Third RCN meeting, Tucson, Arizona
March 1 – 3, 2013

http://pollennetwork.org/

Integrative Pollen Biology RCN Lead PI:

Dr. Alice Cheung, University of Massachusetts, Amherst

Meeting Organizers:

Dr. Ravi Palanivelu, University of Arizona, Tucson

Dr. Jeffery Harper, University of Nevada, Reno

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We also recognize and acknowledge support and cooperation of:

Integrative Pollen Biology RCN Steering Committee
&
iPlant Collaborative (www.iplantcollaborative.org)
&

&
Residence Inn Tucson Airport – Marriott

Pollen RCN Workshop in Tucson - Program

Venue: Residence Inn Tucson Airport, 2660 East Medina Road, Tucson, Arizona Phone: (520-294-5522)

Tucson contact: Ravi Palanivelu <rpalaniv@ag.arizona.edu> Phone: 520-235-2761

Pollen RCN PI: Alice Cheung acheung@biochem.umass.edu

Friday, March 1, 2013

7:00-8:20	***Dinner to be catered by Sapphire Catering in Residence Inn***	
Session I: Chair, Ravi Palanivelu, University of Arizona		
8:20-8:30	Ravi Palanivelu, University of Arizona	Introductory Remarks
8:30-8:50	Mark Johnson, Brown University	Molecular Dialogues Between Pollen and Pistil
8:50-9:10	Andrew McCubbin, Washington State University	Unraveling CDPK functions and pathways in pollen tube growth
9:10-9:30	Matina Donaldson-Matasci, University of Arizona	The role of ecology and communication in pollen foraging by honey bees

Saturday, March 2, 2013

7:00-8:30	***Complimentary breakfast by Residence Inn***	
Session II: Chair, Jeff Harper, University of Nevada, Reno		
8:30-8:40	Jeff Harper, University of Nevada, Reno	Introductory Remarks
8:40-9:00	Sheila McCormick, Plant Gene Expression Center, USDA-ARS/ UC Berkeley	RNA-seq analysis of pollen gene expression
9:00-9:20	Steve Goff, Director, iPlant Collaborative and Martha Narro, Senior Project Coordinator, iPlant Collaborative	iPlant overview, Data Store, Discovery Environment
9:20-9:30	Martha Narro, Senior Project Coordinator, iPlant Collaborative	Bisque
9:30-9:40	Matt Bomhoff, iPlant Collaborative	CoGe
9:40-9:50	Naim Matasci, iPlant Collaborative	Atmosphere
9:50-10:00	iPlant Presenters	Questions and Discussion
10:00-10:20	***Poster Session/Coffee Break***	
10:20-10:40	Salika Dunatunga, University of Arizona	Pollen tube tracker – A module to automatically track multiple, overlapping pollen tube trajectories
10:40-11:00	Ann Loraine, University of North Carolina at Charlotte	Visual analysis of high-throughput sequencing data with Integrated Genome Browser
11:00-12:00	***Poster session/Booth Demonstrations by presenters in the previous session***	
12:00-1:00	***LUNCH to be catered by Sapphire Catering in Residence Inn***	
1:00-3:30	***FREE TIME*** - Group visit to Saguaro National Park East and hike	
3:30-4:00	***Poster Session/Discussion/Coffee Break***	
Session III: Chair Chris Staiger, Purdue University		
4:00-4:20	Ravi Palanivelu, University of Arizona	Characterization of an Arabidopsis mutant defective in pollen tube elongation
4:20-4:40	Chris Staiger, Purdue University	Regulation of actin dynamics
4:40-5:00	Teagen Quilichini, University of British Columbia	Investigating pollen wall trafficking and assembly in Arabidopsis thaliana.
5:00-5:20	***Poster Session/Discussion***	

5:20-5:40	Liliana Elizabeth Garcia Valencia, Universidad Nacional Autónoma de México, México	NaSIPP is a pollen protein specific of self-incompatible Nicotiana species, which interacts with NaStEP, a stigmatic protein essential to pollen rejection
5:40-6:00	Alejandro Tovar-Mendez	Reconstructing an interspecific reproductive barrier in tomato with S-RNase and HT-proteins
6:00-7:00	***DINNER to be catered by Sapphire Catering in Residence Inn***	
Session IV: Chair, Sheila McCormick, Plant Gene Expression Center, USDA-ARS		
7:00-7:20	Anna Edlund, LaFeyette University	Performance variations between pollen tubes
7:20-7:40	Anna Dobritsa, University of Chicago	Walls and gates: Genetics of exine and aperture formation in Arabidopsis pollen
7:40-8:00	Anke Reinders, University of Minnesota	Identification of amino acids important for substrate specificity in sucrose transporters using gene shuffling
8:00-9:00	***Poster Session/Open Discussion,*** BYB	

Sunday, March 3, 2013

7:00-8:10	***Complimentary breakfast by Residence Inn***	
Session V: Chair, Mark Johnson, Brown University		
8:10-8:30	Luis Cárdenas, Universidad Nacional Autónoma de México, México	Reactive oxygen species in root hair cells and pollen tubes, expression of Hyper as a new ROS sensitive molecular probe.
8:30-8:50	Pedro T. Lima, Instituto Gulbenkian de Ciência, Portugal	Pumping and plugging in Arabidopsis pollen tubes
9:10-9:30	Rob Swanson, Valparaiso University	The genetics of post pollination nonrandom mating
8:50-9:10	Guang-Yuh Jauh, Institute of Plant and Microbial Biology Academia Sinica, Taiwan	Profiling of Translatomes of in vivo-grown Pollen Tubes Reveals Genes with Roles in Micropylar Guidance During Pollination
9:30-9:50	Jeff Harper, University of Nevada, Reno	Pollen -- a model for insights into Ca2+ signaling and membrane dynamics

Oral presentations – Session I

Chair: Ravi Palanivelu, University of Arizona

8:20 PM – 9:30 PM Friday March 1, 2013

8:30-8:50, Friday March 1, 2013

A POLLEN TUBE TRANSCRIPTIONAL NETWORK CONTROLS INTERACTIONS WITH FEMALE CELLS REQUIRED FOR SPERM RELEASE IN *ARABIDOPSIS*

Alexander R. Leydon[1], Kristin M. Beale[1], Karolina Woroniecka[1], Elizabeth Castner[1], Jefferson Chen[1], Casie Horgan[1], Ravishankar Palanivelu[2], and Mark A. Johnson[1]
1)Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI 02912, USA.
2)Department of Plant Sciences, University of Arizona, Tucson, AZ, 85721, USA.

Flowering plants have immotile sperm that develop within the pollen grain and are delivered to female gametes by a pollen tube. Pollen tubes interact with several cell types within the pistil and represent a model system for dynamic differentiation of a single cell as it grows through a changing extracellular environment. We identified three pollen tube-expressed MYB transcription factors as possible regulators of pollen tube differentiation. Pollen tubes lacking these transcriptional regulators fail to stop growing in synergids, specialized cells that attract pollen tubes and degenerate upon pollen tube arrival. Mutant pollen tubes also fail to release their sperm cargo. Moreover, we find evidence that signaling from the pollen tube rather than pollen tube rupture triggers synergid degeneration and that signaling requires pollen tube MYB activity. These data indicate that pollen tube transcription during growth in the pistil leads to differentiation required for male-female recognition, synergid degeneration, and release of sperm cells. We define a suite of pollen tube-expressed genes regulated by these critical transcription factors and identify small peptides, carbohydrate active enzymes, and transporters representing likely molecular mediators of pollen-female interactions necessary for flowering plant reproduction.

8:50-9:10 Friday, March 1, 2013

UNRAVELLING CDPK FUNCTIONS AND PATHWAYS IN POLLEN TUBE GROWTH

Gyeong Mee Yoon, Feng Guo and Andrew G. McCubbin
School of Biological Sciences and Centers for Reproductive Biology and Biotechnology, Washington State University,Pullman, Wa 99164

A tip-focused calcium gardient plays a pivotal role in regulating the polar growth of pollen tubes. One of the downstream effectors of this gradient are calcium dependent protein kinases (CDPK's). We have previosuly reported the effects of over-expressing two pollen specific CDPK isoforms on pollen tube growth in *Petunia inflata*. One (*PiCDPK1*) was determined to be critical in the regulation of growth polarity and localized tp the plasma membrane. The second (*PiCDPK2*) inhibited pollen tube extension when over-expressed and localized to internal membrane compartment. Here we update our progress in identifying substrates of these two CDPKs. Two potential substrates have been identified using the yeast 2 hybrid system, both interact with and are phosphorylated by CDPK in pull-down and in vitro phosphorylation assays. The first, termed PiSCP1 is a small peptide which we show co-localizes to peroxsomes with PiCDPK2 but has no homologs of known function. The second is a Rho Guanine Dissociation Inhibitor (RhoGDI) which modulates the activity of Rop/Rac GTPase. This discovery of this latter cadidiate suggests the presence of a feedback loop involving calcium, Rop/Rac, RhoGDI and PiCDPK1, with PiCDPK1 modulating recruitment of Rop/Rac to the plasma membrane. We review evidence for this existence of this feedback loop.

9:10-9:30 Friday, March 1, 2013

THE ROLE OF ECOLOGY AND COMMUNICATION IN POLLEN FORAGING BY HONEY BEES

Matina C. Donaldson-Matasci[1], Nhat R. Nguyen[2], Min C. Shin[2], Anna Dornhaus[1]
1) Department of Ecology & Evolutionary Biology, University of Arizona
2) Department of Computer Science, University of North Carolina, Charlotte

For many pollinating insects, especially bees, pollen is a critical resource that provides essential nutrients for larval development. Generalist pollinators typically collect pollen from a wide variety of plant species, yet the factors affecting this behavior are poorly understood. In honey bees (Apis mellifera), communication is known to play an important role in foraging decisions. Because individuals within a colony can communicate with one another about the location of food, using their famous "waggle dance", communication might influence the choice or the breadth of pollen species that a colony utilizes. To test this hypothesis, we assess the diversity of pollen taxa collected by honey bee colonies across several different environments, while manipulating their ability to communicate about food resources. The traditional method of identifying pollen taxa is a laborious process requiring expert knowledge. In this project, we develop a pollen recognition software algorithm based on machine learning, and compare its performance to that of a human expert. We expect this new technology to develop into a powerful tool for exploring questions not only in bee behavior, but also pollination biology, climate change ecology, and agricultural sciences.

Oral presentations – Session II

Chair: Jeff Harper, University of Nevada, Reno

8:20 AM – 9:30 AM Saturday March 2, 2013

8:40-9:00 Saturday, March 2, 2013

RNA-SEQ ANALYSIS OF *ARABIDOPSIS THALIANA* POLLEN

Ann E. Loraine[1], Sheila McCormick[2],April Estrada[1], Ketan Patel[1], Peng Qin[2]
1) Department of Bioinformatics and Genomics, University of North Carolina at Charlotte, North Carolina Research Campus, Kannapolis, NC 28081
2) Plant Gene Expression Center, USDA-ARS/UC-Berkeley, Albany, CA, 94710

Pollen grains of Arabidopsis thaliana contain two haploid sperm cells enclosed in a haploid vegetative cell. Upon germination, the vegetative cell undergoes rapid growth, extruding a tubular structure (the pollen tube) that carries the sperm to an ovule for fertilization. Most Arabidopsis pollen transcriptome studies have used the ATH1 microarray, which does not assay splice variants and lacks specific probe sets for many genes. To investigate the pollen transcriptome, we performed high-throughput sequencing (RNA-Seq) of Arabidopsis pollen and three-week-old seedlings for comparison. RNA-Seq detected 10,529 protein coding genes expressed in pollen, including 431 assayed by non-specific probe sets only. To illustrate the usefulness of this dataset, I will present various examples, as visualized in The Integrated Genome Browser (http://bioviz.org/igb/). I will also compare and contrast some of these examples with results from pollen and sperm microarray experiments, and/or from promoter-reporter constructs.

10:20-10:40 Saturday, March 2, 2013

POLLEN TUBE TRACKER – A MODULE TO AUTOMATICALLY TRACK MULTIPLE, OVERLAPPING POLLEN TUBE TRAJECTORIES

Ernesto Brau[1], Andrew Predoehl[1], Kobus Barnard[1], Salika Dunatunga[2], Damayanthi Dunatunga[1], Tatsuya Tsukamoto[2], Ravishankar Palanivelu[2], Martha Narro[3], and Nirav Merchant[3]*
1) SISTA, University of Arizona, Tucson, Arizona, 85721
2) School of Plant Sciences, University of Arizona, Tucson, Arizona, 85721
3) iPlant Collaborative, University of Arizona, Tucson, Arizona, 85721
**, Undergraduate student author*

Capability to track pollen tubes in a time-lapse image sequence is of considerable importance and interest, as it will circumvent labor-intensive image analysis. However, an effective parametric model for the trajectories does not exist. We present a general model for tracking smooth trajectories of multiple targets in complex data sets, where tracks potentially cross each other many times. As the number of overlapping trajectories grows, exploiting smoothness becomes increasingly important to disambiguate the association of successive points. Hence we modeled trajectories as independent realizations of Gaussian processes with kernel functions, which allowed for arbitrary smooth motion. Our statistical model accounts for the data as coming from an unknown number of such processes, together with expectations for noise points and the probability that points are missing. For inference we compared two methods: A modified version of the Markov chain Monte Carlo data association (MCMCDA) method, and a Gibbs sampling method which is much simpler and faster, and gives better results by being able to search the solution space more efficiently. In both cases, we compared our results against the smoothing provided by linear dynamical systems (LDS). We tested our approach on pollen tubes growing in a petri dish, each with up to 60 tubes with multiple crossings. We achieved 93% accuracy on image sequences with up to ten trajectories (35 sequences) and 88% accuracy when there are more than ten (42 sequences). This performance surpasses that of using an LDS motion model, and far exceeds a simple heuristic tracker (1). We recently partnered with iPlant Collaborative and adapted this module to the Bisque (Bio-Image Semantic Query User Environment) image management platform and made it widely available to the research community. In the workshop, we will present a detailed tutorial and a demonstration of the 'pollen tube tracker' module.

This work was supported by funding from the National Science Foundation (IOS-073421) and stipend support to S.D. from Integrative Pollen Biology RCN.

Reference:

1) Brau et al (2012). Proceedings of the IEEE Computer Vision and Pattern Recognition. 1137-1144. doi:10.1109/CVPR.2011.5995736.

10:40-11:00 Saturday, March 2, 2013

VISUAL ANALYSIS OF HIGH-THROUGHPUT SEQUENCING DATA WITH INTEGRATED GENOME BROWSER

Ann Loraine [1], *Sheila McCormick*[2], *April Estrada*[1], *Hiral Vora*[1]
1)Department of Bioinformatics and Genomics, University of North Carolina at Charlotte, North Carolina Research Camps, Kannapolis, NC 28081
2)Plant Gene Expression Center, USDA-ARS/UC-Berkeley, Albany, CA, 94710

Visual exploration and analysis of large-scale genomic data sets has many benefits. Interactive visualization software can reveal trends, highlight meaningful outliers, expose errors, promote insight, and communicate meaning. To help scientists explore and analyze data from ultra high throughput sequencing experiments, we added new visual analytics features to Integrated Genome Browser (IGB), a flexible, highly interactive genome browser software tool. In this talk, I'll describe features that aid exploration and analysis of data from RNA-Seq experiments, using data from *Arabidopsis thaliana* pollen as an example. IGB is open source, free software available from http://www.bioviz.org.

Oral presentations – Session III

Chair: Chris Staiger, Purdue University

4:00 PM – 6:00 PM Saturday March 2, 2013

4:00-4:20 Saturday, March 2, 2013

CHARACTERIZATION OF AN ARABIDOPSIS MUTANT DEFECTIVE IN POLLEN TUBE ELONGATION

Tatsuya Tsukamoto[1], Jolene Nelson[1], Gary Drews[2] and Ravishankar Palanivelu[1]
1) School of Plant Sciences, University of Arizona, Tucson, Arizona, 85721
2) Department of Biology, University of Utah, Salt Lake City, Utah

Sexual reproduction in flowering plants is unique in multiple ways. Distinct multicellular gametophytes contain either a pair of immotile, haploid male gametes (sperm cells) or a pair of female gametes (haploid egg cell and homodiploid central cell). After pollination, the pollen tube, a cellular extension of the male gametophyte, transports both male gametes at its growing tip and delivers them to the female gametes to affect double fertilization. The pollen tube travels a long path and sustains its growth over a considerable amount of time in the female reproductive organ (pistil) before it reaches the ovule, which contains the female gametophyte (1).

In a screen for genes critical for reproduction, we isolated a mutant that produced significantly reduced seeds compared to the wild type. Analysis of the this mutant (*fem137*) revealed that mutant ovules were defective in attracting pollen tubes due to noticeably reduced size of the mutant female gametophytes. Transmission efficiency anaysis of *fem137* mutation revealed that this mutation was also transmitted through the male gametophyte at a very reduced rate. Consistent with this result, we uncovered that mutant tubes elongate poorly in vitro and in the pistil, even though the mutant pollen development and germination were on par with wild-type pollen. To further dissect its role in the male and female gametophyte function, we performed map-based cloning and complementation of the FEM137 gene, which putatively encodes a protein involved in biosynthesis of tRNAs and 5S rRNAs. Progress on establishing the role of FEM137 in pollen tube elongation and female gametophyte development will be presented.

This work is supported by funding from the National Science Foundation (IOS-1045314).

References

1) Palanivelu, R., and Tsukamoto, T. (2012). Pathfinding in angiosperm reproduction: pollen tube guidance by pistils ensures successful double fertilization. WIREs Dev Biol. 1:96-113.

4:20-4:40 Saturday, March 2, 2013

TURNING OVER TRACKS: STOCHASTIC DYNAMICS OF THE CORTICAL ACTIN ARRAY

Christopher J. Staiger[‡]*, Jessica L. Henty-Ridilla, and Jiejie Li*
Department of Biological Sciences and the Bindley Bioscience Center, Hansen Life Sciences Research Building, Purdue University, West Lafayette, IN 47907-2064, USA
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The actin cytoskeleton is generally understood to participate in cell elongation and responses to biotic and abiotic stimuli; however, a detailed description and molecular mechanism(s) underpinning filament nucleation, growth and turnover are lacking. We have used variable-angle epifluorescence microscopy (VAEM) to examine the organization and dynamics of the cortical cytoskeleton in growing and non-growing epidermal cells from *Arabidopsis thaliana* hypocotyls. Actin in the cortical array exists as individual actin filaments that are short-lived, as well as longer-lived actin filament bundles. Collectively, the single actin filaments are randomly oriented and amazingly dynamic. Single actin filaments elongate at rates of ~ 2 μm s^{-1}, allowing them to grow from one side of the cell to another in tens of seconds. Instead of depolymerization from ends, actin filaments are disassembled by prolific severing activity. This incessant remodeling of the cortical actin array also features filament buckling and straightening events. We consider several mechanisms for the control of actin dynamics, including rapid polymerization from a large pool of profilin–actin, specific severing and capping activities, and myosin-driven filament-filament interactions. Aspects of this model have been tested using pharmacological agents and with reverse-genetics. Specifically, we have demonstrated a role for the actin depolymerizing factor, ADF4, in severing actin filaments *in vivo*. Moreover, we show that the heterodimeric capping protein, CP, modulates the availability of filament barbed ends and is negatively regulated by the signaling lipid, phosphatidic acid. Our observations, the first to describe single actin filament behavior in plant cells, indicate a mechanism inconsistent with treadmilling, instead resembling the stochastic dynamics of a recently described biomimetic system for actin assembly *in vitro*.

The research on stochastic dynamics is supported by a Collaborative Research Award from the NSF *Arabidopsis* 2010 Program (IOS-1021185) to CJS. We also gratefully acknowledge support for CP research through a grant from the Physical Biosciences Program of the Office of Basic Energy Sciences, US Department of Energy (DE-FG02-09ER15526).

4:40-5:00 Saturday, March 2, 2013

INVESTIGATING POLLEN WALL TRAFFICKING AND ASSEMBLY IN *ARABIDOPSIS* THALIANA

Teagen Quilichini, Lacey Samuels, Carl Douglas
Department of Botany, UBC, Vancouver Canada

Sporopollenin, a tapetum-derived outer pollen wall polymer composed of fatty acids and aromatic compounds, is likely to include polyketide(s) synthesized by ACOS5, PKSA/B, and TKPR1. An Arabidopsis ATP-binding cassette transporter ABCG26 is thought to function in sporopollenin export from tapetum cells, but its substrate remains unknown. Through the analysis of anthers by multi-photon microscopy, abcg26 mutant tapetum cells were found to contain autofluorescent vacuoles not observed in wild type. TEM supports these findings, with enlarged, debris-filled tapetum vacuoles in abcg26. The nature of the putative ABCG26-trafficked metabolites was investigated by examining double mutants affecting the transporter and enzymes required for sporopollenin formation. This analysis revealed the disappearance of tapetum inclusions in double mutants of abcg26 and acos5, pks-a/pks-b, and tkpr1. These data suggest that the proposed aliphatic polyketide component of sporopollenin is transported from tapetum cells by ABCG26. We also observed novel autofluorescent extracellular bodies around tapetum cells in acos5, pks-a/pks-b, and tkpr1, but not in abcg26. We will discuss how elucidating the chemical nature of these bodies informs our understanding of pollen wall trafficking and assembly in Arabidopsis.

5:20-5:40 Saturday, March 2, 2013

NASIPP IS A POLLEN PROTEIN SPECIFIC OF SELF-INCOMPATIBLE *NICOTIANA* SPECIES, WHICH INTERACTS WITH NASTEP, A STIGMATIC PROTEIN ESSENTIAL TO POLLEN REJECTION

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In Solanaceae, self-incompatibility (SI) is controlled by the female determinant (S-RNase) and the male determinant (SLF), which are encoded in the S-locus. However, it has been shown that other non-S-locus linked genes are such as HT-B, 120K and NaStEP are also essential for SI in Nicotiana alata (McClure et al., 1999; Hancok et al., 2005; Jiménez-Durán et al., 2013).
Particularly, we have been involved in study of NaStEP, which codes a protein with protease inhibition activity. Loss of function experiments shows that the suppression of NaStEP disrupts pollen rejection in a S-specific manner. Likewise, NaStEP is taken up by pollen tubes after pollen grain germination. Notably, in transgenic NaStEP suppressed plants, the HT-B protein is degraded inside pollen tubes no matter if the cross is compatible or incompatible, a situation that does not occur in the wild type SI N. alata, where the HT-B protein is only degraded in pollen tubes of self-compatible crosses. All evidences above suggest that NaStEP functions in pollen rejection might be through its interaction with PT proteins. To test it, we searched for PT protein interactors by yeast-two-hybrid. We recovered a cDNA encoding a protein with homology to the C-terminal region of a phosphate transporter, which we called NaSIPP (N. alata Self-Incompatibility Pollen Protein). RNA-blot assays showed that the NaSIPP transcript is highly abundant in mature pollen, but is no detectable in young anthers neither in other organs. Notably, the NaSIPP mRNA is highly abundant in mature pollen of SI Nicotiana species but undetectable self-compatible Nicotiana species. Western-blot analysis and NaSIPP-GFP fusion assays suggests that NaSIPP is associated to the endomembrane system and with a mitochondrial fraction.
We discuss the relevance of this interaction in a pollen rejection scenario.
DGAPA IN210312, CONACYT 81968”

5:40-6:00 Saturday, March 2, 2013

RECONSTRUCTING AN INTERSPECIFIC REPRODUCTIVE BARRIER IN TOMATO WITH S-RNASE AND HT-PROTEINS

Alejandro Tovar-Mendez[1], Aruna Kumar[1], Katsuhiko Kondo[1], Amy Ashford[2], You Soon Baek[2], Lillian Welch[2], Patricia A. Bedinger[2], and Bruce A. McClure[1]
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2) Department of Biology, Colorado State University, Fort Collins, CO 80523-1878, USA

We are investigating intra- and interspecific pollen rejection in Solanum Section Lycopersicon, the tomato family. Self-incompatible (SI) species in the Section display S-RNase-based gametophytic systems that provide for recognition and rejection of self-pollen and pollen from closely related individuals. In this system, the polymorphic S-locus controls specific pollen rejection and encodes both, pollen and pistil S-specificity determinants, the S-locus F-box and S-RNase genes, respectively. Modifier genes are required for SI function but do not contribute to S-specificity per se, for example, small pistil-expressed proteins (HT-proteins) are required for SI. Interspecific compatibility usually follows the SI X SC rule: pollen from SC species is rejected on SI species pistils, but the reciprocal pollinations are compatible. This rule implies that inter- and intraspecific pollen rejection systems are linked. We tested this hypothesis by expressing SI factors in the pistil and testing whether this caused rejection of pollen from SC species. We found that co-expression of HT-proteins and S-RNase caused rejection of pollen from the red- and orange-fruited tomato relatives. The reconstructed barrier mimics the natural barrier separating these species from the rest of the clade.

Oral presentations – Session IV

Chair: Sheila McCormick, Plant Gene Expression Center, USDA-ARS/UC Berkeley

7:00 PM – 8:00 PM Saturday March 2, 2013

7:00-7:20 Saturday, March 2, 2013

PERFORMANCE VARIATIONS BETWEEN POLLEN TUBES

Anna F. Edlund
Biology Department, Lafayette College, Easton, PA 18042

Not all pollen grains that germinate on a receptive stigma surface will find success when elongating through the style and navigating toward the ovules for fertilization. Performance and cell behaviors vary, even among compatible mates, so that a 50/50 mix of pollens hand dusted onto a stigma may result in a 90/10 mix of seeds sired. We compared pollen germination and tube elongation paths and speeds among five ecotypes of Arabidopsis thaliana: Landsberg, Columbia, WS, Shadhara and Bay-O, seeking cell behaviors associated with non-random mating. We found that pollen tubes can escape the grain walls either by passing through apertures in the sporopollenin or by rupturing the walls, and that both the frequency of aperture use, as well as the speed of germination differ between ecotypes. By hand pollinating and watching pollen tubes pour out of the severed ends of styles, we found that ecotypes can differ by hours in their arrival times at the top of the ovary. Curious if forerunner pollen tubes could block the transmitting tissue for slower tubes, we pollinated with sterile pop2 pollen, followed after three hours with wild-type pollen, and found no decrease in seed count. Surprised by the ability of A. thaliana pollen tubes to rupture sporopollenin walls, we surveyed germination behaviors across ~30 Brassicaceae family members, and found 7 other species that can germinate by wall rupture, with species (like ecotypes) differing in their pollen aperture use frequencies.

7:20-7:40 Saturday, March 2, 2013

WALLS AND GATES: GENETICS OF EXINE AND APERTURE FORMATION IN *ARABIDOPSIS* POLLEN

Anna Dobritsa
Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637

Plant pollen grains protect sperm cells inside them with the help of the unique cell walls, exines. Exines have remarkable physicochemical properties and exhibit enormous morphological variations across plant taxa, assembling into intricate and diverse species-specific patterns. Very little is known about the mechanisms through which these complex extracellular structures are synthesized and faithfully deposited at precise sites and assume precise shape within a species. To study the process of exine formation, I took a genetic approach and performed a large-scale forward genetic screen in Arabidopsis that led to the recovery of a large number of exine mutants. As will be illustrated by examples in the talk, these mutants now provide a window into genetic, biochemical, developmental, and evolutionary studies of exine production. In particular, I will concentrate on the *INP1* gene that is required for formation of apertures, the areas on pollen surface where exine deposition is limited.

7:40-8:00 Saturday, March 2, 2013

IDENTIFICATION OF AMINO ACIDS IMPORTANT FOR SUBSTRATE SPECIFICITY IN SUCROSE TRANSPORTERS USING GENE SHUFFLING

Anke Reinders, Ye Sun, Kayla L. Karvonen and John M. Ward
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Plant sucrose transporters (SUTs) function as H+-coupled uptake transporters. Plants generally contain more than one SUT. SUTs are important for phloem loading as well as for uptake of sucrose into sinks. A role of SUTs in pollen has been shown; for instance, OsSUT1 from rice is essential for pollen function. Based on amino acid sequence SUTs can be organized into three clusters: type I, II and III. Type I and II SUTs differ in their substrate specificity. Type I SUTs transport a broad range of glucosides but type II SUTs are much more selective. We investigated the structural basis for this difference using gene shuffling. Differentially conserved amino acid residues from type I SUTs were introduced into OsSUT1, a type II SUT. The OsSUT1 variants were expressed in yeast and variants that were able to transport the fluorescent coumarin β-glucoside esculin were selected by fluorescence-activated cell sorting (FACS). By substituting five amino acids present in type I SUTs we were able to confer esculin uptake ability to OsSUT1. The introduced changes also affected the transport of other substrates so that the OsSUT1 variant had a substrate specificity that was nearly identical to that of type I SUTs.

Oral presentations – Session V

Chair: Mark Johnson, Brown University

8:10 AM – 9:50 AM Sunday March 3, 2013

8:10-8:30 Sunday, March 3, 2013

REACTIVE OXYGEN SPECIES IN ROOT HAIR CELLS AND POLLEN TUBES, EXPRESSION OF HYPER AS A NEW ROS SENSITIVE MOLECULAR PROBE

Luis C·rdenas1, Alejandra Hern·ndez-Barrera1, Rosana S·nchez1, Jes¨s Montiel1, Eric Johnson2, Federico S·nchez1, Carmen Quinto1, Hen-ming Wu2, Alice Cheung2.
1) Instituto de BiotecnologÌa, Universidad Nacional Autúnoma de México, Ap. Postal 510-3 Cuernavaca, Morelos, México. Luisc@ibt.unam.mx.
2) Department of Biochemistry and Molecular Biology, University of Massachusetts, Amherst.

Many responses in animal and plant cells depend from reactive oxugen species (ROS). These ROS can activate calcium channels and receptors involved in signaling processes and metabolism. In plant cells ROS accumulation have been involved in several processes such as: development, hypersensitive response, hormonal perception, gravitropism and stress response. (Mittler and Berkowitz, 2001). In guard cells from Vicia faba regulates the opening of stomata and more recently in root hair cells from Arabidopsis ROS levels generate and maintain an apical calcium gradient. This ROS accumulation plays a key role in root hair tip growth and suggested to play a similar role in pollen tubes (Pei et al., 2000; Foreman et al., 2003).
Herein we report a new molecular probe to depict the ROS dynamic during root hair cell and pollen tube apical growth. Hyper is a new generated GFP fused to the OxyR domain that result in a hydrogen peroxide specific probe. With this probe in root hair cells an apical gradient of H2O2 is observed and support the polar growth, furthermore we were able to visualize the ROS oscillation, which are couple to growth oscillations. In pollen tubes we found a different ROS distribution, however their oscillations were clearer and couple to growth oscillations. In both tip growing cells, the apical domain result the site with the more dynamic ROS changes.

8:30-8:50 Sunday, March 3, 2013

PUMPING AND PLUGGING IN *ARABIDOPSIS* POLLEN TUBES

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When looking at the evolutionary trajectory of pollen tubes (PTs), skewed toward fast growth in more recent clades, there is a clear correlation between speed and the appearance of a cell wall-related novelty in angiosperms – callose plugs. The deposition of these plugs is periodic, suggesting the presence of an internal "sizer" enabling the cell to estimate its dimensions. We are currently focusing on the mechanisms related to the formation of these septa-like structures. Stemming from the observed callose plug phenotypes in tobacco PTs expressing a constitutively active proton pump, we are testing a general hypothesis that ion diffusion, in particular protons (H+), could be the basis for sensing spatial scales inside the cell. We are in the process of characterizing KO mutants for members of the Arabidopsis plasma membrane H+-ATPase (AHA) family, with special focus on reproductive phenotypes. Despite the putative compensation exerted by remaining AHA isoforms, we find an array of defects in mutant plants with direct implications in development and fitness. In particular, PTs from aha1, aha7 and aha9 KO lines show reduced H+ pumping capability and poorer growth rates when compared to wild-type. Combining live imaging of the dynamics of pump localization, internal pH gradients and callose deposition, we hope to assess in the future how the regulation of proton pumping along the cell, the periodicity of plug formation and modulation of expansion rates might intertwine during the PT growth process.

8:50-9:10 Sunday, March 3, 2013

PROFILING OF TRANSLATOMES OF IN VIVO-GROWN POLLEN TUBES REVEALS GENES WITH ROLES IN MICROPYLAR GUIDANCE DURING POLLINATION

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Transcriptome profiling has been used to identify genes expressed in the elongating tubes of pollen, but these studies have primarily focused on pollen tubes cultured in vitro synthetic media. Little is known of the transcriptome of in vivo-grown pollen tubes, due to difficult collection of elongating-pollen tubes within the maternal gynoecium. By using a pollen-specific promoter to generate an epitope-tagged polysomal-RNA complex that can be affinity purified, we obtained the translatome of in vivo-grown pollen tubes from self-pollinated Arabidopsis gynoecia. Translatomes of pollen grains, in vivo and in vitro cultured pollen tubes were assayed by microarray analyses, revealing 519 transcripts enriched in in vivo-grown pollen tubes. Further analysis identified transcripts enriched in those pollen tubes and functional analyses of several available mutants (iv) of these in vivo-enriched genes exposed partial pollination/fertilization and seed formation defects in siliques (iv2, iv4, and iv6). Cytological observation confirmed the involvement of these genes in specialized processes during pollination including pollen tube burst in the embryo sac (IV2), micropylar guidance (IV6 and IV4), and repulsion of other pollen tubes (IV2). The isolation of transcripts engaged with polysomes in elongating pollen tubes has enabled the identification of pollen tube genes important in late stages of fertilization.

9:10-9:30 Sunday, March 3, 2013

THE GENETICS OF POSTPOLLINATION NONRANDOM MATING

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The pollen dusted across flower stigmas is often a mixture, whose proportions do not often match proportions within progeny. In other words, some pollen have greater mating success—a phenomenon called nonrandom mating. Our lab has been studying nonrandom mating in the model plant Arabidopsis thaliana (Arabidopsis). In previous work, we have: (1) developed a system to investigate nonrandom mating in Arabidopsis, (2) demonstrated that Arabidopsis accessions from geographically distinct regions mate nonrandomly, and (3) used quantitative trait locus (QTL) mapping to construct genetic maps of loci responsible for both female and male-mediated nonrandom mating in two populations of recombinant inbred lines. We are currently expanding our genetic understanding of this trait using whole genome association mapping, while identifying candidate genes for the loci we have in hand.

9:30-9:50 Sunday, March 3, 2013

POLLEN -- A MODEL FOR INSIGHTS INTO CA2+SIGNALLING AND MEMBRANE DYNAMICS

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Research in the Harper lab is focused on the role of Ca2+and membrane dynamics in pollen development. In Arabidopsis thaliana, there are at least 6 cyclic nucleotide-gated Ca2+-permeable ion channels (CNGCs). CNGC18 was previously shown to be essential for pollen tube tip growth. Recent studies now indicate that CNGC7 and 8 together are also essential, perhaps functioning with CNGC18 as part of a hetero-multimeric complex. In cngc7/8 knockouts, the pollen grains burst during in vitro germination. A GFP-tagged CNGC7 shows a preferential localization to the plasma membrane flanking the growth cone, similar to what was observed for CNGC18. Unlike CNGC's 18 and 7/8, CNGC16 is not essential under standard growth conditions. However, cngc16 knockouts are nearly male sterile when plants are grown under conditions of hot days and cold nights. These mutants have an attenuated transcriptional response for a subset of stress response genes, providing evidence linking a stress-triggered cyclic nucleotide signal to a CNGC and a downstream stress response pathway. Our working model is that pollen CNGCs have multiple functions, including the formation of Ca2+signals that coordinate growth processes as well as stress response pathways.

Poster Presentations

P-01

LESTIG1, A PARTNER FOR POLLEN RECEPTOR KINASE LEPRK2 IN THE STIGMA, BINDS TO PHOSPHOLIPIDS AND PROMOTES POLLEN TUBE GROWTH

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LeSTIG1 is a small secreted cysteine-rich protein specifically expressed in the stigma and style of tomato. Previously studies showed that LeSTIG1 interacts with the extracellular domain of LePRK2, a pollen-specific receptor kinase from tomato and can promote pollen tube growth *in vitro*. Here, we find that the conserved STIG1 domain in the C-terminus of LeSTIG1 is both necessary and sufficient for its activity. Adding recombinant LeSTIG1 or its C-terminus alone to *in vitro*-germinated pollen tubes stimulated endocytosis in the subapical region, and elevated the reactive oxygen species (ROS) in the cytosol. When ectopically expressed in pollen tubes, LeSTIG1 was found to associate with unknown endomembrane compartments. Besides, a high expression of LeSTIG1 would block normal vesicle trafficking and also central vacuole formation during pollen tube growth. These phenotypes strongly indicate a disorder of phospholipid metabolism in pollen tubes. In fact, detailed deletion analysis of the STIG1 domain revealed the existence of two phospholipid binding sites, which showed different binding affinities towards PI4P and PI3P. Interestingly, the short PI4P binding region is also sufficient for the LeSTIG1-LePRK2 interaction. And pretreatment with PI3P inhibitor wortmannin at low concentration inhibited the growth-promoting effect of LeSTIG1. Taken together, these results highlight novel phospholipid-binding motifs in LeSTIG1 that might underlie its growth-promoting effect, a process mediated by LePRK2 signaling.

P-02

LLFH1-MEDIATED INTERACTION BETWEEN ACTIN FRINGE AND EXOCYTIC VESICLES IS INVOLVED IN POLLEN TUBE TIP GROWTH

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Pollen tube tip growth is an extreme form of polarized cell growth, which requires polarized exocytosis based on dynamic actin cytoskeleton. However, the molecular basis for the connection between actin filaments and exocytic vesicles is unclear. In addition, contrasting spatial patterns for exocytosis at the tip have been reported. Here, we identified a *Lilium longiflorum* pollen-specific formin (LlFH1) and found it involved in pollen tube tip growth. LlFH1 localized at the apical vesicles and plasma membrane via its N-terminus. Overexpression of LlFH1 induced excessive actin cables in the tube tip region, and downregulation of LlFH1 eliminated actin fringe. FRAP analysis revealed that LlFH1 labeled exocytic vesicles exhibited a clear initial accumulation at the shoulder of the apex. Meanwhile, we found that the exocytic site coincided with the leading edge of the actin fringe, indicating the correlation between actin fringe and exocytic vesicles. Time-lapse analysis of pollen tubes simultaneously expressing LlFH1-GFP and Lifeact-mRFP suggested that nascent actin filaments followed the emergence of the apical vesicles, implying that LlFH1 could initiate actin polymerization from the apical vesicles. We therefore propose that the LlFH1 coordinates the interaction between the actin fringe and exocytic vesicle trafficking, which may provide a mechanism for the delivery of exocytic vesicles to the shoulder of the apex during the growth of lily pollen tubes.

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P-03

ENVIRONMENTAL SENSITIVITY OF POLLEN GERMINATION BEHAVIORS IN THE BRASSICACEAE

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Although some pollen tubes must breach solid sporopollenin walls to germinate, most pollen grain walls contain apertures, through which fluid enters and cells exit. Surprisingly, in some species with apertures (including A. thaliana), germinating pollen tubes have the ability to disregard the apertures and to escape directly through the wall instead. These pollen localize changes in the intine and overlying exine precisely to the pollen: stigma contact point, where the pectin-rich intine swells and the inner layer of the sporopollenin wall degrades to allow tube escape. Frequencies of these behaviors vary across species, and across ecotypes within the same species. We asked how responsive such germinating pollen grains were to environmental cues like pollen density, outcrossing/self-pollination, and various chemical factors, and found that we could experimentally alter pollen germination speed, strategy (escape route), and performance (%).

P-04

NASIPP IS A POLLEN PROTEIN SPECIFIC OF SELF-INCOMPATIBLE *NICOTIANA* SPECIES, WHICH INTERACTS WITH NASTEP, A STIGMATIC PROTEIN ESSENTIAL TO POLLEN REJECTION

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In Solanaceae, self-incompatibility (SI) is controlled by the female determinant (*SRNase*) and the male determinant (*SLF*). In addition, other non-*S*-locus linked genes are such as *HT-B*, *120K and NaStEP* are also essential for SI in *N. alata*.
We have been studying *NaStEP,* which is a stigma specific protein of SI *Nicotiana* species, which is also taken up by pollen tubes (PT). *NaStEP* codes a protein with protease inhibition activity. Suppression of NaStEP in transgenic plants disrupts pollen rejection in a *S*-specific manner. In transgenic NaStEP suppressed plants, the HT-B protein is degraded inside PT no matter if the cross is compatible or incompatible, a situation that does not occur in the wild type SI *N. alata.* All evidences above suggest that NaStEP functions in pollen rejection might be through its interaction with PT proteins. To test it, we searched for PT protein interactors by yeast-two-hybrid. We recovered a cDNA encoding a protein with homology to the C-terminal region of a phosphate transporter, which we called NaSIPP (***N. a****lata* **S**elf-**I**ncompatibility **P**ollen **P**rotein). The *NaSIPP* transcript is only detected in mature pollen of SI *Nicotiana* species. Western-blot analysis and NaSIPP-GFP fusion assays suggests that NaSIPP may be associated to the endomembrane system.
DGAPA IN210312, CONACYT 81968

P-05

CYCLIC NUCLEOTIDE-GATED CHANNELS ARE CRITICAL TO POLLEN FERTILITY AND STRESS TOLERANCE

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Cyclic nucleotide gated channels (CNGCs) have been implicated in diverse aspects of plant growth and development, including responses to biotic and abiotic stress, as well as pollen tube growth and fertility. The Arabidopsis genome encodes 20 CNGCs, 6 of which are highly expressed in pollen. Here we provide evidence that *CNGCs 7* and *8* are essential for pollen tube growth and fertilization, while *CNGC16* is critical to a pollen stress tolerance pathway.