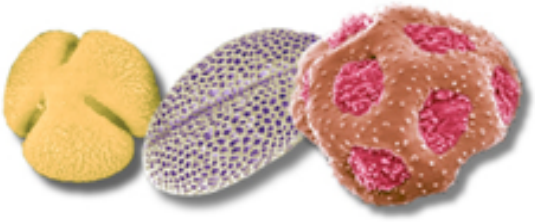
Pollen RCN

Integrative Pollen Biology Research Coordination Network



4th Annual Pollen RCN meeting, May 14 & 15 2014

UNC Charlotte City Center Campus in Charlotte, North Carolina

Meeting Organizers:

Dr. Alice Cheung, University of Massachusetts, Amherst

Dr. Ann Loraine, University of North Carolina, Charlotte

Program Committee:

Dr. Ravi Palanivelu, University of Arizona

Dr. Mark Johnson, Brown University

Dr. Jeffery Harper, University of Nevada, Reno

Integrative Pollen Research Coordination Network:

Funding Support:

National Science Foundation
WHERE DISCOVERIES BEGIN

Website:

http://www.pollennetwork.org

4th Annual Pollen RCN meeting, May 14 & 15 2014 - Program

UNC Charlotte City Center Campus in Charlotte, North Carolina
Pollen RCN PI: Alice Cheung acheung@biochem.umass.edu

Wednesday, May 14, 2014

5:15-7:00pm	**Registration (*pay registration fees and pick up program*)**	
5:15-7:00pm	**Poster session (*posters will be open for viewing through the meeting*)**	
5:45-7:00pm	**DINNER**	
Session I: Chair, Mark Johnson, Brown University		
7:00-7:30pm	William Snell, UT Southwestern	Fertilization across kingdoms: using *Chlamydomonas reinhardtii* and *Plasmodium berghei* to understand sperm molecules in pollen, and vice-versa
7:30-7:50pm	Elizabeth Haswell, Washington University	I'm shocked, shocked! The mechanosensitive ion channel *msl8* is required for pollen grain and pollen tube integrity
7:50-8:10pm	Hsiao-Lin Wang, University of Missouri, Kansas City	Stress-induced siRNAs target the intron sequences that regulate splice site selection in *Brachypodium*
8:10-8:30pm	Cheol-Min Yoo, Noble Foundation	Understanding the role of AGD1 in root hair polarity establishment
8:30-8:50pm	Gloria Muday, Wake Forest University	A genetic approach to understanding the roles of flavonols in tomato pollen and seed development

Thursday, May 15, 2014

7:00-8:30am	**BREAKFAST**	
Session II: Chair, Jeff Harper, University of Nevada, Reno		
8:30-8:50am	Simon Wallace, University of Tennessee	Evolutionary development of the plant spore and pollen wall
8:50-9:10am	Anna Dobritsa, Ohio State University	Aperture formation on Arabidopsis pollen surface
9:10-9:30am	Joseph Williams, University of Tennessee	Repeated evolution of tricellular (and bicellular) pollen
9:30-9:50am	Mark Johnson, Brown University	Exploiting pollen to define gene function within complex families: A pair of β-galactosidases essential for extension of the pollen tube cell wall

9:50-10:20am	**COFFEE BREAK**	
Session III: Chair, Alice Cheung, University of Massachusetts, Amherst		
10:20-10:40am	Sharon Kessler, University of Oklahoma	Functional characterization of CrRLK1L and MLO family members in pollen tube reception
10:40-11:00am	Gabriele Monshausen, Penn State University	Sensing strain - a role for the receptor-like kinase FERONIA in mechanical signaling?
11:00-11:20am	Jian Huang, University of Wisconsin-Milwaukee	TPD1 acts as a protein ligand of the EMS1 receptor kinase to regulate anther cell differentiation in *Arabidopsis*
11:20-11:40am	Christine Chase, University of Florida	Identification of fertility restorers for S male-sterile maize: beyond PPRs
11:40-1:10pm	**LUNCH BREAK**	
Session IV: Chair, Ravi Palanivelu, University of Arizona		
1:10-1:30pm	Jeff Harper, University of Nevada, Reno	Pollen -- a model system for Ca2+signalling and insights into stress tolerance
1:30-1:50pm	Jose Feijo, University of Maryland	Coordination of pollen tube growth by Ca2+ regulated anion fluxes
1:50-2:10pm	Andreas Nebenführ, University of Tennessee	Myosin motors are required for rapid pollen tube growth and normal fertilization efficiency
2:10-2:30pm	Alice Cheung, University of Massachusetts, Amherst	Formin regulation of tip-focused growth in pollen tubes
MEETING ENDS		

Oral Presentations - Session 1

Wednesday, May 14, 2014, 7:00 – 8:50pm

Chair: Dr. Mark Johnson, Brown University

FERTILIZATION ACROSS KINGDOMS: USING *CHLAMYDOMONAS REINHARDTII* AND *PLASMODIUM BERGHEI* TO UNDERSTAND SPERM MOLECULES IN POLLEN, AND VICE-VERSA

Jue Ning[1], Yanjie Liu[1], Gary Vanderlaan[1], Thomas D. Otto[2], Claudia Pfander[2], Frank Schwach[2], Mathieu Brochet[2], Ellen Bushell[2], David Goulding[2], Mandy Sanders[2], Oliver Billker[2], William J. Snell[1]

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The sperm carried within pollen ultimately are released to interact and fuse with female gametes in the ovule. The membrane fusion reaction during gamete fusion occurs in two steps, membrane adhesion and membrane merger. In the green alga *Chlamydomonas*, the malaria organism, *Plasmodium*, and now in *Arabidopsis* and mouse, membrane adhesion is known to be brought about by species-limited proteins, whereas membrane merger requires the conserved sperm protein HAP2 (also called GCS1) in plants and protists. We exploited the ease of obtaining *Chlamydomonas* gametes in distinct physiological conditions to identify mating type *plus* and mating type *minus* gamete transcriptomes to test the idea that additional fertilization proteins would be conserved. One gamete-specific gene in *Chlamydomonas* was related to the *Arabidopsis* gamete-specific GEX1 protein and to an uncharacterized protein in *Plasmodium berghei*. Gene disruption, cell fractionation, immunolocalization, and further sequence analysis showed that GEX1 is part of a larger, previously unrecognized family of nuclear envelope proteins present in protist, plants, and animals whose first-discovered member is the nuclear fusion protein KAR5.Taking advantage of unifying cellular and molecular themes in fertilization across kingdoms should continue to be useful for understanding fundamental events during fertilization in eukaryotes.

I'M SHOCKED, SHOCKED! THE MECHANOSENSITIVE ION CHANNEL MSL8 IS REQURED FOR POLLEN GRAIN AND POLLEN TUBE INTEGRITY

Gregory S. Jensen, Grigory Maksaev, Eric S. Hamilton, Emma January, Angela Schlegel and Elizabeth S. Haswell

Department of Biology, Washington University in Saint Louis, USA

Pollen grains undergo large and rapid changes in water content during development, desiccation, and rehydration on the stigma surface. How cell volume and membrane integrity is controlled during these massive changes in cellular water potential is not well understood. We have recently been studying the contribution of the MscS-Like gene *MSL8* to pollen water relations. MSL8 is a plant homolog of the bacterial mechanosensitive channel MscS, and MscS-Like proteins have been shown to serve as osmotic release valves under osmotic stress in bacteria and in plastids. We have established that MSL8 provides a stretch-activated ion channel activity with a conductance of 60 pS and a modest preference for anions when expressed in Xenopus ooctyes. The *MSL8* gene is expressed exclusively in tricellular pollen, and *msl8* mutants show reduced transmission through the male germline. Pollen from *msl8* mutants loses integrity upon in vitro rehydration, and *msl8* pollen tubes burst immediately after exit from the pollen grain at a higher rate than the wild type. A MSL8-YFP fusion protein localizes to small puncta throughout the mature, dry pollen grain and is subsequently mobilized to the periphery of a germinating tube. These data show that MSL8 is required for pollen grain and pollen tube integrity *in vitro*. We propose that MSL8 provides an anion efflux channel that allows the pollen grain to release ions in response to membrane stretch, thereby controlling the volume changes and membrane deformations associated with rapid rehydration and germination. I will also describe our plans to generate 1) a complete set of pollen-expressed fluorescent markers for subcellular compartments and 2) genetically encoded biosensors of membrane voltage and tension.

STRESS-INDUCED SIRNAS TARGET THE INTRON SEQUENCES THAT REGULATE SPLICE SITE SELECTION IN *BRACHYPODIUM*

Hsiao-Lin V. Wang, Brandon Dinwiddie, Herman Lee, and Julia A. Chekanova

School of Biological Sciences, University of Missouri-Kansas City, MO, USA.

In plants, exposure to abiotic stresses triggers changes in the expression of thousands of genes at both transcriptional and post-transcriptional levels. smRNAs play crucial roles in implementing stress responses to environmental conditions, defining cellular identity, and coordinating developmental programs. Plants also regulate development and respond to environmental stress by regulating pre-mRNA splicing. Although both smRNA pathways and splicing are crucial mechanisms regulating gene expression, the examples of these two pathways intersecting remains limited.

Mechanistically, known smRNAs regulate gene expression by sequence-specific transcript degradation, translational inhibition or transcriptional repression. Here, to investigate the role of smRNAs in stress responses, we examined the smRNA transcriptomes of *Brachypodium distachyon* plants challenged by various abiotic stresses. We found that exposure to different stresses specifically induces a group of novel, endogenous siRNAs (stress-induced, 3'UTR-derived siRNAs, or sutr-siRNAs) that originate from the 3' UTRs of a subset of coding genes. Our bioinformatics analyses also predicted that sutr-siRNAs have regulatory potential and that over 90% of sutr-siRNAs target intronic regions of many mRNAs in *trans*. Importantly, a subgroup of these sutr-siRNAs target the regulatory regions within introns that affect splice-site selection, indicating that these sutr-siRNAs might affect splicing or alternative splicing of the target mRNAs.

Our study suggests that in *Brachypodium*, the group of novel stress-induced sutr-siRNAs may affect splicing to mediate gene expression in response to stresses and this may serve as a general mechanism for regulation of gene expression in plants.

UNDERSTANDING THE ROLE OF AGD1 IN ROOT HAIR POLARITY ESTABLISHMENT

Cheol-Min Yoo and Elison B. Blancaflor

Noble Foundation, Ardmore, Oklahoma

The *Arabidopsis AGD1* mutant, which is disrupted in gene encoding a class 1 ARF-GAP, has root hairs with wavy/spiral growth and two tips originating from one initiation site. Bundling of endoplasmic microtubules and filamentous actin (F-actin) were associated with these defects. Genetic interaction studies between *agd1* and other mutants involved in root hair development indicate that AGD1 functions in common signaling pathways with phosphoinositides and actin in controlling *Arabidopsis* root hair polarity with its effects occurring predominantly during root hair initiation. A functional native promoter-driven AGD1-GFP protein localized to the plasma membrane of initiating root hairs. AGD1 is a multi-domain ARF-GAP. Deletion analysis of the AGD1 protein revealed specific domains for its localization. The pleckstrin homology (PH) domain deleted AGD1-GFP localized to the cytosol, suggesting that phosphoinositide binding is essential for plasma membrane localization. GAP domain deletions on the other hand, redirected AGD1-GFP to the subapical zone of root hairs and produced a synthetic phenotype. Taken together, our results suggest that the GAP domain, in coordination with the PH domain, have regulatory roles in AGD1 localization. This tight control of AGD1 localization by different protein domains might be crucial for sustaining polarized root hair growth.

A GENETIC APPROACH TO UNDERSTANDING THE ROLES OF FLAVONOLS IN TOMATO POLLEN AND SEED DEVELOPMENT

Gloria K. Muday and Greg Maloney

Department of Biology, Wake Forest University

This study utilized a tomato mutant with a defect in anthocyanin synthesis (*anthocyanin reduced, are*), to explore the impact of flavonoids on pollen and seed development. The *are* mutant has a point mutation in the *flavonoid 3-hydroxylase* gene encoding a flavonoid biosynthetic enzyme. The F3H substrate accumulates at higher levels, while the pathway products kaempferol, quercetin, and myricetin, are produced at much lower levels in *are* than wild-type. Seed numbers are reduced in *are* compared to wild-type, especially when plants are grown at higher temperatures. In cleared seeds of *are*, embryos are malformed or are absent. Pollen of *are* has a lower viability compared to wild-type and *in vitro* pollen germination studies show that are pollen have a lower percentage of germination, independent of viability. The levels of reactive oxygen species (ROS) were examined in *are* pollen and pollen tubes by staining with DCF, a general ROS sensor. DCF staining shows that both non-germinated pollen grains and growing pollen tubes of are accumulate much greater concentrations of ROS than wild-type. These data suggest a model in which lowered flavonol antioxidant concentrations in *are* lead to increases in ROS, causing defects in pollen germination leading to infertility and seed abnormalities.

Oral Presentations - Session 2

Thursday, May 15, 2014, 8:30 – 9:50am

Chair: Dr. Jeff Harper, University of Nevada, Reno

EVOLUTIONARY DEVELOPMENT OF THE PLANT SPORE AND POLLEN WALL

S. Wallace, C.C. Chater, Y. Kamisugi, A.C. Cuming, C. H. Wellman, D. J. Beerling and A.J. Fleming

Many theories have been advanced regarding the key innovations required to enable plants to colonize terrestrial habitats. One such proposed innovation is the development of a durable spore wall structure, containing the biopolymer sporopollenin, to withstand desiccation and a UV rich environment. Numerous studies have been published with regards to the molecular genetics of pollen wall (the derived homologue of the spore wall) development in the angiosperm, *Arabidopsis*. However, research into the molecular genetics of spore wall development in basal plants has thus far been extremely limited. In this study, the results of a microarray experiment at early and mid stages of sporogenesis in the moss *Physcomitrella*, have allowed upregulated genes to be compared with those known to be involved in pollen wall development, therefore allowing spore wall candidate genes to be identified. Subsequently, a gene knock-out experiment with *Physcomitrella* demonstrates that one candidate gene, *MALE STERILITY2* (*MS2*), which is involved in sporopollenin biosynthesis in *Arabidopsis* pollen wall development, has a similar function in *Physcomitrella*. However, the moss homologue of *MS2* cannot recover functionality in *Arabidopsis ms2* mutants, indicating that the *MS2* gene is only partially conserved and has evolved in angiosperms as their pollen walls have increased in complexity.

APERTURE FORMATION ON ARABIDOPSIS POLLEN SURFACE

Anna Dobritsa

Department of Molecular Genetics and Center for Applied Plant Science, The Ohio State University, Columbus, OH

Cells rely on a regulated production of extracellular materials to control their morphology, growth, and motility, to promote tissue formation, and to protect themselves from harmful influences. Despite the importance of extracellular structures in development and disease, the question of how cells decide when, where, and in which manner these materials should be produced, deposited, and specifically assembled or modified is far from being understood in any system. Pollen presents a unique and powerful model for studying how controlled formation of extracellular structures is achieved. Pollen grains are surrounded by a complex extracellular structure, exine, which assembles into intricate 3D patterns of enormous morphological diversity among species, yet very conserved within a species. In most plant species, the pollen surface has characteristic areas called apertures, which lack exine and which are species-specific in their number, location, and morphology. This indicates that exine deposition machinery in a given species reliably recognizes particular areas on pollen surface as different from others and does not deposit exine onto these areas. In a forward genetic screen in Arabidopsis I have recovered multiple mutants defective in exine development, including those with abnormal aperture formation. Here I will describe what we have learned about this process based on the analysis of the *inp1* mutants that either lack apertures or have abnormally short apertures and the *lsq* mutants that have ectopic apertures. INP1 protein exhibits a very distinct tripartite localization in the developing pollen, consistent with its direct involvement in specification of aperture position and controls aperture length in a dosage-dependent manner.

REPEATED EVOLUTION OF TRICELLULAR (AND BICELLULAR) POLLEN

Joseph H. Williams, Mackenzie L. Taylor and Brian C. O'Meara

Department of Ecology and Evolutionary Biology, University of Tennessee

Male gametophytes of seed plants are sexually immature at the time they are dispersed as pollen, but approximately 30% of flowering plants have tricellular pollen containing fully formed sperm at anthesis. For nearly a century it has been thought that tricellular pollen had many parallel, but irreversible, origins within angiosperms. Those ideas were immortalized in a classic paper by James Brewbaker (1967; *American Journal of Botany*), in what may have been the first use of an angiosperm-wide phylogenetic comparative analysis to answer an evolutionary developmental question. We readdressed the main premises of that study by modeling the evolution of pollen cell number for 2511 species on a time-calibrated molecular phylogeny of angiosperms. We used recently-developed comparative phylogenetic methods that either, 1) accounted for the effect of species diversification rates on character transition rates (BiSSE method) or, 2) allowed transition rates to vary across the phylogeny (HRM method). Seventy-percent of species had bicellular pollen. BiSSE found a 1.9-fold higher bicellular to tricellular transition rate than in the tricellular to bicellular direction, and bicellular lineages had a 1.8-fold higher diversification rate than tricellular lineages. HRM found heterogeneity in evolutionary rates, with bidirectional transition rates in three of four rate classes. Contrary to expectation, there was ambiguity in the ancestral state. These results indicate that the tricellular state is not irreversible. However, tricellular lineages have both diversified slowly and given rise to bicellular lineages slowly. The slow evolutionary rates of tricellular lineages reflect a linkage between the evolution of sporophyte lifestyles and the developmental evolution of male gametophytes.

EXPLOITING POLLEN TO DEFINE GENE FUNCTION WITHIN COMPLEX FAMILIES: A PAIR OF β-GALACTOSIDASES ESSENTIAL FOR EXTENSION OF THE POLLEN TUBE CELL WALL

Julie Diamond[1], Sumitha Raman[1], Alexander R. Leydon[1], David Kern[1], Laurel Wright[1], Sophia Wang[1], Clare Levy[1], Yue Zhang[1], Mark St. Louis[1], Jefferson Chen[1], Ravishankar Palanivelu[2], Alison DeLong[1], Heven Sze[3], Amit Basu[4], Mark A. Johnson[1]

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[4]Department of Chemistry, Brown University, Providence, RI

Large gene families complicate genetic analysis because functional redundancy masks the phenotypic consequences of loss-of-function of individual family members. We developed a tool called the Arabidopsis Heat Tree Viewer that integrates gene expression data with protein sequence phylogenies and have applied this tool to identify members of gene families likely to be functionally redundant for pollen functions. Since pollen is a single cell and performs multiple critical tasks during flowering plant reproduction it is an excellent system to understand the specific functions of individual members of large gene families. We have applied this approach to glycoside-hydrolases, which are likely important for modulating the dynamic pollen tube cell wall as it extends and comprise multiple large gene families. We found a pair of β-galactosidases that are essential for extension of a morphologically normal pollen tube and that localize to the pollen tube wall as it extends. Future studies will focus on identification of the substrates for these enzymes that are critical for pollen tube extension.

Oral Presentations - Session 3

Thursday, May 15, 2014, 10:20 – 11:40am

Chair: Dr. Alice Cheung, University of Massachusetts, Amherst

FUNCTIONAL CHARACTERIZATION OF CRRLK1L AND MLO FAMILY MEMBERS IN POLLEN TUBE RECEPTION

Sharon A. Kessler, Daniel Jones, Emily Kumimoto, Heike Lindner, and Ueli Grossniklaus

Department of Microbiology and Plant Biology

Plants have evolved elaborate signaling mechanisms to insure that sperm cells are delivered to the female gametophyte during sexual reproduction. During pollination, a pollen grain is recognized at the stigma and germinates to produce a pollen tube that grows through the style and transmitting tract and finally into the micropyle of the ovule, attracted by a signal produced by the female gametophyte. The final step of pollination is the reception of the pollen tube at one of the synergid cells that flank the egg cell followed by the cessation of pollen tube growth and rupture to release the sperm so that double fertilization can occur and viable seeds can be produced. In *Arabidopsis thaliana feronia* (*fer*) and *nortia* (*nta*) mutants, cell-to-cell communication at early stages of pollination is normal, but upon reaching the synergid the pollen tubes continue to grow instead of bursting to release the sperm, leading to infertility. FER and NTA are both members of large gene families, the CrRLK1-like family of receptor-like kinases and the Multiple Resistance O (MLO) family of 7 transmembrane proteins, respectively, but little is known about conservation of function in different members of these protein families. A functional characterization of CrRLK1L and MLO family members using the pollen tube reception phenotype indicates that members of these gene families likely use conserved downstream mechanisms.

SENSING STRAIN - A ROLE FOR THE RECEPTOR-LIKE KINASE FERONIA IN MECHANICAL SIGNALING?

Gabriele Monshausen

Department of Biology, Penn State University

Among the myriad cues that constantly inform plant growth and development, mechanical forces are unique in that they are an intrinsic result of cellular turgor pressure and also imposed by the environment. While the key role of mechanical forces in shaping plant architecture from the cellular level to the level of organ formation is well established, the components of the early mechanical signal transduction machinery remain to be defined at the molecular level. We have found that an Arabidopsis mutant lacking the receptor-like kinase FERONIA has severely altered Ca^{2+} signaling and growth responses to different forms of mechanical perturbation. Ca^{2+} signals are either abolished or exhibit qualitatively different signatures in *fer* mutants exposed to local touch or bending stimulation. Furthermore, mechanically-induced upregulation of known *TCH* genes is significantly decreased in *fer* mutants. In addition to these defects in mechanical signaling, *fer* mutants also exhibit growth phenotypes consistent with impaired mechanical development, including biased root skewing, an inability to penetrate hard agar layers and abnormal growth responses to impenetrable obstacles. Finally, high-resolution kinematic analysis of root growth revealed that *fer* mutants show pronounced spatio-temporal fluctuations in root cell expansion profiles with a time scale of minutes. Based on these results, we propose that FER is a key regulator of mechanical Ca^{2+} signaling and that FER-dependent mechanical signaling functions to regulate growth in response to external or intrinsic mechanical forces.

TPD1 ACTS AS A PROTEIN LIGAND OF THE EMS1 RECEPTOR KINASE TO REGULATE ANTHER CELL DIFFERENTIATION IN *ARABIDOPSIS*

Jian Huang[1], Tianyu Zhang[1], Marisa S. Otegui[2], Heather A. Owen[1] and Dazhong Zhao[1]

[1]Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI 53211, USA, and

[2]Department of Botany, University of Wisconsin- Madison, WI 53706-1381, USA

Somatic and reproductive cell fate determination is tightly controlled during sexual reproduction. The TPD1 and EMS1 are proposed to work as a ligand-receptor pair to regulate tapetal cell fate in *Arabidopsis* anthers. Here, we provide strong evidences to support that TPD1 is a secretary protein. Our subcellular localization studies demonstrate that TPD1 is localized in the trafficking vesicles and the presence of EMS1 is required for TPD1 localization on the plasma membrane. We identified the C-terminal conserved region as its functional domain. We also found a dibasic cleavage site at the C-terminal. The cleavage of TPD1 produces a 12.6KD mature protein. Moreover, the cleavage is required for its secretion. We further confirmed the protein-protein interaction between TPD1 and EMS1 by using Bimolecular Fluorescence Complementation (BiFC) method. A TPD1 binding site is identified at the N-terminal of EMS1 LRR domain. Meanwhile, a 13-amino acid sequence is identified as TPD1 interaction domain. Our gene expression and protein localization studies indicate that EMS1 is highly expressed and localized in tapetal cells. TPD1 is localized in the microsporocytes and tapetal cells, though *TPD1* is only expressed in microsporocytes precursors and microsporocytes. The gene expression/protein localization data and the secretion of TPD1 strongly suggest the cell-to-cell communication between microsporocytes and tapetal cells. To study the effect of microsporocytes on tapetal cell differentiation, we performed experiments to inhibit the secretion of TPD1 or disrupt the function of microsporocytes by gene ablation. Our results suggest that TPD1/EMS1 mediated cell-to-cell communication affects somatic cell proliferation and tapetal cell fate determination.

IDENTIFICATION OF FERTILITY RESTORERS FOR S MALE-STERILE MAIZE: BEYOND PPRS

Yan Wang[1] , Rosalind Williams-Carrier[2] Karen Chamusco[1], Liming Zhao[3], L. Curt Hannah[1] , Susan Gabay-Laughnan[4], Alice Barkan[2], Christine Chase[1]

[1] University of Florida, Horticultural Sciences Department, Box 110690, Gainesville, FL 32611

[2]Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

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Interaction of plant mitochondrial and nuclear genetic systems is exemplified by mitochondria-encoded cytoplasmic male sterility (CMS) under the control of nuclear restorer-of-fertility (restorer) genes. Many restorer genes encode pentatricopeptide repeat (PPR) proteins that arose through adaptive evolution for the silencing of specific CMS gene targets. CMS-S maize is characterized by a pollen cell death phenotype and a novel paradigm for fertility restoration. Restorers are recovered as genetic mutations that rescue CMS-S pollen but condition homozygous-lethal seed phenotypes. Restorer-of-fertility, seed-lethal (*rfl*) mutants recovered from *Mutator* (*Mu*) transposon-active, CMS-S lines were associated with post-transcriptional failure to accumulate mitochondria-encoded respiratory subunits. Illumina sequencing of Mu-flanking regions identified *Mu* insertions in nuclear genes encoding mitochondrial ribosomal proteins and mitochondrial protein complex assembly factors as candidate *rfl* mutations. These mutations therefore rescue CMS-S pollen by disrupting mitochondrial processes that are expendable in pollen but essential for seed development. The genetic dissection of mitochondrial functions is limited by the critical nature of mitochondrial processes. Our research demonstrates that CMS-S fertility restoration affords a novel genetic approach for the study of mitochondrial functions in plants.

Oral Presentations - Session 4

Thursday, May 15, 2014, 1:10 – 2:30pm

Chair: Dr. Ravi Palanivelu, University of Arizona

POLLEN -- A MODEL SYSTEM FOR CA2+SIGNALLING AND INSIGHTS INTO STRESS TOLERANCE

Jeffrey F. Harper, Maryam Ishka, Chong Tang, and Elizabeth Brown. University of Nevada, Reno NV 89557 USA. (*) jfharper@unr.edu.*

Research in the Harper lab is focused on the role of Ca2+ signaling in pollen development. Ca2+ signals have been implicated in regulating pollen grain germination, tube growth, and sperm cell discharge in ovules. In Arabidopsis thaliana, there are at least 6 cyclic nucleotide-gated Ca2+-permeable ion channels (CNGCs) expressed in pollen. Gene knockouts have shown that three of these channels (CNGC18, 7, and 8) are essential for pollen tube tip growth. In contrast, a knockout of CNGC16 has no visible phenotype under normal growth conditions. However, *cngc16* pollen are nearly sterile when plants are grown under conditions of hot days and cold nights. The most stress-sensitive time for *cngc16* pollen development is during germination and the initiation of pollen tube tip growth. Using RNA-seq technology, the expression profiles of *cngc16* pollen grains were compared to wild type. Under heat-stress conditions, pollen grains from both wild type and mutant plants showed nearly 5000 stress-dependent changes in their transcriptomes. However, more than 50% of these changes were different between wild type and mutant. This supports a model in which the mutant fails to develop a normal transcriptional stress response, and is therefore more sensitive to stress conditions. However, even under normal conditions, the two pollen types showed more than 1000 differences. Thus, it is also possible that cngc16 pollen have a “pre-existing condition” that make them more sensitive to death at the onset of a stress response. Consistent with this alternative model, the expression profiles reveal several mRNA differences of potential importance to the biogenesis of a normal cell wall. Defects in cell wall could make pollen more susceptible to bursting under conditions that either increase turgor or desynchronize growth processes during tip growth.

COORDINATION OF POLLEN TUBE GROWTH BY CA2+ REGULATED ANION FLUXES

José A Feijó

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Pollen transcriptomics reveals the expression of of about 7.000 genes in pollen, but theoretical modelling suggests that the cooperation of all of these into the processes of wall surface and cytoplasmic volume production is a minimal condition to explain most of the morphogenic events that characterize these cells. Spatial and temporal integration of extended biochemical and biophysical processes is mandatory, and in the past we have proposed that ion dynamics can be a common regulator of fundamental growth processes. I will report on advances on the biology of Glutamate-Receptor Like Ca2+-channels. These channels are hypothesized to participate on the generation of the Ca2+ focused gradient characteristic of functional pollen tubes. I will also describe a new regulatory loop downstream of the Ca2+ signal, based on the activation of specific Ca2+ dependent kinases (CPK) and the regulation of the anion channel SLAH2. We have developed novel chloride (Cl-) sensing genetic probes, and imaged for the first time the dynamics of the cytosolic concentration of this ion. I will present data that allows the proposition of a feed-back between Cl- and Ca2+ as underlying the regulation of pollen tube growth.

Myosin motors are required for rapid pollen tube growth and normal fertilization efficiency

Stephanie L. Madison, Matthew Buchanan, Tanner Beard, Jeremiah Glass, Andreas Nebenführ

Department of Biochemistry and Cellular and Molecular Biology, University of Tennessee, Knoxville, TN 37996-0840, USA

email: nebenfuehr@utk.edu; web: www.bio.utk.edu/cellbiol

Pollen tubes display prominent reverse fountain streaming that continuously mixes their cytoplasm. It is thought that these rapid intracellular movements are required for efficient tip growth, but experimental evidence for this assumption has been lacking. We have taken a reverse genetic approach to remove the myosin motor proteins responsible for organelle movements in order to investigate their effect on pollen tube growth. Specifically, we have analyzed knock-out mutants in all six pollen-expressed myosin XI genes of *Arabidopsis thaliana*. Some mutants showed slightly reduced seed set or reduced fitness when competing against wild-type pollen, however, most single-gene mutants had little effect on seed set. Loss of two closely related myosin genes, *Myo11C1* and *Myo11C2*, on the other hand resulted in dramatically reduced seed set, suggesting that myosin-driven intracellular movements are required for normal pollen function. The reduced seed set of the double mutant was accompanied by slower growth of pollen tubes in both *in vitro* and *in vivo* experiments. Within mutant pollen tubes, movements of Golgi stacks and peroxisomes were greatly reduced, suggesting that these two myosin motors are central for organelle movements and that cytoplasmic streaming in turn is required for rapid pollen tube growth.

FORMIN REGULATION OF TIP-FOCUSED GROWTH IN POLLEN TUBES

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As one of nature's fastest growing cells, elongating pollen tubes are often considered an excellent system for studying polarized cell growth. The apical cytoplasmic organization in an elongating pollen tube, an apical collection of vesicles and a subapical actin mesh that temporally and spatially keep pace with the extending tube apex, is critical for its tip-focused growth characteristic (1). Group I formins are an intriguing family of actin nucleating proteins. In addition to a cytoplasmic domain that stimulates nascent actin assembly and universally present in all formins, group I plant formins have an N-terminal extension comprised of a transmembrane domain and an extracellular domain. In *Arabidopsis*, there are twenty-one formins, ten of them being group I. The expression level of these formins, as well as for the other better known actin-nucleating proteins, Arp2/3 complex, are generally low, implying that the level of actin nucleation needs to be carefully monitored by the cell. Using two Arabidopsis formins, FH1 and FH5, we demonstrated that this N-terminal extension anchors the cytoplasmic actin nucleating domain to the cell membrane and that these formins stimulate actin assembly from the cell surface (2,3). FH5 is among the most prominent pollen-expressed formin (3); FH3 is a pollen-specific formin (4). We showed previously that FH5 plays a key role in the assembly and maintenance of the subapical actin mesh and in maintaining the apical vesicular and subapical actin organization (2). Functionally redundancy among formins has frustrated a genetic approach to dissect their functions; also, a non-growth phenotype is not useful towards understanding growth. Here, we show that double T-DNA insertion mutations, one in *Fh5* and one in *Fh3*, results in significantly reduced pollen tube growth rates and compromised tip-focused growth. We also demonstrate the actin cytoskeleton basis that underlies the *fh5 fh3* pollen tube growth phenotype.

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Poster Presentations

CATION/H^+ EXCHANGERS ON DYNAMIC MEMBRANES AFFECT FERTILIZATION AND SEED DEVELOPMENT

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Flowering plants are the most dominant plants on earth due to innovations that ensure reproductive success. However, the molecular bases of these innovations are poorly understood. Several predicted cation/H^+ exchangers (CHX) localized to endosomes had a role in pH homeostasis and protein trafficking in yeast, though their roles in plants were obscure. Based on the crystal structure of the *E. coli* Na^+/H^+ antiporter NhaA, we used 3D homology model-guided mutagenesis of the CHX17 transmembrane domain to identify conserved and novel residues important for activity. Results support the idea that CHX17 can sense pH and act as a K^+/H^+ antiporter. CHX17, CHX18 or CHX19 genes are expressed in vegetative cells, pollen, micropylar endosperm, or sperm. Seed set was reduced 65% in *chx17*$^{-/-}$*/18*$^{-/-}$*/19*$^{-/-}$ mutants. Reciprocal crosses showed that *CHX18* and *CHX19* are critical for male transmission. Triple mutant pollen tubes reached all ovules, indicating that tube growth and guidance in vivo were not compromised. However mutant pods consisted of 33% unfertilized ovules, 11% embryo or endosperm only, and 55% seeds at different embryo stages. These studies of triple mutants indicate that pH and K^+ homeostasis in dynamic endomembranes play critical roles for successful fertilization, for proper seed development, or both. [Supported by DOE, Div Chem Sci, Geosci. & Biosci., BES to HS]

TOWARDS AN UNDERSTANDING OF THE ROLE OF METABOLITE TRANSPORTERS IN POLLEN

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Among the genes expressed in pollen those encoding membrane transporters are overrepresented, yet for many their role is unclear. In mature pollen the vegetative cell contains carbohydrate reserves (e.g. starch, sucrose, fructose, glucose). These reserves are sufficient to support pollen survival and germination but pollen tubes take up carbohydrate secretions from the stylar canal to support growth. The Major Facilitator Superfamily (MFS) is a superfamily of transporters with members in every organism, from bacteria to humans. In plants the MFS has undergone a large expansion and in Arabidopsis encompasses more than 100 members. For most of these plant transporters little or no functional data are available. Generally they are predicted to be involved in the uptake of small metabolites across the plasma membrane or in compartmentation of metabolites in organelles. Genes encoding transporters belonging to the MFS that are specifically or preferentially expressed in pollen were selected. The aim is to identify genes encoding MFS proteins with important functions in pollen, to determine at what stage, during development or during germination and tube growth, their function is required, and to understand the biochemical function of these transporters. Initial experiments are focused on analyzing the membrane localization pattern of selected MFS transporters.

MICROSPORES ABORTION IN GMS CHINESE CABBAGE

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The genetic male sterility (GMS) in Chinese cabbage is conditioned by three alleles at same site and characterized by microspores abortion at the tetrad stage of development. The molecular and cellular basis of this phenotype was investigated by comparing microspore development in male sterile anther and fertile anther of near-isogenic line. Aniline blue stain revealed that callose around tetrad delayed degradation and microspores could not be released in male sterile anther. Real-time PCR showed that callose synthase expressed high in both male sterile and fertile anthers and no difference between them. Callase A6, however, expressed differently. It was high in the male fertile anther before microspores release and did not express after microspores release; It was, low in the male sterile anther but persistent. TUNEL assays showed that tapetum PCD occurred differently at the tetrad stage in male sterile anthers. Therefore we put forward a hypothesis that abnormal tapetum PCD influenced callase transportation from tapetum to locule and failed to degrade callose around tetrad. Microspores could not develop further and abortion occurred.