, individually, to various mutant lines. For dominant alleles, the F1 (selected for mutant phenotype) progeny was backcrossed for 5 generations (BC5). For recessive alleles backcrosses were made to individual F2 plants expressing the mutant phenotype which was repeated until reaching the BC5 generation. NIL populations have also been made. The two Ligon lintless NIL lines are being used to identify genes involved in elongation of cotton fiber in Dr. David Fang's Lab. The fuzzless seed mutants will be evaluated this summer using RNA-Seq. This labor intensive project was begun back in the mid-1990's as a side project to multiple physiological studies; it has now progressed far enough to actually benefit numerous research projects in cotton.

P12. Identification and Characterization of a MAP Kinase-Containing Complex

Carleton Bequette and Johannes Stratmann, Department of Biological Sciences, University of South Carolina, Columbia, SC

Mitogen-activated protein kinase (MAPK) cascades are crucial components of signal transduction pathways, which are involved in transmission of extracellular stimuli to a wide range of cellular responses. These protein phosphorylation cascades consist of three functionally linked kinases: MAPK kinase kinase, MAPK kinase, and MAPK. In plants, MAPK signaling pathways are involved in responses to many environmental stresses and in development. Prior research has identified specific components of MAPK cascades, however, it is largely unknown how MAPK signaling modules are organized in response to a specific stimulus, which induces a specific output response. Using size exclusion chromatography and subsequent immunoblotting, we have identified MAPKs and MAPKKs in high molecular weight (~300-600kDa) fractions of tomato, tobacco, and Arabidopsis leaf tissue. This indicates that MAPKs and MAPKKs associate with other proteins and form a multiprotein complex (MC). After MAPK activation, all active MAPKs are present as free proteins and do not associate with the MC. However, in the presence of a phosphatase inhibitor, active MAPKs associate with the MC. This indicates that the MC plays a role in MAPK dephosphorylation. Our long-term goal is to identify the constituents of the MC.

P13. Immunohistochemical Investigation of Cotton Carpel Tissue Exposed to Xylanolytic Hydrolases of *Aspergillus flavus*

Jay E. Mellon[1] and Kevin C. Vaughn[2]. [1]USDA, ARS, SRRC, New Orleans, LA. [2]4358 River Rd. S., Salem, OR.

Cotton carpel tissue (35-45 dpa) that had been treated with a mixture of xylanolytic hydrolases derived from *Aspergillus flavus* was subjected to immunocytochemical analysis. Microscopic examination of treated tissues revealed severe degradation of the secondary wall structure. Control tissue cells revealed the presence of high concentrations of xylans/arabinoxylans throughout the cell wall, as well as significant concentrations of arabinogalactan proteins in secondary wall structure. Carpel cells treated with a mixture of *A. flavus*-produced xylanolytic hydrolases showed a much reduced presence of labeling by xylan-specific antibodies on the inner wall surface, suggesting a severe loss of these plant polysaccharides in the secondary wall structure. Carpel exposure to a purified 14-kD endoxylanase from *A. flavus* also resulted in a severe reduction of xylans from secondary wall structure, although penetration of the tissue was not as dramatic. Arabinogalactan proteins were not as severely affected by the xylanolytic hydrolases. Comparison of control tissue with hydrolase-treated tissue stained with toluidine blue revealed an apparent reduction in wall thickness, supporting the conclusion of secondary wall structure degradation. Interestingly, the pectins could only be detected in the samples treated with xylanolytic enzymes, indicating that the pectins were being masked by xylans. These results are consistent with the conclusion that the xylanolytic hydrolase complex of *A. flavus* is a critical factor for host cell wall maceration and may represent another important fungal virulence factor.

P14. Does Inhibiting Phloem Loading in *Verbascum phoenicium* Induce Apoptosis in Intermediary Cells?

Christie Hightower and Ashlee McCaskill, Department of Biology, North Georgia College and State University, Dahlonega, GA

Inhibition of phloem loading in Verbascum phoenicium via RNA interference induces ultrastructural changes in the intermediary cells of transgenic plants. The characteristics of these cells are very similar to those of cells undergoing apoptosis. To determine if apoptosis has been induced, the DNA composition of the intermediary cells will be analyzed using the TUNEL technique.

P15. Exploring an Energy Sensor Protein Complex

Janet L. Donahue[1], Jenna L. Hess[2], Elitsa Ananieva[3], S. Phoebe Williams[4], F. Les Erickson[5], and Glenda E. Gillaspy[6]. [1]Dept. of Biochemistry, Virginia Tech, Blacksburg, VA, [2]Alliance Biosciences, Inc., Richmond, VA, [3]Dept. of Human Nutrition, Foods and Exercise, Virginia Tech, Blacksburg, VA, [4]Dept. of Biochemistry, Virginia Tech, Blacksburg, VA, [5]Dept. of Biological Sciences, Salisbury University, Salisbury, MD, [6]Dept. of Biochemistry, Virginia Tech, Blacksburg, VA

The sucrose nonfermenting-1-related kinase (SnRK1) is an important energy sensor that is highly conserved among eukaryotes. We have previously shown that SnRK1.1 is degraded by the proteasome, and one component from the inositol signaling system (5PTase13) functions to protect SnRK1.1 from degradation by the proteasome when the energy status of a seedling is low. Inositol signaling is an important mechanism plants use to respond and adapt to environmental stress. We report a new 5PTase13 interactor we call P80 that also interacts with SnRK1.1. Seedlings with a P80 loss-of-function have reduced root growth in low energy conditions that is reversed by the addition of sucrose. When grown in soil, p80 mutants have altered development and senesce early, phenotypes reminiscent of a shortened lifespan. The P80 phenotypes are also associated with changes in SnRK1.1 stability in p80 mutants, suggesting that P80 regulates SnRK1.1 stability via interaction with components of the proteasome. To test this possibility we are using transient expression in *Nicotiana benthamiana* followed by immunoprecipitation of tagged and endogenous proteins to characterize P80 interactors. Specifically, we are testing whether P80 binds to the Cullin 4 (CUL4)/ DNA Damage Binding 1a (DDB1a) substrate adaptor complex for the 26S Proteasome, as is predicted by the presence of an aspartic acid-tryptophan-aspartic acid motif in P80. In addition, we are testing whether P80 interacts with a deubiquinating enzyme (DUB), as predicted by proteomics data from humans and yeast.

P16. Control of Mycotoxigenic Fungi with Synthetic Antimicrobial Peptides

Jeff W. Cary[1], Kaniah Rajasekaran[1], J. Jaynes[2] and Deepak Bhatnagar[1], [1]USDA, ARS, SRRC, New Orleans, LA, [2]Integrative Biosciences, Tuskegee, AL

Development of disease-resistant transgenic crops is difficult because host plant-pathogen interactions are complex and often crop/variety or pathogen/strain-specific. Furthermore, identifying resistance factors to mycotoxigenic fungi such as *Aspergillus spp.* is exacerbated by the fact that this group of fungi is saprophytic and does not follow typical host-pathogen relationships. Our primary objective is to control *A. flavus* which produces the toxic and carcinogenic group of compounds termed aflatoxins during growth on oil-rich seed crops such as cottonseed, corn, peanut and tree nuts.

In this regard, synthetic peptides are useful in controlling a broad spectrum of plant pathogens including the difficult-to-control fungal pathogen, *Aspergillus flavus*. Recent advances in combinatorial chemistry and automated peptide synthesis have paved the way for rational design of stable, potent, and novel synthetic peptides with target-specific biological activity. Some of these lytic, synthetic peptides have already been expressed in transgenic plants including cotton with varying degrees of success against fungal and bacterial plant pathogens. Here we provide examples of different classes of synthetic peptides that are effective against mycotoxigenic fungi including *A. flavus*. The β-sheet peptides D4E1, AGM181 and AGM182, and the α-helical peptide AGM184, demonstrated *in vitro* inhibitory activity against *A. flavus*, *Fusarium verticillioides*, and *Verticillium dahliae*. We have also reported on transgenic cottons expressing the synthetic peptide D4E1 which showed inhibitory activity *in planta* against *A. flavus* and other cotton pathogens. Our long-term objectives are to develop transgenic cottons expressing potent synthetic peptide gene(s) to minimize the aflatoxin problem in cottonseed and other susceptible crops.

P17. Expression and Localization of *Arabidopsis thaliana* Proteins Involved in Metal Metabolism

Shannon R. Alford and Steven Richardson, Department of Chemistry, Presbyterian College, Clinton, SC

Plants are sessile organisms and cannot flee from an onslaught of deleterious offenders in their environment. Our research is focused on understanding how plants respond to external stimuli at the molecular level. Specifically we are interested in the ability of plants to take up metals from the environment and maintain vigor, rather than suffer from metal toxicity. Our interest stems from recent research and field applications of plants used in phytoremediation, a bioremediation process that uses plants to remove contaminants in the environment. Metal contamination is a well-documented problem for industrialized countries. To progress the science of how plants can be better engineered for phytoremediation, we are studying the basic molecular biology of plant response to metals. We are therefore examining the subcellular localization of proteins involved in metal response and expression analysis of the associated genes in Arabidopsis. We are generating stably transformed plants with protein:GFP fusions of proteins involved in selenium and cadmium response and promoter:GUS fusions of the respective gene promoters. We plan to examine the expression of these metal-responsive genes under varying physiological conditions to elucidate more precise responses to these stimuli. Our findings will further the knowledge of the molecular basis of plant responses to metals and educate future scientists in the field of plant molecular biology and biochemistry.

P18. Subcellular Localization of Regulatory Enzymes in Inositol Signaling

S. Phoebe Williams[1], Joonho Park[2], Imara Y. Perera[3], Victor Raboy[4], and Glenda E. Gillaspy[5]. [1]Department of Biochemistry, Virginia Tech, Blacksburg, VA, [2]Department of Biochemistry, Virginia Tech, Blacksburg, VA, [3]Department of Plant Biology, North Carolina State University, Raleigh, NC, [4]USDA, Agricultural Research Services, Aberdeen, ID, [5]Department of Biochemistry, Virginia Tech, Blacksburg, VA

Myo-inositol phosphates (InsP) are critical signaling molecules used by eukaryotes, with roles in nutrient and abiotic stress sensing/signaling. One group of these signaling molecules has diphospho- or triphospho- (PPx) moieties at one or more positions on the inositol ring. This signaling pathway has been characterized in yeast but remains a novel area of research in plants. Previous studies show that plants synthesize large quantities of InsP and we have determined that PPx-InsPs are detected in seeds. Two groups of enzymes are responsible for the synthesis and regulation of PPx-InsP, VIP kinases and Nudix hydrolases (NUD). Subcellular regulation of pools of InsP is critical to their role as signaling molecules. We report the subcellular localization of their regulatory proteins, VIPs and NUDs, using transient expression of GFP fusion proteins in *Nicotiana benthamiana*. This information will be used to determine when and where these enzymes are regulating PPx-InsPs.

P19. Alteration of Energy Sensing Pathways in *Arabidopsis thaliana*

Padma Rangarajan[1], Phoebe Williams[1], Elitsa Ananieva[2] , Jenna E. Hess[3], Janet L.Donahue[1], and Glenda E.Gillaspy[1]. [1]Dept. of Biochemistry, Virginia Tech, Blacksburg, VA-24061. [2]Dept. of Human Nutrition, Food and Exercise, Virginia Tech, Blacksburg, VA-24061. [3]Allied Biosciences Inc., Richmond, VA

The *myo*-inositol signaling pathway in plants allows them to sense external environmental stimuli and respond to them. This signaling pathway depends on the dynamic levels of the second messenger, inositol 1,4,5 trisphosphate ($InsP_3$), which in turn is regulated by inositol polyphosphate 5-phosphatases (5PTases). Previous studies have shown that the sucrose nonfermenting-related kinase 1, SnRK1, an important energy sensor, is a binding partner of 5PTase13, and a novel protein called P80. P80 is predicted to be a component of the Cullin4 (CUL4) E3 ubiquitin ligase complex that marks proteins for destruction by adding ubiquitin groups. *p80* mutant seedlings have reduced root growth under low energy conditions that is reversed by the addition of sucrose. When grown in soil, *p80* mutants have an altered phenotype with early senescence. The levels of SnRK1 in *p80* mutants is reduced in low energy conditions in comparison to wild-type *Arabidopsis* seedlings suggesting that P80 is involved in the regulation of SnRK1, and that this reduction in SnRK1 leads to the altered *p80* mutant phenotype. To test whether exogenous SnRK1 can rescue *p80* mutants we have expressed a SnRK1.1:green fluorescent protein (GFP) transgene under control of the 35S CaMV promoter in both wildtype and *p80* mutants. The resulting transgenic plants are being characterized with respect to their phenotypes and expression levels of SnRK1.1:GFP. Analysis of exogenous SnRK1.1:GFP expression in *p80* mutants will facilitate delineation of the mechanism plants use to control energy sensing and metabolism.

P20. Manipulation of Mir156 Genes Leads to Modified Plant Morphology and Enhanced Abiotic Stress Tolerance in Transgenic Creeping Bentgrass (*Agrostis stolonifera* L.)

Zhigang Li, Dayong Li, Qian Hu, Man Zhou, and Hong Luo, Department of Genetics and Biochemistry, Clemson University, Clemson, SC

MicroRNAs (miRNAs) are short single-stranded molecules arising from primary miRNA transcripts (pri-miRNAs) encoded by miRNA genes. As a group of post-transcriptional regulators, they regulate target gene expression by binding to the complementary sequences on messenger RNA transcripts, usually resulting in translational repression or target degradation and gene silencing. In plants, miRNAs have important functions in plant growth, development and stress responses. Based on the miRBase (version 13.0c), rice miR156 family includes twelve members, of which the full length cDNAs of the miR156b, c, d, h and j have been identified in the database (KOME). In this study, we focus on miR156b/c and miR156d to investigate the role different members of the miR156 family play in plant development and plant response to abiotic stress in perennial grass species. Transgenic creeping bentgrass plants overexpressing the full-length cDNAs of miR156b/c, and miR156d were produced respectively. All transgenics exhibited dramatic morphological changes. Compared to wild-type controls, miR156b/c transgenics exhibited significantly increased tiller numbers and narrow leaves, whereas, miR156d transgenics displayed significantly decreased tiller numbers and wide leaves. The leaves of miR156b/c transgenics also have higher nitrogen content. Overexpression of miR156b/c and d led to enhanced drought tolerance in transgenic plants, which is associated with less water consumption rates and increased water retention capabilities. The data obtained point to the great potential of manipulating miR156 genes in transgenic crops for enhanced performance.

P21. Possible Role of Nonphotochemical Quenching in Chloroplast Movement

Judy Schmalstig, Lauren Johnson, Rose Appel and Kenneth Jainandan. Dept. of Biology, Rollins College, Winter Park, FL.

Chloroplast avoidance movement, mediated via phototropin, is one mechanism used to prevent photodamage to excess light. Nonphotochemical quenching (NPQ) leads to thermal dissipation of excess energy. NPQ mutants, *npq4* and *npq7* of *Arabidopsis thaliana* L. showed a lower response than the WT to chloroplast avoidance to strong blue light. Further, blue-induced chloroplast movement was reduced by simultaneous green light (550 nm) in the WT but not in *npq4* and *npq7*. Green light also reduced blue-light induced chloroplast movement in duckweed (*Landoltia punctata* (G. Mey.) Les & D.J. Crawford). An action spectrum with duckweed revealed three peaks of this activity: 510, 550 and 590 nm. Reports in several species have shown a spectral absorbance change at 505 nm and 535 nm in leaves after exposure to strong light, perhaps due to a conformational change of a zeaxanthin bound to a light-harvesting protein. We propose that strong green light is interfering with NPQ via altering the blue-induced conformational change of protein-bound zeaxanthin thus leading to a reduction in chloroplast avoidance movement.

P22. **Investigating the Role of Reactive Oxygen Species (ROS) in Elongating Cotton Fiber**

Michael R. Stiff[1], S.L. Moles[1], and Candace H. Haigler[1,2] Department of Crop Science[1] and Department of Plant Biology[2], North Carolina State University, Raleigh, NC.

The cotton fiber is an important textile fiber and an excellent single-celled model for the study of cell biology. A better understanding of how the cotton fiber elongates could reveal strategies for added-value such as altering cell wall properties towards greater fiber strength and length. Reactive oxygen species (ROS) have been implicated in the development of plant cell types with similar highly-elongated morphology, e.g., root hairs and pollen tubes. This work aims to determine how ROS function in elongating cotton fibers by observing ROS subcellular localization during elongation using the fluorescent indicator dihydrofluorescein diacetate (H_2FDA). Fluorescence was observed in the dense cytoplasm of narrow tips, the nucleus, and other organelle-sized compartments during live-cell monitoring of 3 DPA cotton fiber by confocal microscopy indicating the presence of ROS. Fluorescence was significantly brighter when 3 DPA fibers were treated with 25 μM H_2O_2, but not in the presence of higher concentrations of H_2O_2 (50 or 75 μM) indicating that fluorescence was dependent on ROS concentration. Fibers at 3, 4, 5 and 7 DPA all showed similar levels of H_2FDA-induced fluorescence. Although ROS has been observed in cotton fiber during elongation and the transition to secondary wall deposition, this is the first analysis of ROS content in 3 and 4 DPA fiber. Work is ongoing to further elucidate the subcellular localization of ROS in the elongating cotton fiber. We thank Cotton Incorporated, Cary, NC for support of this research.

P23. Hotspots in Viral siRNA Accumulation in Maize

Sizolwenkosi Mlotshwa[1], Cameron Johnson[2], Amy Wahba Foreman[1], Gail J. Pruss[1], Venkatesan Sundaresan[2], Kay Scheets[2], Lewis H. Bowman[1] and Vicki Vance[1]. [1]Department of Biological Sciences, University of South Carolina, Columbia, South Carolina; [2] Section of Plant Biology, College of Biological Sciences, University of California Davis, Davis, California; [3]Department of Botany, Oklahoma State University, Stillwater, Oklahoma

RNA silencing is a sequence-specific RNA degradation mechanism that serves as an antiviral defense pathway in plants. Most plant viruses have single stranded RNA genomes and replicate via double stranded RNA (dsRNA) replication intermediates. The viral dsRNA triggers antiviral silencing in the host. It is processed by Dicer-like ribonucleases to produce short interfering RNAs (siRNAs) that incorporate into a RNA-induced silencing complex (RISC). Within RISC, the viral siRNA acts as a guide to direct the complex to complementary target RNAs, which are then destroyed. In this way, viruses provide the molecular tools (siRNAs) that lead to their own destruction, providing a potent and specific antiviral defense. Here we report an analysis of the population of viral siRNAs that accumulate during infection of maize with three different viruses: maize dwarf mosaic virus (MDMV), maize chlorotic mottle virus (MCMV) and maize necrotic streak virus (MNeSV). In each case, we find that viral siRNAs comprise a large proportion of the total small RNAs in infected cells and that viral siRNAs are generated along both strands of the entire genome. However, the analysis identified a few regions of the viral genome that generated very high levels of siRNAs. The characteristics of these "hotspot" viral siRNAs will be discussed. The data raise the intriguing possibility that these abundant viral siRNAs mediate an additional level of antiviral silencing by targeting host genes that are required for efficient viral replication.

2012 Kriton – Hatzios Symposium

How Plants Sense Their Environment: the Role of Chloroplasts in the Perception of And Response To Environmental Stress

Klaus Apel, Boyce Thompson Institute for Plant Research, Ithaca NY

Plants are exposed to environmental changes that may adversely affect their growth, development and productivity. Plants grown in the field are challenged by multiple environmental stress factors the relative impact of which may constantly change. To tackle experimentally the complexity of plant-environment interactions we focus our study on chloroplasts that play a key role in sensing environmental changes. Stress conditions such as high light or drought interfere with the photosynthetic electron transport and cause an enhanced generation of reactive oxygen species (ROS). We use the conditional *flu* mutant of Arabidopsis that produces one of these ROS, singlet oxygen ($^1O_2$), in plastids in a controlled manner to define a signaling pathway that transmits environmental cues from the plastid to the nucleus and initiates the plant's response to stress. The release of $^1O_2$ in chloroplasts triggers growth inhibition and programmed cell death. The genetic basis of these $^1O_2$-mediated stress responses was demonstrated by inactivating the plastid protein EXECUTER1. In *ex1/flu* double mutants $^1O_2$-mediated stress responses are abrogated, even though similar amounts of $^1O_2$ are released in the double mutant as in the parental *flu* line. $^1O_2$-mediated signals do not operate via an isolated linear pathway, but rather merge in a signaling network that absorbs various other cues that may modulate consequences of $^1O_2$-mediated signaling. How these different environmental signals are integrated and determine the plant's response to adversity is largely unknown and is the subject of our current work.

A Feeling for the Organelle: Multiple Roles for Mechanosensitive Channels in the Chloroplast Envelope

Elizabeth Haswell, Washington University in St. Louis

My group is interested in the molecular mechanisms by which molecules, cellular structures, and organisms perceive mechanical force. Our current research program includes the structural and functional analysis of a family of ten mechanosensitive (MS) ion channels in the model plant *Arabidopsis thaliana*. This family of MS channels was identified on the basis of their similarity to a bacterial MS channel, MscS, which contributes to survival of extreme osmotic downshock in *E. coli*. Two of these MscS-Like (MSL) proteins in Arabidopsis, MSL2 and MSL3, are localized to the inner chloroplast envelope and are required for normal plastid shape and size. I will describe 1) our analysis of interactions between MSL2, MSL3 and the chloroplast division machinery; 2) evidence that MSL2 and MSL3 are required to relieve hypoosmotic stress in leaf epidermal plastids; and 3) the evolutionary implications of these studies. In addition, I will describe recent results indicating that MSL2 and MSL3 serve to integrate plastidic osmotic stress with cytoplasmic dehydration stress pathways.

Chloroplast Genomics and Genetic Engineering

Henry Daniell, University of Central Florida

Chloroplast transformation has several unique advantages including high levels of expression and transgene containment via maternal inheritance of chloroplast genomes. The highest levels of expression in the published literature for engineering agronomic traits or human therapeutic proteins or industrial products were indeed achieved using this concept. Because of exclusive homologous recombination and species-specific proteins that bind to regulatory sequences, it is essential to use endogenous intergenic spacer regions and regulatory elements in chloroplast vectors for successful transformation and high levels of transgene expression. Therefore, in the past few years, >30 crop chloroplast genomes have been fully sequenced using BAC libraries or rolling circle amplification of purified chloroplast DNA. These newly sequenced chloroplast genomes include important crops like soybean, potato, tomato, grape, cocoa, cotton, coffee, cassava, chickpea, barley, sorghum, or trees like peach, chestnut, cocoa or citrus.

Tobacco chloroplast genome was engineered first to confer herbicide, insect or disease resistance, drought or salt tolerance or phytoremediation. More recently, chloroplast genomes of major crops including cotton, wheat and soybean, vegetables, tubers, fruits and trees have been transformed. Significant advances have been made in expressing vaccine antigens against human bacterial, viral and protozoan pathogens in chloroplasts and animal studies demonstrated their efficacy against pathogen or toxin challenge. Most importantly, oral delivery of vaccine antigens bioencapsulated in plant cells was shown to be more efficacious than injectable vaccines or in developing oral tolerance against autoimmune disorders (diabetes, hemophilia), in addition to the advantages of low cost because of elimination of expensive purification, cold storage/transportation and sterile delivery by injections. Chloroplast genome was also modified to express industrial enzymes for biofuel production. One of these enzymes, beta-glucosidase, released active hormones from their inactive conjugates stored within chloroplasts and almost doubled plant biomass. Recent advances in this field will be presented.