PROGRAM AND ABSTRACTS

FRONTIERS OF SEXUAL PLANT REPRODUCTION III

OCTOBER 17 – 19, 2008

MARRIOTT UNIVERSITY PARK

TUCSON, ARIZONA, USA

MEETING ORGANISERS:

Dr. Ravi Palanivelu, University of Arizona, Tucson, USA

Dr. Dmitry Belostotsky, University of Missouri, Kansas City, USA

MEETING OVERVIEW

Friday, October 17, 2008

7:30 am – 8:30 am	Breakfast
8:30 am - 9 am	Opening Remarks by Dr. Vicki Chandler, Director, BIO5 Institute, University of Arizona.
9 am - 12:15pm	**Session I: Pollen Development and Tube Growth (Marriott Sabino/Pima rooms)**
12 noon - 1 pm	Lunch break
1 pm - 2 pm	Poster session (*please stand by your poster if it is even numbered*)
2 pm – 5 pm	**Session II: Female Gametophyte Development and Function (Marriott Sabino/Pima rooms)**
5 pm - 6 pm	Refreshments and poster session (*please stand by your poster if it is odd numbered)*
6 pm – 7 pm	Dinner break
7 pm – 10 pm	**Session III: Endosperm and Imprinting (Marriott Sabino/Pima rooms)**

Saturday, October 18 2008

7:30 am – 9 am	Breakfast
9 pm – 12 noon	**Session IV: Pollen Pistil Interactions (Marriott Sabino/Pima rooms)**
12 noon – 1 pm	Lunch break
1 – 2 pm	Transport to Sonoran Desert Museum
2 pm - 4 pm	Free time - Enjoy the desert museum!
4 pm – 7 pm	**Session V: Evolution (Desert Museum Warden Oasis Theater)**
7 - 10 pm	Banquet dinner in desert museum

Sunday, October 19, 2008

7:30 am – 8:30 am	Breakfast
8:30 am - 11:30 am	**Session VI: Novel Systems and Emerging Technologies (Marriott Sabino/Pima rooms)**
11:30am - 1 pm	Lunch break
	END OF MEETING

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ORAL PRESENTATIONS OVERVIEW

Friday, 17 October 2008, 9-12 pm

Pollen Development and Tube Growth Session (Chair: Zhenbiao Yang)

9:00 AM **Dazhong Zhao, U of Wisconsin-Milwaukee**
REGULATIONN OF CELL DIFFERENTATION BY THE EMS1 MEDIATED SIGNALING IN *ARABIDOPSIS*

9:10 AM **ASN Reddy, Colorado State University**
ISOLATION AND FUNCTIONAL ANALYSIS OF A POLLEN-SPECIFIC CALMODULIN-BINDING PROTEIN

9:25 AM **John Fowler, Oregon State University**
DIVERSITY AND CONSERVATION IN MAIZE POLLEN: PHENOTYPES AND TRANSCRIPTS

9:40 AM **Anja Geitmann, U of Montreal, Canada**
THE DYNAMICS OF POLLEN TUBE GROWTH - A HARMONIC OSCILLATOR MODEL BASED ON CELLULAR MECHANICS

9:55 AM **Alice Cheung, U of Mass, Amherst**
ACTIN ASSEMBLY AND POLLEN TUBE GROWTH.

10:10 AM COFFEE BREAK

10:40 AM Peter Hepler, U of Massachusetts, Amherst
EXOCYTOSIS LEADS AND ENDOCYTOSIS FOLLOWS THE INCREASE IN GROWTH RATE IN OSCILLATING POLLEN TUBES

10:55 AM Guang-Yuh Jauh, IPMB, Taiwan
LLLIM1, AN ACTIN BINDING AND BUNDLING PROTEIN, REGULATES THE ACTIN DYNAMICS THROUGH CALCIUM AND PROTON SIMULTANEOUSLY IN ELONGATING POLLEN TUBES

11:05AM Heven Sze, University of Maryland
CATION/PROTON ANTIPORTERS ARE ESSENTIAL FOR POLLEN TUBE GUIDANCE

11:20 AM Yan Zhang, PGEC/UC Berkeley
DYNAMIC MEMBRANE LOCALIZATION OF ARABIDOPSIS POLLEN RECEPTOR KINASE 2A REGULATES POLARIZED GROWTH OF POLLEN TUBES

11:35 AM Zhenbiao Yang, UC Riverside
THE REGULATION AND FUNCTION OF THE DYNAMICS OF TIP-LOCALIZED ROP GTPASE ACTIVITY IN THE CONTROL OF POLARIZED POLLEN TUBE GROWTH.

Friday, 17 October 2008, 2-5 pm

Female Gametophyte Session (Chair: Gary Drews)

2:00 PM Chuck Gasser, UC Davis
PATTERNING PROCESSES IN OVULE DEVELOPMENT

2:15 PM Kay Schnietz, Tech U of Munich, Germany
THE ARABIDOPSIS ATYPICAL RECEPTOR-LIKE KINASE STRUBBELIG MEDIATES INTER-CELL-LAYER SIGNALING DURING FLORAL DEVELOPMENT

2:30 PM Lucia Colombo, U of Milan, Italy
TARGET GENES OF THE OVULE IDENTITY COMPLEX IN *ARABIDOPSIS*

2:45 PM Matt Evans, Stanford University
REGULATION OF EMBRYO SAC DEVELOPMENT BY *INDETERMINATE GAMETOPHYTE1*

3:00 PM Venkatesan Sundaresan, UC Davis
PATTERNING OF THE FEMALE GAMETOPHYTE IN ARABIDOPSIS

3:15 PM Dongfang Wang, U of Arizona
REGULATORY NETWORKS CONTROLLING FEMALE GAMETOPHYTE DEVELOPMENT

3:25 PM COFFEE BREAK

3:50 PM Anna Koltunow, CSIRO, Australia
IDENTIFICATION OF THE LOCUS CONTROLLING THE AVOIDANCE OF MEIOSIS DURING GAMETOPHYTE FORMATION IN APOMICTIC *HIERACIUM*

4:05 PM Rita Groß-Hardt, U of Tubingen, Germany
WHEN FEMALES GO EGG-CESSIVE: REGULATION OF GAMETIC CELL FATE IN ARABIDOPSIS

4:20 PM Stefanie Sprunck, U of Regensburg, Germany
EXPRESSION PROFILING OF ISOLATED EMBRYO SAC CELLS TO IDENTIFY GENES WITH KEY ROLES IN EMBRYO SAC FUNCTION AND DOUBLE FERTILIZATION

4:35 PM Arp Schnittger, IBMP, CNRS, France
(EMBO Young Investigator Lecture)

DISSECTING REGULATORY CIRCUITS DURING THE REPRODUCTIVE LIFE PHASE OF FLOWERING PLANTS

4:50 PM Yuki Hamamura, Nagoya University, Japan
REAL TIME IMAGING OF DOUBLE FERTILIZATION IN *ARABIDOPSIS THALIANA*

Friday, 17 October 2008, 7-10 pm

Endosperm and Imprinting Session (Chair: Ramin Yadegari)

7:00 PM Gary Drews, U of Utah
ROLE OF *AGL62* IN ENDOSPERM DEVELOPMENT

7:15 PM Hugh Dickinson, U of Oxford, UK
EPIGENETIC SUBFUNCTIONALISATION – CLUES TO THE ORIGIN OF IMPRINTING?

7:30 PM Paolo Sabelli, U of Arizona
MAIZE ENDOSPERM DEVELOPMENT: ROLE OF THE RETINOBLASTOMA PATHWAY

7:45 PM Frédéric Berger, TLSL, Singapore
RETINOBLASTOMA AND ITS BINDING PARTNER MSI1 CONTROL IMPRINTING IN *ARABIDOPSIS*

8:00 PM Mary Gehring, FHCRC, Seattle
GENOMIC ANALYSIS OF METHYLATION PATTERNS IN ARABIDOPSIS EMBRYO AND ENDOSPERM

8:10 PM COFFEE BREAK

8:35 PM Ueli Grossniklaus, U of Zurich, Switzerland
EPIGENETIC REPROGRAMMING OF THE PATERNAL GENOME IN *ARABIDOPSIS*

8:50 PM Paul Grini, U of Oslo, Norway
GENETIC DISSECTION OF PARENT OF ORIGIN EFFECTS IN SEED DEVELOPMENT

9:05 PM Matthew Bauer, UC Berkeley
DEMETER REGULATES PLANT GENE IMPRINTING BY ACTIVE DNA DEMETHYLATION

9:20 PM Claudia Köhler, ETH, Zurich, Switzerland
(EMBO Young Investigator Lecture)

IMPRINTING CONTROL MECHANISMS IN ARABIDOPSIS

9:35 PM Tetsu Kinoshita, NIST, Ikoma, Japan
CONTROL OF GENOMIC IMPRINTING BY *ALARM CLOCK1* GENE IN *ARABIDOPSIS*

<u>Saturday, 18 October 2008, 9-12 pm</u>

Compatible and Incompatible Pollinations Session (Chair: Alice Cheung)

9:00 AM Betty Lord, UC Riverside
SCA (LILY STIGMA/STYLE CYSTEINE-RICH ADHESIN) AND A SCA-LIKE LTP IN ARABIDOPSIS FUNCTION IN PLANT REPRODUCTION

9:15 AM Mark Johnson, Brown University
HAP2 IS A SPERM-EXPRESSED COMPONENT OF A DEEPLY CONSERVED FERTILIZATION MECHANISM

9:30 AM Patricia Bedinger, Colorado State University
UNILATERAL INCONGRUITY IN TOMATO: ROLE OF SELF-INCOMPATIBILITY FACTORS

9:45 AM Tetsuya Higashiyama, Nagoya University, Japan
POLLEN TUBE ATTRACTANTS DERIVED FROM THE SYNERGID CELL

10:00 AM Rob Swanson, Valparaiso University
GENETIC MAPPING OF FEMALE NONRANDOM MATING LOCI IN *ARABIDOPSIS THALIANA*

10:10 AM COFFEE BREAK

10:40 AM Teh-Hui Kao, Penn State University
THE ROLE OF THE *S-LOCUS-F-BOX* GENE IN S-RNASE-BASED SELF-INCOMPATIBILITY

10:55 AM Hidenori Sassa, Yokohama U, Japan
CHARACTERIZATION OF MULTIPLE F-BOX GENES LINKED TO THE *S* LOCUS OF APPLE AND JAPANESE PEAR

11:05 AM Daiki Matsumoto, Kyoto University
PRUNUS MAY HAVE A DISTINCT RECOGNITION MECHANISM IN ITS S-RNASE BASED GAMETOPHYTIC SELF-INCOMPATIBILITY SYSTEM

11:15 AM Jerry Kermicle, U of Wisconsin
GENES GOVERNING CROSS INCOMPATIBILITY BETWEEN MAIZE AND TEOSINTE

11:25 AM Bruce McClure, U of Missouri
ENDOCYTOSIS IN S-RNASE-BASED SELF-INCOMPATIBILITY

11:40 AM Noni Franklin-Tong, U of Birmingham, UK
SELF-INCOMPATIBILITY IN *PAPAVER*: IDENTIFICATION OF THE

POLLEN SELF-INCOMPATIBILITY DETERMINANT

Sat, 18 October 2008, 4:15-7 pm (Sonoran Desert Muesem)

Evolution of Reproductive Development (Chair: Ned Friedman)

4:15 PM Spencer Barrett, U of Toronto
SEX-RATIO VARIATION IN DIOECIOUS PLANT POPULATIONS

4:30 PM Kathleen Kay, UC Santa Cruz
POLLEN–PISTIL INTERACTIONS AS REPRODUCTIVE ISOLATING MECHANISMS: EVIDENCE FROM SPECIATION STUDIES OF THE SPIRAL GINGERS

4:45 PM Jeffery Conner, Michigan State University
TESTING FOR STABILIZING AND CORRELATIONAL SELECTION ON CONSERVED FLORAL TRAITS USING EXPANDED PHENOTYPIC VARIATION

5:00 PM Pamela Diggle, U of Colorado
PHENOTYPIC PLASTICITY, GENETIC ASSIMILATION, AND THE EVOLUTIONARY DIVERSIFICATION OF SEXUAL SYSTEMS IN *SOLANUM*

5:15 PM COFFEE BREAK

5:40 PM Chiara Airoldi, U of Leeds, UK
EVOLUTION OF C-FUNCTION GENES. SMALL PROTEIN CHANGES THAT LEAD TO BIG FUNCTIONAL DIFFERENCES

5:50 PM Joe Williams, U of Tennessee
UNDERSTANDING EARLY PATTERNS OF POLLEN TUBE GROWTH IN RELATION TO CARPEL CLOSURE AND FLORAL DIVERSIFICATION AMONG ANGIOSPERMS

6:05 PM Ned Friedman, U Colorado
OVERCOMING A CENTURY OF DOGMA ABOUT THE REPRODUCTIVE FEATURES OF THE FIRST ANGIOSPERMS: INSIGHTS FROM *HYDATELLA* AND OTHER WATER LILIES

6:15 PM Elvira horandl, U of Vienna, Austria
EVOLUTIONARY IMPLICATIONS OF SELF-COMPATIBILITY AND MENTOR EFFECTS IN THE APOMICTIC *RANUNCULUS AURICOMUS* POLYPLOID COMPLEX (RANUNCULACEAE)

6:30 PM Josh Kohn, UC San Diego
EVOLUTIONARY DYNAMICS OF THE PAPAVERACEAE S-LOCUS

6:45 PM Simon Hiscock, U of Bristol, UK
REPRODUCTION AND HYBRID SPECIATION IN *SENECIO*: THE OXFORD RAGWORT STORY

Sunday, 19 October 2008, 8:30-11:30 am

Novel Systems and Emerging Technologies (Chair: José A. Feijó)

8:30 AM Jeff Harper, U of Nevada, Reno
POLLEN TIP GROWTH AND FERTILIZATION: CALCIUM PUMPS, CHANNELS, AND KINASES

8:45 AM Aurelien Boisson –Dernier, UC San Diego
THE PEROXIN LOSS-OF-FUNCTION MUTATION ABSTINENCE BY MUTUAL CONSENT DISRUPTS MALE-FEMALE GAMETOPHYTE RECOGNITION

9:00 AM Glenn Hicks, UC Riverside
A POLLEN-BASED SCREEN FOR BIOACTIVE CHEMICALS TO DISSECT ENDOMEMBRANE TRAFFICKING

9:15 AM Shannon Stewman, U of Chicago
IN VITRO AND IN SILICO STUDIES OF THE DYNAMICS OF POLLEN TUBE–OVULE INTERACTIONS

9:25 AM Daniel Philippe Matton, U Montreal, Canada
OVULE DEVELOPMENT and POLLEN TUBE GUIDANCE ARE AFFECTED in the *SOLANUM CHACOENSE* MAPKKK *ScFRK1* MUTANT

9:40 AM José A. Feijó, U of Lisbon, Portugal
OF MALE GAMETOPHYTES AND THEIR URGES FOR MODELS

9:55 AM COFFEE BREAK

10:15 AM Scott Russell, U of Oklahoma
EXPRESSION PROFILE OF THE RICE MALE GAMETOPHYTE

10:30 AM Steve Wolniak, U of Maryland
CELL FATE DETERMINATION AND BASAL BODY FORMATION IN *MARSILEA* SPERMATIDS

10:45 AM Clay Carter, U of Minnesota
FUNCTIONAL ANALYSIS OF THE *ARABIDOPSIS THALIANA* NECTARY TRANSCRIPTOME

10:55 AM Jörg Becker, U of Lisbon, Portugal
COMPARATIVE TRANSCRIPTOMICS OF ARABIDOPSIS THALIANA SPERM CELLS

11:10 AM Keith Slotkin, Cold Spring Harbor Laboratory
EPIGENETIC TRANSPOSABLE ELEMENT REACTIVATION IN *WILD-TYPE* POLLEN

Oral Presentations - Session I

Pollen Development and Tube Growth Session

Friday, October 17, 2008, 9am – 12noon

Marriott Sabion/Pima rooms

Chair: Dr. Zhenbiao Yang

POSTER TALK **P1-01**

REGULATIONN OF CELL DIFFERENTATION BY THE EMS1 MEDIATED SIGNALING IN *ARABIDOPSIS*

Dazhong Zhao, *Gengxiang Jia, Xiaodong Liu, Jian Huang, Yao Wang and Heather A. Owen*
Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI 53211, USA

Sexual reproduction requires specification of cells with distinct fates in plants and animals. So far, little is known about the molecular mechanisms underlying cell fate determination during sexual reproduction in plants. In flowering plants, an anther contains highly specialized reproductive and somatic cells. Recently anther has emerged as a prime model system for the study of cell fate determination and receptor-linked signaling. Our previous study showed that the *Arabidopsis ems1* (also known as *exs*) mutant anthers produce excess reproductive microsporocytes at the expense of somatic tapetal cells, indicating that there is a trade-off between somatic and reproductive cells. The *tpd1* mutant has a similar phenotype to that of *ems1*. The *EMS1* gene encodes a leucine-rich repeat receptor-like kinase (LRR-RLK), while *TPD1* encodes a small, putatively-secreted protein. Here we demonstrate that ectopic expression of *TPD1* causes abnormal differentiation of somatic and reproductive cells in anthers. In addition, the ectopic TPD1 activity requires functional EMS1. The secretion of TPD1 is also required for its normal function. Our further biochemical evidence suggests that TPD1 may serve as a ligand for the EMS1 receptor kinase to signal cell fate determination during anther cell differentiation. Both monocots and eudicots possibly use the same mechanism to control anther development, since an apparent ortholog to EMS1 exists in rice. Our results also revealed that a rice *TPD1-like* gene may play a similar role to the TPD1. A model for signaling anther cell fate determination will be discussed as well.

GENETIC CONTROL OF MALE GERMLINE DEVELOPMENT IN FLOWERING PLANTS

David Twell, Lynette Brownfield, Said Hafidh, Michael Borg, Anna Sidorova
Department of Biology, University of Leicester, Leicester LE1 7RH United Kingdom

Recent advances in our understanding of landmark events in pollen development are largely based on progress achieved using genetic and transcriptomic approaches. These new datasets and molecular tools complement progress in genetic analyses that is now beginning to uncover some of the key molecular mechanisms that pattern pollen development.

A central outstanding question in pollen development is how the vegetative cell exits the cell cycle and differentiates to form the pollen tube, while the generative cell divides to form functional twin sperm cells? Genetic analysis has led to the identification of a suite of *Arabidopsis* mutants that specifically block cell division in the male germline and that allow the formulation of molecular models of male germline development. Recent progress involves the identification of an SCF complex in male germ cells that targets CDK inhibitor proteins for proteasome-dependent degradation. Thus, SCF^{FBL17} acts as a male germline proliferation factor, but is not involved in cell differentiation. The R2R3 Myb regulatory protein DUO1, coordinates these processes by activating germline-specific gene expression and Cyclin B1;1 expression to commit differentiating germ cells to mitosis. DUO1 is therefore a key male germline fate determinant that regulates twin-sperm cell production and the expression of germline specific genes, including AtGCS1/HAP2 that is essential for gamete fusion and double fertilization.

Further progress in understanding the essential switch between germ and non germ cell lineages will continue to benefit from the integration of genetic and genomic technologies, offering new opportunities to build complex functional models of male gametophyte development.

ISOLATION AND FUNCTIONAL ANALYSIS OF A POLLEN-SPECIFIC CALMODULIN-BINDING PROTEIN

Maxim Golovkin, Sung-Bong Shin, Farida Safadi and A.S.N. Reddy
Department of Biology and Program in Molecular Plant Biology, Colorado State University, Fort Collins, CO, USA

Calcium and calmodulin have been implicated in pollen germination and tube growth. However, the mechanisms by which they regulate pollen germination and tube growth are largely unknown. To understand calcium/calmodulin-mediated signaling pathways in pollen function, we performed an in vitro protein-protein interaction screen using a labeled calmodulin and isolated a novel pollen-specific calmodulin-binding protein from maize. In Arabidopsis, three proteins related to this maize protein were found and all three bound calmodulin. However, only one (*NPG1*) of these was expressed specifically in pollen whereas the other two were expressed in pollen as well as in other tissues. Transgenic plants containing GFP fused to the *NPG1* promoter showed reporter gene expression only in mature and germinating pollen. Localization of GFP-NPG1 during different stages of pollen germination revealed uniform cytosolic distribution of GFP along the growing pollen tube. To analyze the function of *NPG1* we isolated a mutant in which *NPG1* was disrupted by a T-DNA insertion. Segregation and molecular analyses with the *NPG1* knockout mutant, and a genetic cross with a male sterile mutant indicated that the mutant allele is not transmitted through the male gametophyte. Analysis of pollen development in the knockout mutant by light microscopy showed normal pollen development. Pollen carrying *npg1* allele in the *quartet* background has confirmed that *NPG1* is dispensable for pollen development. However, germination studies with pollen from the mutant in the *quartet* background have shown that pollen carrying a mutant allele do not germinate. These studies indicate that *NPG1* is not necessary for microsporogenesis but is essential for pollen germination. Our current studies are aimed at understanding the mechanism(s) by which NPG1 regulates pollen germination.

DIVERSITY AND CONSERVATION IN MAIZE POLLEN: PHENOTYPES AND TRANSCRIPTS

Fowler, John E.[1]*, Vejlupkova, Zuzana*[1]*, Cooper, Laurel D.*[1]*, Qu, Annie*[2]*, and Watrud, Lidia S.*[3]*.* [1]*Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR USA;* [2]*Dept. of Statistics, Oregon State University, Corvallis, OR USA;* [3]*US EPA NHEERL Western Ecology Division, Corvallis, OR USA*

In addition to its crucial role in seed production, pollen serves as a vector for gene flow between plant populations. Recently, pollen was identified as a mechanism for introduction of transgenes into non-transgenic populations. To investigate the genetic basis for pollen fitness, which likely influences pollen-mediated gene flow, we are documenting phenotypic and transcriptomic variation in pollen from a set of diverse maize lines. At the phenotypic level, we have measured pollen competitive ability (PCA) in 19 lines using a pollen-mixing method employed previously by Sari-Gorla, Ottaviano, Pé and co-workers. We found a range of significant differences in PCA among maize inbreds. Furthermore, pollen from three out of four hybrids tested ranked as the most competitive, consistent with earlier work indicating correlation between sporophytic vigor and pollen fitness (reviewed in Ottaviano & Mulcahy 1989). At the transcriptomic level, microarrays were used to assess gene expression in seedling, mature pollen and germinated pollen samples from two different inbred lines (B73 and W22). As expected, a large number of probes (~10,000) showed significant differences between pollen and seedling; a smaller number of probes (~1000) showed significant differences between the two inbred lines in both pollen and seedling, although these were largely non-overlapping sets. Quantitative RT-PCR experiments are being used to validate these results. Interestingly, at a stringent statistical cutoff, the microarray data found no significant differences between mature and germinated pollen. Finally, we have used our data to help identify a set of conserved pollen-enriched genes in maize, rice and Arabidopsis.

THE DYNAMICS OF POLLEN TUBE GROWTH - A HARMONIC OSCILLATOR MODEL BASED ON CELLULAR MECHANICS

Anja Geitmann[1], Rabah Zerzour[1], Jens Kroeger[2]
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[2]Department of Physics, McGill University, 3600 rue University, Montréal, Québec, H3A 2T8, Canada

Pollen tubes grow extremely fast and often in oscillatory manner. Numerous aspects of this phenomenon have been investigated in the past and various models have been proposed to explain its mechanism. A frequently cited requirement to explain oscillatory behavior is the presence of a pacemaker that dictates the rhythm and provides the initial trigger. The nature of the pacemaker has not been identified, however.
All biological systems must obey the laws of physics. Therefore, we propose a model that emphasizes the mechanical aspect of the growth process. It is not mutually exclusive with most aspects of previously proposed models but it distinguishes itself by the fact that it does not require the existence of a pacemaker to trigger or sustain oscillations. This model is based on the mechanical relationship between turgor pressure and tensile resistance in the apical cell wall. The equilibrium between these two forces can be considered to be analogous to that of a mechanical harmonic oscillator - such as a weight attached to a spring. We show experimentally that just as in the spring model it only takes a single mechanical disturbance to displace the components of this oscillator from their equilibrium position and cause the system to oscillate.

To assess the dynamic changes in the mechanical cell wall properties over time, we used micro-indentation to measure cellular stiffness at the growing apex. We observed that prior to a rapid growth phase the cellular stiffness is reduced at the apex and it increases during a pulse - consistent with a process of strain hardening. These findings confirm the important role of the mechanical properties of the cell wall for cellular growth processes.

ACTIN ASSEMBLY AND POLLEN TUBE GROWTH

Alice Y. Cheung. Department of Biochemistry and Molecular Biology, University of Massachusetts, Amherst, MA 01003.

We are interested to understand how cellular and molecular factors that underlie the polarized pollen tube growth process interact to achieve the intracellular design that drives apical growth. An aspect of our recent efforts is to examine the role of actin assembly on the actin cytoskeleton, vesicular trafficking and the tip growth process. We have shown several years ago that a subset of the formin family of actin nucleating protein stimulates actin assembly from the pollen tube cell membrane. As transmembrane proteins with an extracellular domain, these formins potentially provide direct linkages to mediate extracellular cues to the actin cytoskeleton to regulate the tip growth process. Our discussion here will focus on an apical membrane-localized formin and its role in orchestrating the actin and vesicular organization in the apical and subapcial cytoplasm and the importance of regulated actin assembly to the tip growth process. Our results suggest formins and other actin modifying activity together maintain the needed level of nascent actin assembly and modification to attain the actin cytoskeleton organization that is normally observed in elongating pollen tubes.

EXOCYTOSIS LEADS AND ENDOCYTOSIS FOLLOWS THE INCREASE IN GROWTH RATE IN OSCILLATING POLLEN TUBES

Sylvester T. McKenna[2], Joseph G. Kunkel[1], Maurice Bosch[4], Caleb Rounds[1], Luis Vidali[1], Lawrence J. Winship[3], and Peter K. Hepler[1]
1. Department of Biology, University of Massachusetts, Amherst, MA 01003
2. Department of Biology, Long Island University, Brooklyn, NY 11201
3. Department of Sciences, Hampshire College, Amherst, MA 01002
4. Institute of Biological, Environmental & Rural Sciences (IBERS), Aberystwyth SY23 3EB, UK.

Exocytosis and endocytosis have been examined during oscillatory growth in pollen tubes of lily and tobacco. For exocytosis we have employed three markers: 1) changes in cell wall thickness using Nomarski differential interference contrast (DIC) optics; 2) changes in apical cell wall fluorescence in cells stained with propidium iodide (PI); and 3) changes in apical fluorescence in cells expressing pectin methyl esterase linked to GFP (NtPPME1-GFP). For endocytosis we have used the styryl dye, FM4-64. Our results show that both processes oscillate during pollen tube growth, with exocytosis leading and endocytosis following the increase in growth rate. Phase analysis reveals that exocytosis leads growth by $-120\text{-}130^0$, while endocytosis follows growth exhibiting a lag of $+10\text{-}15^0$ at the apical membrane surface, which becomes progressively more delayed in regions removed from the apex. Surprisingly, exocytosis is not closely correlated temporally with changes in intracellular calcium, whereas endocytosis is. Not only does exocytosis lead growth, but the amount of material secreted strongly predicts the rate and extent of the subsequent growth surge. Exocytosis, as an anticipatory event, emerges as a prime candidate for the initiation of oscillatory pollen tube growth.

Supported by the NSF, Grant No. MCB-0516852.

POSTER TALK **P1-02**

LLLIM1, AN ACTIN BINDING AND BUNDLING PROTEIN, REGULATES THE ACTIN DYNAMICS THROUGH CALCIUM AND PROTON SIMULTANEOUSLY IN ELONGATING POLLEN TUBES

Huei-Jing Wang1, 2, Ai-Ru Wan1, and Guang-Yuh Jauh1
1Institute of Plant and Microbial Biology, Academia Sinica, Nankang, Taipei, Taiwan.
2Institute of Life Science, National Defense Medical Center, Taipei, Taiwan.

Actin microfilaments are crucial for polar-cell tip growth, and their configurations and dynamics are regulated by the actions of various actin-binding proteins (ABPs). A home-made lily (*Lilium longiflorum*) pollen tube cDNA microarray was used to screen pollen tube transcripts whose expression levels were upregulated after pollen germination and/or style-secreted exudate treatment. Two cDNAs encoding partial fragments of the LIM domain-containing protein shared high similarity to Arabidopsis AtWLIM1. We explored the function of this lily pollen-enriched LIM-domain-containing protein, LlLIM1, in regulating the actin dynamics in elongating pollen tube. Cytological and biochemical assays verified LlLIM1 functioning as an ABP, promoting F-actin bundle assembly and protecting F-actin against LatB-mediated depolymerization. Overexpressed LlLIM1 significantly disturbed pollen tube growth and morphology, with multiple tubes protruding from one pollen grain and co-aggregation of FM4-64-labeled vesicles and Golgim apparatuses at the subapex of the tube tip. Moderate expression of LlLIM1 induced an oscillatory formation of asterisk-shaped F-actin aggregates that oscillated with growth period but in different phases at the subapical region. These results suggest that the formation of LlLIM1-mediated over-stabilized F-actin bundles interfered with endomembrane trafficking to result in growth retardation. Co-sedimentation assays revealed that the binding affinity of LlLIM1 to F-actin was simultaneously regulated both by pH and Ca^{2+}: LlLIM1 showed a preference for F-actin binding under low pH and low Ca^{2+} concentration. The potential functions of LlLIM1 as an ABP sensitive to pH and calcium in integrating endomembrane trafficking, oscillatory pH and calciumcircumstances to regulate tip-focused pollen tube growth are discussed.

CATION/PROTON ANTIPORTERS ARE ESSENTIAL FOR POLLEN TUBE GUIDANCE

Yongxian Lu, Xiyan Li,. Salil Chanroj, Senthil Padmanaban, Kevin W Bock, Heven Sze
Department of Cell Biology & Molecular Genetics, Biosciences Res.Bldg., University of Maryland, College Park, MD 20742. (hsze@umd.edu)

The dynamics of Ca^{2+}, K^+ and H^+ fluxes observed during pollen tube growth *in vitro* illustrate the critical roles of ion transporters, though their specific roles in male fertility are largely unknown. Transporter genes that are preferentially-expressed in pollen include Ca^{2+} pump ACA9, K^+ channel SPIK, CNGC18, and cation/proton exchangers CHX. Others demonstrated that pollen from mutant *spik*, *cngc18* or *aca9* showed reduced tube growth, though we found mutant of *chx-a* or *chx-b* was normal. Reciprocal crosses of *Aabb* with wild-type indicated a failure in transmission by *ab* pollen. To visualize mutant pollen *in vivo*, *aaBB* and *Aabb* plants were obtained, where the T-DNA insertion in '*a*' harbored a Lat52 promoter-GUS. Pollen from *aaBB* discharged GUS into ovules, however pollen from *Aabb* plants showed blue tubes mainly in the transmitting tract. Introducing a wild-type B_T transgene into Aabb plants recovered $aabbB_T$ plants in the T2 generation. Thus CHX-A or CHX-B does not affect tube elongation but is essential for guidance. CHX-B protein fused to GFP was localized to PVC/vacuole in leaf protoplast. The role of A or B is unclear, though a related gene expressed in yeast conferred tolerance to basic pH when K^+ is low. CHX20 affects guard cell movement, and its association with endomembranes suggests a role of pH and K^+ in vesicle trafficking and membrane dynamics during osmoregulation. We propose a working model where CHX-A and CHX-B functions are linked to signaling events that shift the axis of polarity thus turning pollen tip growth towards the ovule. (Supported by NSF & DOE)

DYNAMIC MEMBRANE LOCALIZATION OF ARABIDOPSIS POLLEN RECEPTOR KINASE 2A REGULATES POLARIZED GROWTH OF POLLEN TUBES

Yan Zhang[1,], Junmin He[1], Emily Fox[1,2,3], Anja Kombrink[1,2,4], Colleen Lau[1,2,5] and Sheila McCormick[1,]*
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The plant-specific GTPase ROPs are central to the polarized growth of pollen tubes, through which immotile sperm are delivered to the female gametophyte for double fertilization. Its crucial role in polarity control indicates the existence of a dynamic activation, on which little is known. We demonstrated previously that an Arabidopsis PRK2a acts as a scaffolding protein to recruit the activator of ROPs, ROPGEFs, to the plasma membrane. Here we show that the membrane localization of PRK2a was dynamically regulated by endocytosis. PRK2a co-localized with a subpopulation of ARA6/ARA7-positive endosomes when overexpressed and PRK2a overexpression disturbed the structural integrity of these endosomes. The internalization of PRK2a did not involve clathrin-coated pits but require adaptor protein 2 complex and actin cytoskeleton system. We then tested the possibility that PRK2a is internalized via lipid-raft mediated endocytic route. Using an established lipid raft marker, we first showed that lipid rafts were enriched at the apical plasma membrane of growing pollen tubes, and then tested the effect of co-expressing caveolin1 on PRK2a internalization. In metazoan cells, the expression of a non-phosphorylatable mutant cav1-Y14F inhibits lipid raft-mediated internalization of diverse receptors. Co-expressed cav1-Y14F but not cav1 increased the plasma membrane occurrence of PRK2a, causing a more severe ballooning tip phenotype than when only PRK2a was expressed. We propose that lipid raft-mediated internalization of PRK2a is a key mechanism by which ROP activation by RopGEFs is dynamically regulated during pollen tube growth.

THE REGULATION AND FUNCTION OF THE DYNAMICS OF TIP-LOCALIZED ROP GTPASE ACTIVITY IN THE CONTROL OF POLARIZED POLLEN TUBE GROWTH.

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Polarized pollen tube growth is controlled by the ROP1 GTPase signaling dynamically to the apical dome of pollen tubes. The localized ROP1 signaling is essential for pollen tube elongation, while the spatiotemporal dynamics of ROP1 activity modulates the polarity of pollen tube elongation. We are interested in understanding the mechanisms by which the localized ROP1 promotes pollen tube elongation and as well as those by which the dynamics of the ROP1 activity is achieved. Our recent results suggest that the ROP1-dependent dynamics of apical actin microfilament is important for coordinating vesicular trafficking required for tip growth such as polarized accumulation and exocytosis of vesicles. Our data support the hypothesis that the spatiotemporal dynamics of ROP1 activity at the tip is primarily controlled by a balance between the lateral amplification of localized ROP1 activity and the global inhibition of this activity. This balancing act not only explains the polarized tip growth is controlled but also the oscillatory growth of pollen tubes. Recent data supporting this hypothesis will be discussed.

Oral Presentations- Session 2

Female Gametophyte Session

Friday, October 17, 2008 2pm – 5pm

Marriott Sabino/Pima rooms

Chair: Dr. Gary Drews

PATTERNING PROCESSES IN OVULE DEVELOPMENT

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Genetic and molecular methods are being utilized to elucidate the mechanisms underlying ovule morphogenesis in Arabidopsis. We have focused in particular on initiation and laminar growth of integuments, the lateral structures developing on the flanks of an ovule primordium. Several genes regulating this process encode proteins belonging to families that are associated with ab/adaxial polarity determination in the primary lateral organs of angiosperms. *INNER NO OUTER* (*INO*) plays an essential role in initiation and asymmetric growth of the outer integument and encodes an ovule-specific member of the YABBY family of transcription factors. *ABERRANT TESTA SHAPE* (*ATS*) encodes a member of the KANADI protein family and is required for the separation of the inner and outer integuments and for growth of the inner integument. Other KANADI proteins are required for outer integument development. In primary lateral organs YABBY and KANADI proteins act in opposition to adaxial determinants such as the Class III HD-Zip proteins (HD-ZipIII). We find that loss and gain of function mutants in HDZipIII genes also have effects on ovule development, but that there are apparent differences in the interactions between these adaxial factors and the abaxial factors in ovules in comparison to primary lateral organs. BASIC PENTACYSTEINE (BPC) proteins were found to bind to specific motifs in a regulatory element of the *INO* promoter. Lines mutant for multiple BPC genes exhibit pleiotropic phenotypes including aberrant ovules, implicating these proteins as additional factors important for ovule development. Supported by grants from U. S. National Science Foundation (IBN-0419531, MCB-0517104).

THE ARABIDOPSIS ATYPICAL RECEPTOR-LIKE KINASE STRUBBELIG MEDIATES INTER-CELL-LAYER SIGNALING DURING FLORAL DEVELOPMENT

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In plants important questions relate to the mechanisms that control signaling between the histogenic cell layers of apical meristems and developing organs. The Arabidopsis putative atypical leucine-rich repeat receptor-like kinase STRUBBELIG (SUB) regulates amongst others integument development, floral morphogenesis and root hair patterning. We provide evidence that *SUB* is posttranscriptionally regulated, acts in a non-cell-autonomous fashion, functions in a radial inside-out signaling process, and mediates cell morphogenesis and cell fate across clonally distinct cell layers in developing ovules as well as floral and root meristems. In addition we used forward genetics to identify genes with a function in *SUB*-dependent signaling. Plants with a defect in *DETORQEO* (*DOQ*), *QUIRKY* (*QKY*) and *ZERZAUST* (*ZET*) exhibit a *sub*-like phenotype. Thus, *SUB*, *DOQ*, *QKY* and *ZET* define the *STRUBBELIG-LIKE MUTANT* (*SLM*) class of genes. Molecular cloning of *QKY* revealed that the predicted QKY protein may function in membrane trafficking. Taken together, the data suggest that *DOQ*, *QKY* and *ZET* contribute to *SUB*-dependent organogenesis and shed light on the mechanisms, which are dependent on signaling through the atypical receptor-like kinase SUB.

TARGET GENES OF THE OVULE IDENTITY COMPLEX IN *ARABIDOPSIS*

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In the model species *Arabidopsis thaliana*, ovule identity is controlled by the action of the MADS-box genes *SEEDSTICK* (*STK*), *SHATTERPROOF1* (*SHP1*), *SHP2* and *AGAMOUS* (*AG*) .Among these genes, *STK* is specifically expressed in the ovule while the *SHP* and *AG* genes are also expressed in the developing carpel. Protein interaction experiments demonstrated that the ovule identity factors assemble in protein complexes in presence of the MADS-box proteins SEPALLATA (SEP). In order to better elucidate the molecular mechanisms controlling ovule development we are interested in the identification and characterization of genes regulated by the ovule identity MADS-box protein complex.

Arabidopsis ovule primordia were isolated through Laser Microdissection System, .RNA was extracted and used to hybridise Affymetrix microarrays. This analysis led to the identification of a set of transcription factors that are expressed during early stages of ovule development. Subsequently, this gene set was analysed for the presence of multiple MADS-box binding sites in their putative regulatory regions. A subset of genes that were positive in this bioinformatics screen were further analysed by Chromatin Immunoprecipitation (ChIP) experiments using an antibody specific for STK. This allowed us to identify two REM genes as direct targets of the ovule identity factor STK. Expression analysis showed that these genes are developmentally regulated and broadly expressed genes however in situ hybridisation on developing flowers revealed that their expression in wild-type plants is restricted to the ovule starting from stage 8 of flower development. In the *stk shp1 shp2* triple mutant both *REM* are not expressed in the developing ovules. We will present the characterization of the *rem* mutants which allowed us to understand their roles during ovule development.

REGULATION OF EMBRYO SAC DEVELOPMENT BY *INDETERMINATE GAMETOPHYTE1*

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Embryo sac development begins with an early free-nuclear proliferation phase before cellularizing and differentiating into four different cell types. The four cell types occupy stereotypical positions along the micropylar-chalazal axis with the synergids and egg at the micropylar pole, the antipodal cells at the chalazal pole, and the central cell in the middle of the embryo sac. Micropylar-chalazal asymmetry is detectable in the mature embryo sac and earlier, before cellularization, even before the first free nuclear division of the functional megaspore. Little is known about the mechanisms regulating polarity of the embryo sac. The maize *indeterminate gametophyte1* (*ig1*) gene and its Arabidopsis ortholog, *ASYMMETRIC LEAVES2* (*AS2*), are expressed in embryo sacs and required for normal abaxial/adaxial polarity of lateral organs in the sporophyte. Loss-of-function *indeterminate gametophyte1* (*ig1*) embryo sacs undergo extra rounds of free nuclear divisions and have nuclei in abnormal positions in the syncytial embryo sac. The extra nuclei lead to the production of extra egg cells, extra central cells, and extra polar nuclei as well as other abnormalities. Consequently, *ig1* mutant plants produce several classes of abnormal seed. A putative role for *ig1* in the regulation of micropylar-chalazal polarity is being examined, and genetic and genomic tools are being used to identify targets of *ig1* in the embryo sac.

PATTERNING OF THE FEMALE GAMETOPHYTE IN ARABIDOPSIS

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The female gametophyte or embryo sac consists of only four cell types, including two gametes, that are the result of a precise developmental program. Due to the relative inaccessibility of the developing gametophyte and the limited availability of informative mutants, there has been little information about the genes and functions that establish developmental pattern in the embryo sac. From a large-scale screen for gametophytic mutations in Arabidopsis, we have identified an unusual mutation, *eostre*, in which the embryo sac has an additional egg cell in place of a synergid cell. The *eostre* mutant has revealed that transcriptional regulators of the BELL and class II Knox homeodomain families, and interacting proteins of the OVATE family, affect cell specification through their effects on nuclear positioning in the embryo sac prior to cellularization. In addition, experimental evidence has been obtained to suggest that signaling by the hormone auxin plays a key role in position based cell identity in the embryo sac. Manipulation of auxin concentrations and auxin responses result in switches of cell identities, consistent with a model in which cell type specification in the embryo sac is determined by levels of auxin.

POSTER TALK **P2-01**

REGULATORY NETWORKS CONTROLLING FEMALE GAMETOPHYTE DEVELOPMENT

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We have undertaken a comprehensive approach to determine functions for genes expressed in the female gametophyte (FG) with the long-term goal of identifying the gene regulatory networks controlling FG cell specification and differentiation. The FG is an integral part of the plant life cycle and plays critical roles in essentially every step of the angiosperm reproductive process. Using mRNA-based assays, we have identified a set of genes including members of transcription factor gene families expressed in each of the FG cells. FG cell-specific patterns of gene expression have been characterized using *promoter:Reporter* and *protein-Reporter* lines. To determine whether any of these genes have functions in FG cell specification or differentiation, we have analyzed a corresponding set of knock-out and knock-down mutant alleles for any defects in FG development and function. We will report on our efforts to gain a more comprehensive understanding of gene-regulatory networks operating during FG development.

IDENTIFICATION OF THE LOCUS CONTROLLING THE AVOIDANCE OF MEIOSIS DURING GAMETOPHYTE FORMATION IN APOMICTIC *HIERACIUM*

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Asexual seed formation, or apomixis, in the daisy-like plant *Hieracium* initiates when a somatic cell within the ovule, termed an 'Aposporous Initial' (AI), undergoes mitosis to form an unreduced embryo sac. The nearby meiotic events of sexual reproduction are suppressed. Seed initiation is fertilization-independent, thus the embryo retains the genotype of the female parent. Two dominant and independent loci are known to control the avoidance of meiosis (*apomeiosis*) during gametophyte development and autonomous embryo development (*parthenogenesis*), respectively. A nested set of gamma deletion mutants have been generated in apomictic *Hieracium* termed *loss of apomeiosis* (*loa*) because they are unable to form AIs. However, parthenogenesis remains intact resulting in seedlings with lower ploidy relative to the parental apomict. The development of markers linked with the *loa* locus has led to the generation of a BAC contig spanning over 1 Mb of DNA in the wild type apomict. FISH analysis has revealed the locus resides on a single chromosome, indicating the *LOA* locus is simplex (hemizygous) in nature. To narrow down the genomic region containing *LOA*, we have generated a segregating F1 population from a cross between the apomictic plant (as a pollen donor) with a sexual plant, and investigated the linkage between segregants retaining various markers and the ability to form AIs. Our progress towards the identification of sequences containing the *apomeiosis* locus in *Hieracium* will be discussed.

WHEN FEMALES GO EGG-CESSIVE: REGULATION OF GAMETIC CELL FATE IN ARABIDOPSIS

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Specification and regulation of gametic cell fate is key to reproductive success. The female gametophyte of *Arabidopsis* differentiates gametic cells and flanking accessory cells. In a screen for regulators of egg cell fate we isolated a total of three mutants, *lachesis* (*lis*), *clotho* (*clo*), and *atropos* (*ato*) that show deregulated expression of an egg cell marker. We have previously shown that in *lis* mutants, which are defective for the splicing factor PRP4, accessory cells can differentiate gametic cell fate. We now found that *CLOTHO/GAMETOPHYTIC FACTOR 1* (*CLO/GFA1*) is necessary for the restricted expression of egg and central cell fate and hence for reproductive success. Surprisingly, infertile gametophytes can be expelled from the maternal ovule tissue, thereby preventing the needless allocation of maternal resources to sterile tissue. *CLO/GFA1* codes for the *Arabidopsis* homologue of Snu114, a protein considered an essential component of the spliceosome. Furthermore, we demonstrate that *ATO* codes for the *Arabidopsis* homologue of SF3a60, a protein implicated in pre-spliceosome formation. Our results thus establish that the restriction of gametic cell fate is specifically coupled to the function of various core spliceosomal components. Possible mechanistic implications will be discussed.

EXPRESSION PROFILING OF ISOLATED EMBRYO SAC CELLS TO IDENTIFY GENES WITH KEY ROLES IN EMBRYO SAC FUNCTION AND DOUBLE FERTILIZATION

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The female gametophyte (embryo sac) is essential for sexual reproduction of flowering plants. Genetic, molecular, and *in vitro* studies have shown that the different cell types of the embryo sac are actively involved in multiple stages of the double fertilization process. It has become evident that double fertilization requires extensive intercellular signaling between the different cells of the female gametophyte, the surrounding maternal tissues and the male gametophyte. Nevertheless, the molecular processes by which the female gametophyte cells acquire their unique features and functions are not understood. Over the past years considerable progress has been made in the identification of a number of *Arabidopsis* female gametophytic mutants and in analysing the respective transcriptomes. However, this does not give information about the distinct gene expression profiles or the physiological state of the individual cell types of the female gametophyte.
We have isolated the individual cell types of the embryo sacs of different plant species and compared their specific expression profiles. This allows to investigate different cellular aspects such as cell cycle and transcriptional activity as well as signal transduction, RNA silencing, and methylation pathways at the individual cell level. We will report here our current progress in comparing the transcriptomes of the different embryo sac cells as well as the identification of cell-type specific genes and their function as key regulators in embryo sac function and double fertilization.

DISSECTING REGULATORY CIRCUITS DURING THE REPRODUCTIVE LIFE PHASE OF FLOWERING PLANTS

(EMBO Young Investigator lecture)

Alexander Ungru[1], Manoj Kumar[1], Reza Shirzadi[2], Catherine Schalk[3], Hugues Barbier[3], Moritz K. Nowack[1], Paul E. Grini[2], and Arp Schnittger[1,3,4]
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Seeds development in flowering plants is typically the outcome of a double fertilization progress and requires a tight interplay between the two sexually derived structures, the embryo and the endosperm, as well as the surrounding maternal tissues. In addition, to a spatial coordination, there is also the requirement for proper temporal control of gene expression patterns, e.g. certain genes, especially in developing endosperm, are privilegedly expressed from one parental genome, i.e. they are imprinted. We have recently identified a mutant in the key cell cycle regulator CDKA;1, the Arabidopsis homolog of the yeast cdc2/CDC28 kinase. Mutant cdka;1 pollen contains only a single sperm cell allowing a genetic dissection of double fertilization and seed development. Using this single-sperm pollen, we could show that there is a new signal emanating from the fertilization of the egg cell that triggers endosperm development. Here, we present that there exist a large genetic variation with respect to this signaling process in the species Arabidopsis thaliana. By using two recombinant inbred line populations we have identified at least two principle components and one complex quantitative trait locus that influence this signaling process. By further exploiting this genetic variation we could dissect the dependencies of embryo development on endosperm development during early seed development. Our data show an unexpectedly large degree of independency of embryo growth but also lay open its developmental restrictions with respect to endosperm size. Thus, the combination of single fertilization and natural variation appears to be a powerful tool to dissect gene regulatory circuits during gametophyte and seed development.

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POSTER TALK **P2-02**

REAL TIME IMAGING OF DOUBLE FERTILIZATION IN *ARABIDOPSIS THALIANA*

Yuki Hamamura[1, 2], Mathieu Ingouff[3], Chieko Saito[4], Masahiro M. Kanaoka[1], Narie Sasaki[1], Akihiko Nakano[2, 4], Frédéric Berger[3], Tetsuya Higashiyama[1]
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Double fertilization is a unique reproductive system to angiosperms. During double fertilization, one of the two sperm cells fertilizes with the egg cell and the other fertilizes with the central cell of the female gametophyte. Because the female gametophyte is deeply embedded in the ovule, it is difficult to observe the process of double fertilization in the living material. Here, we developed a system to observe the entire process of double fertilization in the in vitro Arabidopsis system. First, we made several cell-specific fluorescent markers that had enough signal intensities for real-time imaging system. In addition to this, we developed a state-of-the-art disk-scan confocal scanning laser microscope equipped with a high-sensitive camera, ahigh-speed piezo Z-axis drive allowing rapid recoding of z-stacks, and a prism to monitor two colors at the same time. We could observe the behavior of sperm nuclei and the interaction between male and female gametophytic cells. Sperm cells were rapidly released into the embryo sac and delivered to the region between the egg and central cell within 30 seconds after pollen tube discharge. The fusion of male and female gametophytic cells occurred about 10 minutes after pollen tube discharge. The mechanism of double fertilization will be discussed based on direct observation.

Oral Presentations - Session 3

Endosperm and Imprinting Session

Friday, October 17, 2008, 7pm – 10pm

Marriott Sabino/Pima rooms

Chair: Dr. Ramin Yadegari

ROLE OF *AGL62* IN ENDOSPERM DEVELOPMENT.

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Endosperm development consists of two main phases: an initial syncytial phase, during which the endosperm nuclei undergo many rounds of mitosis without cytokinesis to produce a multi-nucleate cell (a syncytium), followed by a cellularized phase, during which cell walls form around the endosperm nuclei. The molecular processes controlling the syncytial-cellular transition are not understood. In *agl62* seeds, the endosperm cellularizes prematurely, indicating that *AGL62* is required for suppression of cellularization during the syncytial phase. *AGL62* encodes a Type I MADS domain protein that likely functions as a transcription factor. During seed development, *AGL62* is expressed exclusively in the endosperm. During wild-type endosperm development, *AGL62* expression is strong during the syncytial phase and then declines abruptly just before cellularization. By contrast, in mutant seeds containing defects in some *FERTILIZATION-INDEPENDENT SEED* (*FIS*)-class Polycomb group genes, endosperm fails to cellularize and *AGL62* expression fails to decline. Together, these data suggest that *AGL62* functions in a pathway that controls the syncytial-cellular transition during endosperm development.

EPIGENETIC SUBFUNCTIONALISATION – CLUES TO THE ORIGIN OF IMPRINTING?

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Imprinting of key regulatory genes results in the uniparental expression of transcripts in the endosperm of flowering plants. The mechanism by which transcription of 'silenced' alleles is inhibited varies, and may involve polycomb protein complexes, non-coding RNAs and methylation at specific regions (differentially methylated regions[DMRs]).
The Arabidopsis *Fie* gene, which encodes a polycomb group transcription factor is expressed preferentially from the maternal alleles early in endosperm development. Maize has two such loci, *Fie1* and *Fie2* formed as a result of a complex allotetraploidy event. Both genes are only expressed from the maternal alleles in the endosperm, although the duration of this imprinting-controlled expression differs between the two genes. We have shown that monoallelic expression is likely to be regulated by DMRs, and surprisingly, while the DMRs of *Fie1* receive their asymmetric methylation in the gametes, differential methylation of these regions in *Fie2* takes place in the primary endosperm cells after fertilisation. Most other flowering plants possess a single *Fie* locus, so to discover why such recently generated and perhaps sub-functionalised genes are imprinted by two different processes, we have used transgenesis to investigate the properties of the two maize *Fie* genes in an attempt to ascertain which, if any, is the homolog of the single *Fie* gene of related species such as sorghum and rice.
We have also studied the adaptive value of this apparent sub-functionalisation. A combination of an increasing understanding of how these two genes are imprinted, and analysis of their expression throughout the plant life history, suggests that different imprinting mechanisms have been acquired to permit more effective silencing of the paternal copy of one of these genes in the developing endosperm.

MAIZE ENDOSPERM DEVELOPMENT: ROLE OF THE RETINOBLASTOMA PATHWAY

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In the endosperm of cereals such as maize, a phase of rapid growth and accumulation of storage compounds coincides with the endoreduplication cell cycle, in which multiple rounds of DNA synthesis seemingly occur in the absence of chromatin condensation, mitosis and cytokinesis. The outcome is the generation of large, polyploid cells filled with starch and storage proteins. However, the exact role of endoreduplication in endosperm development is unknown. We have manipulated key cell cycle regulators in order to up- or down-regulate endoreduplication. Previous experiments in which cyclin-dependent kinase A (CDKA) was inhibited by expression of a dominant-negative allele resulted in a ~50% inhibition of endoreduplication. In addition, we have down-regulated retinoblastoma-related 1 (RBR1) during endosperm development by RNAi. Although RBR1 RNA was decreased by only 50%, the encoded protein was undetectable indicating that RBR1 silencing was achieved by both a reduction in transcript levels and translational interference. The closely related RBR2 gene was also down-regulated. As expected, known E2F targets involved in DNA replication initiation were up-regulated in this mutant, while endoreduplication was stimulated up to ~40%. A third member of the retinoblastoma family, RBR3, was up-regulated, and experiments on embryogenic callus demonstrated that RBR3 is repressed by RBR1, suggesting a compensatory mechanism between RBR1 and RBR3 that sustains pocket protein activity. Investigation of the interplay between RBR1 and RBR3 revealed a novel role of RBR3 in controlling the cell cycle and E2F-dependent gene expression.

RETINOBLASTOMA AND ITS BINDING PARTNER MSI1 CONTROL IMPRINTING IN *ARABIDOPSIS*

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Parental genomic imprinting causes preferential expression of one of the two parental alleles. In mammals differential sex-dependent deposition of silencing DNA methylation marks during gametogenesis initiates a new cycle of imprinting. Parental genomic imprinting has been detected in plants and relies on DNA methylation by the methyltransferase MET1. However, in contrast to mammals, plant imprints are created by differential removal of silencing marks during gametogenesis. In *Arabidopsis*, DNA demethylation is mediated by the DNA glycosylase DEMETER (DME) causing activation of imprinted genes at the end of female gametogenesis. Based on genetic interactions we show that in addition to DME, the plant homologues of the human Retinoblastoma (Rb) and its binding partner RbAp48 are required for the activation of the imprinted genes *FIS2* and *FWA*. This Rb-dependent activation is mediated by direct transcriptional repression of MET1 during female gametogenesis. We have thus identified a new mechanism required for imprinting establishment, outlining a new role for the Retinoblastoma pathway, which may be conserved in mammals.

POSTER TALK **P3-01**

GENOMIC ANALYSIS OF METHYLATION PATTERNS IN ARABIDOPSIS EMBRYO AND ENDOSPERM

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Cytosine methylation is a common DNA modification that maintains silencing of transposable elements and influences gene transcription. The potential dynamism of DNA methylation during plant growth and development has not been fully explored. At present, only two genes are known for which DNA methylation changes are tied to alterations in gene expression during development. These are the endosperm-imprinted genes *FWA* and *MEA*, which are expressed exclusively from the maternally-inherited alleles in that tissue. *FWA* and *MEA* are less methylated in the endosperm compared to the embryo due to demethylation in the central cell before fertilization. We have profiled genome-wide methylation patterns in Arabidopsis torpedo-stage seeds by dissecting out the embryo and endosperm, isolating methylated DNA with a 5-methylcytosine antibody, and sequencing the DNA using Solexa high-throughput sequencing technology. These data offer the opportunity to 1) determine how dynamic DNA methylation patterns are across sister tissues 2) potentially discover new imprinted genes by identifying genes that are less methylated in the endosperm compared to the embryo and 3) determine what types of sequences are subject to methylation changes.

EPIGENETIC REPROGRAMMING OF THE PATERNAL GENOME IN *ARABIDOPSIS*

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In flowering plants, seed development is initiated by double fertilization, where two pairs of gametes fuse to form the embryo and endosperm, respectively. Over the last decade, it has become apparent that epigenetic processes play a crucial role in seed development. On the one hand, maternal effect genes, some of which are regulated by genomic imprinting, illustrate the importance of parent-of-origin effects on seed development. On the other hand, studies in *Arabidopsis* (Vielle-Calzada *et al*. (2000) *Nature* 404:91) and maize (Grimanelli *et al.* (2005) *Plant Cell* 17:1061) have shown that for a large number of genes no transcripts derived from the paternal allele can be detected during the first days after fertilization, suggesting a wide-spread maternal control over early seed development. However, there have also been reports of early expression of the paternal alleles for some genes, suggesting that the time of paternal activation may vary between different loci (Weijers D *et al.* (2001) *Nature* 414:709, Meyer and Scholten (2007) *Curr. Biol.* 17:1686). We have investigated the regulation of paternal genome activation in *Arabidopsis*. Our studies have shown that even for genes with very early paternal expression, activation occurs gradually, with each of the loci studied showing a distinct timing of activation and activation kinetics. Paternal genome activation is dependent on maternal factors, which define epigenetic pathways that both repress and activate paternal alleles. We propose that the interplay between activating and repressing maternal factors defines the distinct timing and kinetics of paternal genome activation for each locus.

GENETIC DISSECTION OF PARENT OF ORIGIN EFFECTS IN SEED DEVELOPMENT

Shirzadi, R.[1]*, Andersen, E.D.*[1]*, Bjerkan, K.N.* [1]*, Kaur, M.*[1]*, Nowack, M.K.*[2]*, Aalen, R.B.*[1]*, Schnittger A.*[2,3] *and Grini P.E.*[1,4]
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Seed development is the product of double fertilization of the egg cell and the central cell by two sperm cells from the pollen and requires a coordinated interplay of the transcriptional programs in the embryo, the endosperm and the maternal seed coat. Parent of origin dependent expression plays an important role in this process, and disruption of imprinted genes can lead to a parental specific abortion of the seed. We are using a mutant in the *Arabidopsis Cdc2/CDC28* homologue, *CDKA;1* as a tool to dissect the involvement of parental gene programs in seed development. *cdka;1* mutant pollen fails to undergo the second pollen mitosis, resulting in pollen with a single sperm cell that exclusively fertilizes the egg cell. Although not fertilized, the central cell in *cdka;1* ovules with fertilized egg cells is triggered to initiate endosperm proliferation. Moreover, viable seedlings can develop from seeds lacking a paternal contribution to the endosperm, given the mother is mutant for the Polycomb-group (PcG) chromatin remodeling factors *MEA*, *FIS2* and *FIE*. In order to identify further factors involved in cross talk between the components of the seed and parent of origin specific regulation of seed development, we have generated microarray transcriptional profiles of seeds with uniparental endosperm. Genes that are regulated when the paternal genome is missing could identify paternal specific transcripts (down-regulated) or maternal targets of paternal repressors (up-regulated). Our data show that we can identify novel imprinted genes and thus genetically dissect the role of parent-of-origin effects in seed development.

DEMETER REGULATES PLANT GENE IMPRINTING BY ACTIVE DNA DEMETHYLATION

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Alleles of imprinted genes are expressed differently depending on their parent of origins. Imprinting regulates a number of genes essential for normal development in mammals and angiosperms. The endosperm, an important site of imprinting in plants, has functions analogous to the mammalian placenta and supports seed growth and development. Failure to imprint certain genes results in embryo abortion and seed death. Using the model plant Arabidopsis, we discovered MEDEA imprinting is epigenetically regulated. Epigenetic information resides in chromatin, in which two interdependent processes regulate chromatin conformation: the chemical modification (methylation and acetylation) of histone proteins around which the DNA is wrapped, and the methylation of cytosine (5-methylcytosine). Both mechanisms contribute to the regulation of gene imprinting. The Arabidopsis DEMETER (DME), a novel DNA glycosylase, excises 5-methylcytosine via base excision repair pathway, leads to replacement of cytosine. DME specifically demethylates and activates expression of the maternal MEDEA (MEA) allele in the central cell of the female gametophyte. DME is not present in sperm cells and does not activate expression of the paternal MEA allele. The endosperm, generated from the fertilized central cell, inherits an active maternal MEA allele and a silenced paternal MEA allele. The active maternal MEA allele encodes a SET-domain Polycomb group protein that, in turn, methylates histones at the paternal MEA allele, causing the chromatin to be condensed, and ensuring that the paternal MEA allele is silenced.

IMPRINTING CONTROL MECHANISMS IN ARABIDOPSIS

(EMBO Young Investigator lecture)

Grigory Makarevich, Corina B.R. Villar, Aleksandra Erilova, Claudia Köhler
Institute of Plant Science, ETH Zurich, Universitätsstr. 2, CH-8092 Zurich, Switzerland, koehlerc@ethz.ch

Genetic information needs to be inherited, from cell to cell and from generation to generation. Genetic information is not only DNA encoded, but to a not yet fully appreciated extend encoded by DNA and histone modifications. These marks establish the "epigenome" and are decisive for cell fate development. Polycomb group (PcG) proteins are key players in keeping cellular memory. Some PcG proteins form complexes that insert histone methylation marks into chromatin causing transcriptional repression. The first identified PcG complex in plants is the FERTILIZATION INDEPENDENT SEED (FIS) complex. The FIS PcG complex is most likely repressing genes that should only become expressed after fertilization. *PHERES1* (*PHE1*) is a direct target gene of the FIS complex and is repressed by the FIS complex before and after fertilization. Specifically, the maternal copy of *PHE1* is targeted and repressed by the FIS complex, the paternal copy remains expressed. Thus, *PHE1* expression depends on the parent-of-origin, a phenomenon known as genomic imprinting. We are investigating the nature of the imprint and how this imprint is established and maintained. Our progress in elucidating imprinting mechanisms in plants is going to be presented.

POSTER TALK **P3-02**

CONTROL OF GENOMIC IMPRINTING BY *ALARM CLOCK1* GENE IN *ARABIDOPSIS*

Yoko Ikeda 1, Yuki Kinoshita 1, Tetsuji Kakutani 2, Tetsu Kinoshita 1
1 GCOE, Plant Reproductive Genetics Group, Nara Institute of Science and Technoolgy, Ikoma, Japan
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Genomic imprinting refers to the unequal expression of maternal and paternal alleles of a gene depending on the parent-of-origin, and is a phenomenon that has evolved in both placental mammals and flowering plants. In *Arabidopsis*, imprinting of the maternally expressed genes *MEDEA*, *FIS2* and *FWA* is established in the maternal central cell, which is the progenitor cell of endosperm before fertilization, by the activity of *DEMETER,* a DNA demethylase gene, while the male gametophyte allele is held silent. This "one-way" activation mechanism of genomic imprinting is unique to flowering plants (T. Kinoshita et al. Science 2004 303: 521-523). A process of imprinting likely begins with the recognition of imprinted genes, followed by DNA demethylation and remodeling of silent chromatin to the active state. To gain insight into this DNA demethylation and hence establishment of genomic imprinting, we have carried out genetic screening using *Arabidopsis* plant. We have isolated *alac* (*alarm clock for FWA imprinting*) mutant that is defective in *FWA-GFP* expression, and found that the gene controls all maternally expressed imprinted genes in the central cell and the endosperm.

Oral Presentations - Session 4

Compatible and Incompatible Pollinations Session

Saturday, 18 October 2008, 9-12 pm

(Marriott Pima/Sabino rooms)

Chair: Dr. Alice Cheung

SCA (LILY STIGMA/STYLE CYSTEINE-RICH ADHESIN) AND A SCA-LIKE LTP IN ARABIDOPSIS FUNCTION IN PLANT REPRODUCTION

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Lily pollen tubes grow adhering to an extracellular matrix (ECM) produced by the transmitting tract epidermis in a hollow style. SCA, a small (~ 9.4 kDa), basic protein, plus low-esterified pectin from this ECM are involved in the pollen tube adhesion event. Here, we purified two SCA isoforms (SCA1: 9370 Da, SCA3: 9484 Da) from lily stigma with a partial separation and studied their pollen tube adhesion activities in relation to protein structure. SCA1 displayed a larger internal hydrophobic cavity in our structural homology and molecular dynamics (MD) modeling and higher activity in *in vitro* pollen tube adhesion assays, compared to SCA3. This structure-function relation suggests that SCA1 may participate in pollen tube growth and adhesion by interacting with a putative ligand such as a lipid or a membrane-receptor. In an attempt to understand the role of SCA-like LTPs in *Arabidopsis thaliana* reproduction, we utilized SALK TDNA insertion lines. One mutant showed significantly delayed pollen tube growth and abnormal tube tip morphology. Our reciprocal cross-pollinations to wild-type showed that male sterility was responsible for low seed set in the mutant. In addition, the *LTP* mutation resulted in a subtle effect on normal pistil function in seed production. Our results suggest that this SCA-like LTP plays a role in both pollen and pistil function in Arabidopsis reproduction.

HAP2 IS A SPERM-EXPRESSED COMPONENT OF A DEEPLY CONSERVED FERTILIZATION MECHANISM

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Pollen tubes carry two sperm to the female gametophyte where one fuses with the egg to form the embryo and the other fuses with the central cell to form the endosperm. This process of double fertilization is at the core of seed crop production and is as mysterious as it is fascinating. We know very little about the mechanisms required for interactions between sperm and the female gametes that lead to fusion and initiation of development. We discovered *hap2-1* in a screen for mutations in Arabidopsis that disrupt the function of the male gametophyte. *hap2-1* pollen tubes are defective in pollen tube guidance, but deliver sperm to ovules at reduced frequencies. However, *hap2-1* sperm are completely incapable of fertilizing either the egg or the central cell. Recently, it has been show that the HAP2 ortholog is essential for gamete fusion in the green alga, *Chlamydomonas reinhardtii,* and in the protozoan parasite, *Plasmodium falciparum*. These results, combined with the conservation of HAP2 in plants and other organisms, lead us to propose that HAP2 is an anciently conserved component of a widely used fertilization mechanism. We are taking a number of approaches to understand the biochemical function of HAP2, which has a transmembrane domain but no other domains of known function. Our primary hypothesis is that HAP2 interacts with proteins expressed by the egg and central cell and that these interactions are required for gamete fusion.

UNILATERAL INCONGRUITY IN TOMATO: ROLE OF SELF-INCOMPATIBILITY FACTORS

Paul A. Covey[1], Katsuhiko Kondo[2], Aruna Kumar[2] ,Lilli Welch[1], Esther van der Knaap[3], Bruce A. McClure[2] and Patricia A. Bedinger[1]
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Self-Incompatibility (SI), wherein self pollen is rejected by styles, is widespread in plants and functions to prevent inbreeding. In gametophytic SI, RNases encoded at the *S*-locus (S-RNases), are the female SI determinant. Other factors in addition to *S*-locus genes are required for SI, including the asparagine-rich HT proteins. Interspecific pollen rejection is less well understood than intraspecific SI. Often, interspecific pollinations are only successful in one direction; this phenomenon is known as unilateral incongruity or incompatibility (UI). The role of SI proteins in UI appears to be complex. In tomato, genetic studies have directly implicated the *S*-locus in UI. However, there are clear examples in the tomato clade where UI is S-RNase independent. The mode of pollen tube rejection was examined in interspecific crosses to assess the role of SI genes in UI in wild tomato species. We find that there are at least two modes of interspecific pollen rejection that can be distinguished at the morphological level – rapid (in the upper 15% of the style) and slow (in the lower half of the style). Neither mode of interspecific pollen rejection necessarily depends on high levels of S-RNases. Two HT-family genes, *HT-A* and *HT-B*, are tightly linked and map to a UI QTL on Chromosome 12. While the *HT-A* gene appears to be functional in all wild tomato accessions tested, the *HT-B* gene contains a point mutation that should eliminate expression in all tested accessions of *S. habrochaites*, regardless of whether plants were self-compatible or self-incompatible.

POLLEN TUBE ATTRACTANTS DERIVED FROM THE SYNERGID CELL

Tetsuya Higashiyama; Division of Biological Science, Graduate School of Science, Nagoya University; Nagoya, Japan

The concept of a pollen tube attractant was proposed in the late nineteenth century when pollen tubes were found to grow toward excised pistil tissues on medium. Since then, for about 140 years, plant biologists have tried to identify pollen tube attractants. However, no molecule has been convincingly demonstrated to be the true attractant that actually controls the navigation of pollen tubes in the pistil (Higashiyama and Hamamura, Sex. Plant Reprod., 2008). We developed the in vitro *Torenia* system, whereby pollen tubes growing through a cut style are attracted to a protruding embryo sac and cause double fertilization. By using this system, the synergid cell was shown to emit some diffusible attractant(s) (Higashiyama et al., Science, 2001). The attractant molecule was species preferential even in closely relating species, implying that the molecule had rapidly evolved (Higashiyama et al., Plant Physiol., 2006). Thus, genes expressed in the synergid cell might provide insights into the attractant. We are now investigating genes expressed in the synergid cell of *Torenia*, by collecting isolated synergid cells. Some gene products are likely to be candidates of the pollen tube attractant. We will demonstrate whether the candidates show the necessary and appropriate conditions required by the true attractant or not.

POSTER TALK **P4-01**

GENETIC MAPPING OF FEMALE NONRANDOM MATING LOCI IN *ARABIDOPSIS THALIANA*

Toups, Megan 1, Preuss, Daphne 1, Swanson, Robert 2
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2 Valparaiso University, Biology Department, Neils Science Center, Valparaiso, IN, 46383
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Because of their lack of mobility, flowering plants are sometimes thought to be passive and undiscriminating mates, accepting with equal anonymity whatever sperm should come their way. Empirical studies demonstrate this not to be the case. In many flowering plants in diverse taxa, both self-compatible and self-incompatible, when pollen of mixed genetic lineages compete for fertilization, the progeny from the competition differ in proportion from the initial pollen deposition. This phenomenon is called nonrandom mating. This phenomenon is of potentially great evolutionary importance. The winners of the competition are able to pass their genes on to the next generation. We have developed a system to investigate nonrandom mating in the well-characterized and genetically tractable flowering plant *Arabidopsis thaliana*. We show that *Arabidopsis* mates nonrandomly in competitions between genetically distinct accessions, such as the Columbia accession and the mutagenized Landsberg *erecta* accession. We are currently working to demonstrate this phenomenon in geographically isolated accessions collected from Central-Asia (Shahdara), Europe (Bay-0), Candada (Van-0) and Russia (Est-1). Using Columbia and Landsberg recombinant inbred lines, we demonstrate that nonrandom mating shows transgressive segregation. We have also constructed a preliminary genetic map identifying five loci on two chromosomes that are involved in female mediated nonrandom mating.

THE ROLE OF THE *S-LOCUS-F-BOX* GENE IN S-RNASE-BASED SELF-INCOMPATIBILITY

Teh-hui Kao
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My lab is using *Petunia inflata* as a model to study S-RNase-based self-incompatibility (SI). Two polymorphic genes at the *S*-locus, *S-RNase* and *PiSLF (P. inflata S-Locus)*, control pistil and pollen specificity, respectively. To determine how PiSLF interacts with S-RNase in the cytoplasm of pollen tubes to result in inhibition of self pollen tubes, we have shown that (1) PiSLF, PiCUL1-G (a cullin) and PiSBP1 (a RING-HC protein) could constitute a novel E3 ligase complex, with PiSBP1 playing the dual roles of SKP1 and RBX1 in the canonical SCF complex (a class of E3 ligase complex); (2) a PiSLF interacts with its non-self S-RNases more strongly than with its self S-RNase *in vitro*; (3) S-RNases are degraded in pollen-tube extracts via the ubiquitin-26S proteasome pathway, albeit not in an *S*-dependent manner; (4) PiSBP1, in conjunction with ubiquitin activating enzyme (E1) and conjugating enzyme (E2), ubiquitinates S-RNase *in vitro*. We propose that the PiSLF/PiCUL1-G/PiSBP1 complex preferentially interacts with non-self S-RNases to mediate their ubiquitination and degradation, and that PiSBP1, acting as a mono-subunit E3 ligase, is responsible for basal degradation of all S-RNases. We have examined the interactions of various truncated forms of $PiSLF_2$, chimeric proteins between $PiSLF_1$ and $PiSLF_2$, and chimeric proteins between $PiSLF_2$ and a PiSLF-like protein, with S-RNase *in vitro*. A biochemical model for PiSLF and S-RNase interactions under compatible and incompatible pollinations, as well as under competitive interaction, has been developed, and we are currently using *in vivo* approaches to examine the validity of this model.

CHARACTERIZATION OF MULTIPLE F-BOX GENES LINKED TO THE *S* LOCUS OF APPLE AND JAPANESE PEAR

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Apple (*Malus domestica*) and Japanese pear (*Pyrus pyrifolia*) belong to subfamily Maloideae of Rosaceae, and exhibit S-RNase-based gametophytic self-incompatibility (GSI). The pollen-part determinant of *S* specificity of GSI was recently identified as F-box gene, *SFB/SLF*, in three families, Rosaceae, Solanaceae and Plantaginaceae. Besides *SFB/SLF*. several F-box genes have been identified in the *S* locus regions of *Petunia* (Solanaceae), *Antirrhinum* (Plantaginaceae), and *Prunus* (Rosaceae). However, homology between *SFB*/*SLF* and *SFB/SLF*-like genes in a species were low. We recently sequenced the S^9 haplotype region of apple for 317 kb, and found two related F-box genes (*SFBB*s) in the region. They showed *S* haplotype-specific polymorphisms and pollen-specific expression. No other F-box genes were found in the 317 kb region. In Japanese pear, three *SFBBs* were isolated from S^4 haplotype. In a mutant of S^4 haplotype S^{4sm}, which has deletion of the S^4-RNase region and impairs pistil part SI function, the $SFBB^4$s were retained, being consistent with the hypothesis that they are involved in pollen-part *S* specificity. Recently, Okada et al (2008) determined the extent of deletion of the S^{4sm} haplotype, and found that the S^{4sm} haplotype lacks an F-box gene (termed S_4F-*box0*) which is homologous to *SFBB*. It was recently suggested that pollen with S^{4sm} haplotype is rejected not only by S^4 but also by S^1 pistils. The 'relaxed' pollen-part specificity of the S^{4sm} haplotype is possibly related to the deletion of S_4F-*box0*.

POSTER TALK **P4-02**

PRUNUS MAY HAVE A DISTINCT RECOGNITION MECHANISM IN ITS S-RNASE BASED GAMETOPHYTIC SELF-INCOMPATIBILITY SYSTEM

Daiki Matsumoto and Ryutaro Tao
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S-RNase and F-box genes are commonly found as the pistil and the pollen determinants, respectively of the *S* locus of three plant families, Rosaceae, Solanaceae, and Plantaginaceae. Therefore, a common working hypothesis for the self and non-self recognition in the Rosaceae, Solanaceae, and Plantaginaceae was proposed involving the ubiquitination and subsequent degradation of non-self S-RNases. In this model, SFB/SLF functions as a component of $SCF^{SFB/SLF}$, which ubiquitinates all non-self S-RNases to make them for degradation (general interaction) but interacts specifically (*S* haplotype-specific interaction) with the self S-RNase to leave it active, leading to the arrest of self pollen tube growth. However, recent molecular studies on self-compatible (SC) pollen-part mutant (PPM) *S* haplotypes in *Prunus* (Rosaceae) suggested that the recognition mechanism of the S-RNase-based GSI in *Prunus* may differ from the mechanism(s) in the Solanaceae and Plantaginaceae. In this report, we summarize the differences in the recognition system of self-incompatibility in *Prunus* and others.

POSTER TALK **P4-03**

GENES GOVERNING CROSS INCOMPATIBILITY BETWEEN MAIZE AND TEOSINTE.

Jerry Kermicle, Lab. Of Genetics, University of Wisconsin, USA.

Some populations of annual teosinte (*Zea mays* ssp. *mexicana)* grow as weeds in maize fields, flower in synchrony with the maize, but are not fertilized by it. Major genes govern the incompatibility. For two identified previously, *Ga1-s* and *Tcb1-s*, presence of a strong barrier allele in the pistil requires presence of the same or a related allele in pollen for fertilization to occur. (Just the opposite of self-incompatibility, that is.) A third cross incompatibility system was identified among teosinte populations. It resembles *Ga1-s* and *Tcb1-s* in that a major locus confers both the pistil barrier and pollen competence. The pistil barrier allele is dominant and pollen competence is determined by genotype of the individual pollen grain. This locus proved allelic to *Ga2*, identified earlier among maize genetic stocks by a weaker allele that causes transmission ratio distortion. Populations of Mexican maize that are sympatric to weedy teosintes carry either an allele that is pollen competent but which lacks the pistil barrier (*Ga2-male*), or the phenotypically null allele *ga2*. Presence of *Ga2-s* in teosinte and *ga2* in sympatric maize would prevent maize from fertilizing teosinte. This particular combination was not found at the five collection sites tested. Cross recognition between the three incompatibility systems is weak at most, and incompatibility for any one locus overrides (is epistatic to) compatibility for the other two. Because the three provide reproductive isolation, they are candidate speciation genes.

ENDOCYTOSIS IN S-RNASE-BASED SELF-INCOMPATIBILITY

Bruce McClure, Christopher B. Lee, Sunran Kim, and Aruna Kumar
Division of Biochemistry, Interdisciplinary Plant Group, Christopher S. Bond Life Sciences Center, University of Missouri, 1201 East Rollins Street, Columbia, MO 65211 USA

The genetics of S-RNase-based self-incompatibility (SI) are simple: a multi-allelic *S*-locus determines compatibility, and individual pollen tubes are rejected if their *S*-haplotype matches either of the two *S*-haplotypes in the diploid pistil. However, the mechanism of pollen rejection has not proven to be this simple at the molecular and cellular levels. Although it is now established that S-RNase determines *S*-specificity in the pistil and the *S*-locus F-box (SLF) protein determines specificity in the pollen, it is also clear that these *S*-specificity determinants alone are not sufficient for SI. Several non-*S*-specific factors are required on the pistil side, and a number of pollen proteins that interact with SLF are probably also implicated in SI. Furthermore, there is a topological problem relating to how S-RNase from the pistil extracellular matrix (ECM) gains access to the cytoplasmic compartment. We have observed large amounts of S-RNase associated with pollen tube vacuoles and also that HT-B, a pistil factor known to be required for SI, appears to be degraded in compatible pollen tubes. While S-RNase sequestration could provide a mechanism for compatible pollen tubes to evade S-RNase cytotoxicity, the topological problem remains. A better understanding of how pistil ECM components move through the pollen tube endomembrane system will help resolve this problem. This talk will describe pollen proteins that bind to ECM components and that could be involved in endocytosis and retrograde transport in pollen tubes as well as evidence that uptake of pistil proteins occurs through fluid phase endocytosis.

SELF-INCOMPATIBILITY IN *PAPAVER*: IDENTIFICATION OF THE POLLEN SELF-INCOMPATIBILITY DETERMINANT

Michael J. Wheeler, Barend H. J. de Graaf, Natalie Hadjiosif, Ruth M. Perry, Natalie S. Poulter, Kim Osman, Sabina Vatovec, F. Christopher H. Franklin and Vernonica E. Franklin-Tong
School of Biosciences, University of Birmingham, Edgbaston, Birmingham. B15 2TT, UK

Self-incompatibility (SI) is an important mechanism used by many angiosperms to prevent inbreeding. A multi-allelic *S* locus allows discrimination between "self" (incompatible) pollen from "non-self" (compatible) pollen. In *Papaver rhoeas,* the pistil *S* locus product (S protein) is a small novel protein that interacts with incompatible pollen, triggering a Ca^{2+}-dependent signalling network. We have made progress in elucidating mechanisms involved in mediating SI, which include rapid depolymerization of the actin and microtubule cytoskeletons; phosphorylation of a soluble inorganic pyrophosphatase, Pr-p26.1; activation of a MAPK, p56; and programmed cell death (PCD) involving several caspase-like activities.

I will focus on describing the cloning and identification of three alleles of *PrpS*, and the evidence that it is the *Papaver* pollen *S* locus determinant. *PrpS* is is specifically expressed in pollen and tightly linked to the pistil *S* gene. It displays the polymorphism expected of an *S* locus component. It encodes a novel ~20 kDa protein with no homology to proteins in existing databases. It has three predicted transmembrane domains and is localized to the plasma-membrane, suggesting that it is likely to be a transmembrane receptor. Importantly, we have obtained evidence that PrpS is functionally involved in SI using *PrpS* antisense oligonucleotides to alleviate inhibition of incompatible pollen in an *S*-specific manner. Our identification of *PrpS* as the *Papaver* pollen *S* locus determinant represents a major advance in our understanding of SI and strongly supports our hypothesis that *Papaver* SI is triggered by a receptor-ligand interaction.

Oral Presentations - Session 5

Evolution of Reproductive Development

Saturday, 18 October 2008, 4:15-7 pm

Sonoran Desert Muesem

Chair: Dr. Ned Friedman

SEX-RATIO VARIATION IN DIOECIOUS PLANT POPULATIONS

Spencer C.H. Barrett, Ivana Stehlik & Jannice Friedman
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Diverse ecological and genetic mechanisms can cause deviations from the expected 1:1 ratio of females and males in dioecious plant populations. Most reports of deviations from equality involve male-biased ratios, as a result of differences in gender-based mortality and flowering schedules. Here, we discuss factors influencing female-biased sex ratios in the wind-pollinated alpine plant *Rumex nivalis*. Use of a sex-specific marker enabled dissection of the life cycle to determine stages where female bias occurs. Bias was evident during the gametophytic, progamic and sporophytic stages with increasing amplification of female bias to flowering. A relation between pollination intensity and female bias was established under both garden and field conditions supporting an early hypothesis by Correns (1922) that selective fertilization as a result of differences in fertilization success of female versus male gametophytes (certation) contributes towards sex-ratio bias in *Rumex* species. Because of the role of pollination intensity in modulating sex-ratio bias we predicted that maternal parents in close proximity to males would produce more strongly female-biased progeny sex ratios. We tested this prediction in six alpine populations in Switzerland by measuring the distance between focal females and neighboring males and assessing pollen loads and seed sex ratios of maternal parents. In four of the six populations, females positioned in close proximity to males captured more pollen and exhibited more female-biased sex ratios. Our results demonstrate that demographic aspects of the maternal mating environment can influence progeny sex ratios leading to biased sex ratios.

POLLEN–PISTIL INTERACTIONS AS REPRODUCTIVE ISOLATING MECHANISMS: EVIDENCE FROM SPECIATION STUDIES OF THE SPIRAL GINGERS

Kathleen M. Kay, Department of Ecology and Evolutionary Biology, University of California – Santa Cruz, Santa Cruz, CA, USA

The evolution of reproductive isolating barriers is central to the speciation process. While pollen-pistil incompatibility has been studied extensively in the context of SI, plant breeding, and basic plant functional biology, little is known about the role postpollination barriers play in the diversification of wild plants. I examined pollen–pistil interactions in *Costus pulverulentus* and *Costus scaber* (Costaceae), two recently diverged Neotropical understory herbs that co-occur and share hummingbird pollinators. The hummingbird pollinators transfer pollen between these *Costus* species, but hybrids are rarely found in nature. By performing pollinations between populations of *Costus pulverulentus* and *Costus scaber* from three sites across the species' geographic ranges, I found that pollen–pistil incompatibilities acting prior to fertilization have evolved only between locally sympatric populations, while geographically distant populations within the region of sympatry and allopatric populations remain fully interfertile. The population-level specificity of the incompatibility supports the hypothesis that it has evolved by reinforcement, or direct natural selection to prevent potentially maladaptive hybridization between incipient species. I also conducted a comparative study of isolating mechanisms across the genus *Costus*. I found lower seed set due to pollen–pistil incompatibility between species pairs that co-occur and share pollinators compared to species pairs that are otherwise isolated, regardless of genetic distance. Understanding the barriers that contribute to reproductive isolation will better elucidate the conditions under which speciation is likely to occur and the role of natural selection in speciation, and can motivate studies of the genetic basis of speciation.

TESTING FOR STABILIZING AND CORRELATIONAL SELECTION ON CONSERVED FLORAL TRAITS USING EXPANDED PHENOTYPIC VARIATION

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Keith Karoly and Vanessa Koelling: Department of Biology, Reed College, Portland, OR, USA

A key problem in evolutionary biology is that adaptations have little variation (i.e., they are conserved) because selection has removed unfit variants. Thus, it is difficult to provide evidence for the adaptive or functional value of the trait. We used artificial selection to increase the variance in two anther position traits in wild radish, *Raphanus raphanistrum*. Response to selection was rapid for both traits, in spite of potential constraints caused by strong pleiotropic correlations. Experimental arrays of plants with expanded variation for each trait created from these selection lines were placed in the field, and natural selection based on male fitness of these plants was estimated for both traits. Results showed evidence for stabilizing/correlational selection on anther exsertion, supporting the hypothesis that this trait is an adaptation for effective pollination. However, plants artificially selected for a greater difference between the heights of the four long and two short anthers actually had higher male fitness than random-mated controls.

PHENOTYPIC PLASTICITY, GENETIC ASSIMILATION, AND THE EVOLUTIONARY DIVERSIFICATION OF SEXUAL SYSTEMS IN *SOLANUM.*

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For over a century, it has been hypothesized that selection can act in such a manner as to turn an environmentally stimulated phenotype (the result of plasticity) into a fixed (constitutively produced) phenotype; a process known as genetic assimilation. This process, by which "acquired characters" become converted, over generations, into “inherited characters” remains controversial from both a theoretical and empirical perspective. Within the genus *Solanum*, the fixed production of male flowers has evolved from a phenotypically plastic response to the resource demands of fruit maturation. Experimental manipulation of fruit production shows that for the majority of species examined, the production of male flowers occurs only, or is significantly increased, on plants bearing fruit; that is male flower production is a phenotypically plastic response to fruit set. In several species, however, male flowers are produced constitutively. Architectural analysis shows that the constitutive phenotypes are qualitatively the same as the fruit induced phenotype of the phenotypically plastic species. Phylogenetically based ancestral state reconstructions show that plasticity is plesiomorphic within two distinct lineages and that constitutive species are nested within clades of plastic species. Therefore, evolution of these derived species represents the fixation of the fruit-induced phenotype and has all of the features of evolution via genetic assimilation.

POSTER TALK **P5-01**

EVOLUTION OF C-FUNCTION GENES. SMALL PROTEIN CHANGES THAT LEAD TO BIG FUNCTIONAL DIFFERENCES.

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Gene duplication is a major force driving evolution of new gene functions. Following duplication of the floral c-function, one copy retained the ability to specify stamens and carpels, while the other adopted a new function. In *Antirrhinum* the main C-function was retained by *PLENA (PLE)* while *FARINELLI (FAR)* is only involved in male development. In contrast the *Arabidopsis* ortholog of *FAR*, *AGAMOUS (AG)* retained c-function acitivity. To understand the differences in AG and FAR function, each was overexpressed in *Arabidopsis*. The overexpression of AG converts sepals into carpelloid structures and petals into stamens whereas overexpression of *FAR* can only convert petals into stamens. Despite the high protein similarity between AG and FAR, FAR cannot drive carpel development. To investigate which changes in the FAR protein lead to subfunctionalization we produced transgenic *Arabidopsis* plants overexpressing chimerical proteins with different combinations of AG and FAR domains. We identified a single amino acid that is able to confer the FAR phenotype into an AG protein background. This small difference in the amino acid sequence is located in the part of the protein involved in multimeric complex formation. Several lines of evidence suggest that interactions with the SEPALLATA proteins during multimeric complex formation is the key to the functional differences, explaining how a single amino acid change can completely disrupt the ability of a C-fuction gene to drive carpel development.

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UNDERSTANDING EARLY PATTERNS OF POLLEN TUBE GROWTH IN RELATION TO CARPEL CLOSURE AND FLORAL DIVERSIFICATION AMONG ANGIOSPERMS.

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Angiosperms are notable for their rapid reproductive process relative to most gymnosperms. The evolutionary transition from slow to fast reproduction primarily involved shortening of the pollination to fertilization life history stage, whereas the duration of post-fertilization development was conservative. Acceleration of the fertilization process in early-divergent angiosperms (*Amborella, Nuphar, Austrobaileya* and others) is characterized by much faster pollen tube growth rates than those of any gymnosperm. The ancestral pollen tube pathway is reconstructed as being quite short, entirely secretory, and within an open carpel (with several early transitions to partial carpel closure). All early-divergent angiosperms studied to date have novel callose-walled pollen tubes that can form callose plugs, whereas no gymnosperm has a strongly callosic pollen tube wall structure. Among early-divergent angiosperms, pollen tube growth rates and pollen tube pathway lengths are within the bottom 1 % and 3% of known angiosperm maxima, respectively. Thus, the initial acceleration of pollen tube growth rate and the origin of a callose-walled growth pattern within secretions were innovations that were followed by multiple origins of carpel closure, longer pollen tube pathways (styles, and/or deep and multi-seeded ovaries), and much faster pollen tube growth rates. Callose walls and plugs allow pollen tubes to travel longer distances in less time with a lower potential of cell death than do the primarily cellulosic walls of gymnosperm pollen tubes. As such, pollen tube growth innovations initiated a spectacular exploration of novel life histories, affecting both flower and fruit form and the duration of reproductive cycles in flowering plants.

OVERCOMING A CENTURY OF DOGMA ABOUT THE REPRODUCTIVE FEATURES OF THE FIRST ANGIOSPERMS: INSIGHTS FROM *HYDATELLA* AND OTHER WATER LILIES

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For over a century, the flowering plant family Hydatellaceae was thought to belong to the Poales, a highly derived monocot order that includes the grasses. New molecular phylogenetic evidence shows that this obscure group of minute aquatic plants is closely related to Nymphaeales, which themselves were recently discovered to be one of the most ancient extant lineages of angiosperms. Female gametophyte development and endosperm ploidy *Hydatella inconspicua* (recently renamed *Trithuria inconspicua*) precisely match the unique and potentially plesiomorphic patterns characteristic of most early divergent angiosperm lineages (Nymphaeaceae, Cabombaceae, Austrobaileyaceae, Illiciaceae, Schisandraceae). At maturity, the female gametophyte is four-celled and four-nucleate, with two synergids, an egg cell and a uninucleate central cell; consequently, endosperm in *Hydatella* is diploid. As with all members of the Nymphaeales, endosperm in Hydatellaceae is minimally developed and perisperm is the major embryo-nourishing tissue within the seed. Remarkably, *Hydatella* exhibits a maternal seed-provisioning strategy that is unique among flowering plants, but common to all gymnosperms: pre-fertilization allocation of nutrients to the embryo-nourishing tissue. This exceptional case of pre-fertilization maternal provisioning of an ovule/seed in *Hydatella* may well be an apomorphic feature of Hydatellaceae alone. If so, it is more strong proof that the earliest phases of flowering plant evolution were marked by a significant degree of developmental lability and a tremendous diversification of reproductive features. Alternatively, pre-fertilization maternal resource allocation to ovules/seeds, and specifically to a maternally derived perisperm, in *Hydatella* could represent a plesiomorphic and transitional condition associated with the origin of flowering plants from gymnospermous ancestors. The prospect that the first angiosperms might have used both a perisperm and an endosperm to nourish the embryo within a seed and that the maternal plant allocated reserves to this perisperm before fertilization, is thoroughly congruent with the data derived from Hydatellaceae and its newfound phylogenetic position.

POSTER TALK **P5-02**

EVOLUTIONARY IMPLICATIONS OF SELF-COMPATIBILITY AND MENTOR EFFECTS IN THE APOMICTIC *RANUNCULUS AURICOMUS* POLYPLOID COMPLEX (RANUNCULACEAE)

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Apomixis has often been regarded as advantageous for colonization because of uniparental reproduction. Unidirectional introgression of apomixis into sexual taxa should theoretically replace sexuality in mixed populations (Hörandl 2006, New Phytol. 171: 525–538). In pseudogamous apomicts, uniparental reproduction requires self-compatibility (SC) for production of viable seeds. Self-compatibility, reproductive fitness and mentor effects have been studied in diploid sexual species and their polyploid derivatives of the *Ranunculus auricomus* complex. Diploid sexual taxa are completely self-incompatible, autopolyploid sexual cytotypes are predominantly self-incompatible. The observed breakdown of self-incompatibility in the allohexaploid apomicts may be explained by initial partial SC inherited from semi-selfcompatible ancestors and strong selection for SC genotypes (Hörandl, Int. J. Pl. Sci. in press). Introgression of apomixis is being studied in experimental pollinations of diploid sexuals with pollen from polyploidy apomicts. Analysis of seeds via flow cytometric seed screen suggests that both mentor effects (induced selfing of sexual individuals) but also failure of endosperm development and seed abortion block introgression of apomixis into sexuals. It is concluded that higher reproductive fitness and mentor effects help to maintain sexual populations, whereas SC in apomicts is advantageous in unstable environments, and also for colonization events. Results suggest that SC in connection with pseudogamous apomixis is an important factor for the observed distribution patterns of apomicts and sexuals (geographical parthenogenesis).

EVOLUTIONARY DYNAMICS OF THE PAPAVERACEAE S-LOCUS.

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We have begun to explore the evolutionary relationships among putative S-allele sequences from Papaveraceae native to California. Using primers designed from the four published stylar S-alleles of *Papaver* we have sequenced putative S-alleles from *Argemone munita*, *Platystemon californicus*, and *Romneya coulteri*. Apparent S-locus sequence diversity is lower in these taxa than in *Papaver* and the evolutionary relationships among putative S-allele sequences show lower levels of ancestral polymorphism shared among genera than is found at the stylar S-locus of other families. In crosses, putative S-allele sequences are strongly correlated with mating phenotype but there is evidence of linked duplications of the stylar S-locus in at least two of these taxa which may account for the somewhat unusual evolutionary dynamics observed.

REPRODUCTION AND HYBRID SPECIATION IN *SENECIO*: THE OXFORD RAGWORT STORY

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Oxford ragwort (*S. squalidus* L. [Asteraceae]) is a self-incompatible daisy that was introduced into Britain in the late 17th Century from Sicily via the Oxford Botanic Garden. After being cultivated in the garden for nearly 150 years it ‘escaped’ and became noticeable growing in old college walls around the city. The rise of the railways during the 19th Century and the expansion of the railway network from Oxford provided *S. squalidus* with further suitable habitats, particularly the clinker beds, which allowed it spread rapidly to most parts of Britain. Today there are just a few parts of Scotland and Wales where Oxford ragwort cannot be found. This unique population history raises many important questions about the mating system of *S. squalidus*. During its invasive spread across the British Isles *S. squalidus* has hybridized repeatedly with native *S. vulgaris* (groundsel) to form new *Senecio* taxa: *S. x baxteri* (sterile triploid F1 hybrid), *S. cambrensis* (Welsh ragwort, fertile allohexaploid), *S. eboracensis* (York groundsel, fertile tetraploid) and *S. vulgaris* var. *hibernicus* (radiate groundsel, fertile tetraploid). This makes *Senecio* an excellent model system in which to study the process of abrupt hybrid speciation in plants.

For the past ten years my group has been studying the reproductive biology of *S. squalidus* across the UK, and its native Sicily, together with the genetic and genomic processes associated with homoploid and allopolyploid speciation following S. squalidus’ hybridization with *S. vulgaris*. Here I will describe some of our most recent findings on reproduction and speciation in *Senecio*.

Oral Presentations- Session 6

Novel Systems and Emerging Technologies

Sunday, 19 October 2008, 8:30-11:30 am

Marriott Pima/Sabiono rooms

Chair: Jose Feijo

POLLEN TIP GROWTH AND FERTILIZATION: CALCIUM PUMPS, CHANNELS, AND KINASES.

Jeffrey F. Harper, Sabine Frietsch, Shawn M. Romanowsky, Candace Myers, Claudia Rato da Silva, Amy Curran, Yoshimi Barron, Meral Tunc. University of Nevada, Biochemistry, MS200, 1664 North Virginia St, 89557 Reno, CA, USA. jfharper@unr.edu

Calcium signals play a central role in pollen development. Using *Arabidopsis* as a model, we have identified mutations disrupting Ca pumps (e.g. ACA9, (Schiøtt et al., 2004, PNAS 101:9502), cyclic nucleotide gate channels (e.g. CNGC18, Frietsch et al., 2007, PNAS 104:14531), and Ca dependent protein kinases (CPKs) (e.g. CPK17 and 34), providing genetic insights into how Ca signals regulate pollen tube growth, chemotropism, and fertilization. ***CNGC Mutations***: Gene disruptions for a Ca permeable *cngc18* are male sterile. Mutant pollen tubes are "kinky" and fail to reach the ovary. A GFP tagged CNGC18 can rescue mutant pollen, and displays a plasma membrane localization focused at the pollen tube tip. A similar block in pollen transmission was found for a double mutation of *cngc7* and *8*. A working model is that all three CNGCs (18, 7 and 8) function as part of the same tip localized channel complex to control the rate and direction of tip growth. In contrast, evidence indicates that a different subgroup of pollen expressed CNGCs may function in a separate pathway to mediate stress tolerance. ***CPK Mutations***: Gene disruptions for *cpk17* and *34* result in a nearly complete male sterile phenotype. Mutant tubes have a normal morphology, but are very short and impaired in their tropism towards ovules. Pollen CPKs have many potential regulatory targets, including Ca pumps, vesicle trafficking machinery, and CNGCs. While it remains a considerable challenge to understand the complexities of calcium signaling in plants, pollen provides an excellent model system.

THE PEROXIN LOSS-OF-FUNCTION MUTATION **ABSTINENCE BY MUTUAL CONSENT** *DISRUPTS MALE-FEMALE GAMETOPHYTE RECOGNITION*

Aurélien Boisson-Dernier[1], Tae-Houn Kim, Sabine Frietsch, Marie B. Dizon and Julian I. Schroeder.
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In animals and plants, fertilization relies on complex and specialized mechanisms that achieve the precise delivery of the male gamete to the female gamete and their subsequent union. In plants, the male gametophyte or pollen tube carries two sperm cells over a long distance through the maternal tissues to the female gametophyte. During this long assisted journey, a multitude of signal exchanges between the pollen tube, the maternal diploid tissues and the haploid embryo sac take place that culminate in pollen tube reception, the process through which the pollen tube release the sperm cells into the female gametophyte. Here, we report the isolation and characterization of the *Arabidopsis* mutant *abstinence by mutual consent* where pollen tube reception is impaired only when an *amc* pollen tube reaches an *amc* embryo sac leading to pollen tube overgrowth and the absence of sperm release. Moreover, *AMC* is strongly expressed in both male and female gametophytes during fertilization but strongly down-regulated during subsequent embryo development. We further show that YFP-AMC fusion localized to peroxisomes and that AMC functions as a peroxin that mediates protein import into peroxisomes. The identification of *AMC* (Boisson-Dernier et al., 2008) as a gene required for pollen tube reception with essential roles in both male and female gametophytes, points towards a key role for peroxisomes in gamete recognition and successful sperm release.

Boisson-Dernier, A., Frietsch, S., Kim, T.H., Dizon, M.B., and Schroeder, JI. (2008). The Peroxin Loss-of-Function Mutation *abstinence by mutual consent* Disrupts Male-Female Gametophyte Recognition. *Current Biology*.18(1):63-68.

A POLLEN-BASED SCREEN FOR BIOACTIVE CHEMICALS TO DISSECT ENDOMEMBRANE TRAFFICKING.

Stéphanie Robert[2], Georgia Drakakaki[1], Michelle Brown[1], Anna-Maria Szatmari[2], Jiri Friml[2], Eugenia Russinova[2], Natasha V. Raikhel[1], and Glenn R. Hicks[1]

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Although it is known that proteins are delivered to and recycled from the plasma membrane (PM) via endosomes, the nature of the compartments and pathways responsible for cargo and vesicle sorting and cellular signaling is poorly understood. Such highly dynamic processes are not easily approached genetically. To define and dissect specific recycling pathways, rapid-acting chemical affecters of proteins involved in vesicle trafficking, especially through endosomes, would be invaluable. Thus, we identified chemicals affecting essential steps in PM/endosome trafficking by utilizing the intensely localized PM transport at the tips of germinating tobacco pollen tubes. We screened diverse chemical libraries for those that interfered with pollen germination and tip growth. We found that many also had effects in Arabidopsis roots for which there are several well-characterized marker proteins that cycle to and from the plasma membrane. The compound endosidin 1 (ES1) interfered selectively with endocytosis of PIN2, AUX1 and BRI1 allowing us to define compartments in Arabidopsis root cells through which these markers transit during endocytosis. These results indicate the value of using a chemical biology approach to understand mechanisms of endomembrane trafficking. We have now greatly expanded our chemical screening approach in order to obtain a suite of probes to dissect endomembrane trafficking and sorting processes. Recent results from our screens will be discussed.

POSTER TALK **P6-01**

IN VITRO AND IN SILICO STUDIES OF THE DYNAMICS OF POLLEN TUBE–OVULE INTERACTIONS

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Plant biologists have long speculated about the mechanisms that guide pollen tubes to ovules. Recent semi-in vitro experiments, where dissected ovules were arranged around a cut style on an artificial medium, have shown that ovules secreted unidentified guidance cues that appear to guide pollen tubes in the late stages of fertilization. Combining microscopy and modeling we have extended this work to study how ovules secrete these cues and how pollen tubes respond to them. To study the dynamics of secretion, we varied the amount of time ovules were in contact with the media before being encountered by pollen tubes. With particle tracking, we quantified how the tubes grew in relation to the ovule micropyles and found that pollen tubes reoriented in a manner consistent with each tube sensing a gradient across its tip. Our data also suggested that ovules continue to secrete their cues throughout the experiment. To refine our understanding, we developed a stochastic model of this guidance in which the mean direction of growth is influenced by the difference in the fraction of theoretical receptors bound by a ligand across the two sides of the tube tip. We modeled the cues as diffusing on the surface of the medium and modeled their secretion as continuous. Using fitting and simulation, we showed that this model recapitulates many of the features seen in vitro, and estimates the diffusion constant of the guidance cue. Implications for guidance in vivo are discussed.

OVULE DEVELOPMENT and POLLEN TUBE GUIDANCE ARE AFFECTED in the *SOLANUM CHACOENSE* MAPKKK *ScFRK1* MUTANT

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The *Solanum chacoense* Fertilization-Related Kinase 1 is a member of the MEKK subfamily of plant MAPKKK that is specifically expressed in ovules. *ScFRK1* mRNAs accumulate predominantly in the egg apparatus cells of the embryo sac and *ScFRK1* mRNA levels decrease rapidly following pollination. Transgenic plants down regulated in *ScFRK1* mRNA expression showed no abnormal phenotypes in vegetative tissues but produced parthenocarpic seedless fruits upon pollination. This phenotype could be linked to formation of aberrant embryo sacs, which did not progress further than the functional megaspore stage in affected transgenic lines. Since embryo sac integrity is a prerequisite for pollen tube guidance, we devised a semi *in vivo* pollen tube growth system to assess the ability of *ScFRK1* mutant ovules to attract pollen tubes. As expected, guidance was severely affected, confirming the involvement of the egg apparatus cells as the source of attracting molecules. In WT plants, acquisition of attraction competence was determined to be developmentally regulated and not pollination-induced. Species-specificity of the attraction signal was tested and was robust even with very close *S. chacoense* relatives from the Solanum *Petota* section, where natural interspecific hybridization is quite common among many species. This suggests a high discrimination level for the micropylar guidance phase. This also suggests that the attractant must be highly polymorphic, hinting towards a proteinaceous nature. This was further demonstrated with the use of crude and purified protein extracts. Proteomic as well as genomic strategies to isolate this guidance factor will also be presented.

OF MALE GAMETOPHYTES AND THEIR URGES FOR MODELS

José A. Feijó

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Despite copious amounts of detailed physiological and molecular data, the mechanistic regulation of growth in pollen tubes still lacks a consensual integrative model. While transcriptomics reveals the presence of about 7.000 genes, theoretical modeling shows that cooperation of all of these into two processes- wall surface and cytoplamic volume production- is condition enough to generate apical growth as we know it. Spatial and temporal integration of extended biochemical and biophysical processes is mandatory, and in the past we have propose and demonstrated that "ion dynamics"- which we define specifically as the regulation of ion membrane fluxes and cytosolic free ion concentration- is maybe a common denominator of this integrative processes, and follows the behavior of a dynamical attractor on the space phase (1,2). To test the hypothesis that membrane activity is sufficient condition for the formation of the intracellular patterns of cytosolic ion concentration, we are developing stringent 3-D theoretical modeling of ion fluxes and cytosolic diffusion based on the current knowledge of the system. These models are calibrated experimentally by comparison with the patterns observed in different species that show variations on the patterns of ion coreography. This calibration shows that while the current knowledge about membrane fluxes is sufficient to explain cytosolic pH patterning, it is not for cytolosic Calcium, where intracellular sequestering must play a role in the stabilization of the pattern. We'll then review the current knowledge that implies that, if in pollen tubes ion fluxes are polarized, then carriers for protons, calcium, chloride and potassium should also show non-linear patterns in space (polarized distribution of carriers) and in time (oscillatory and chaotic behaviors) (3). We''ll defend the need of extending this trend in order to be able to generate more comprehensive and testable models, and will illustrate with our recent work on the first proton pump P-ATPase from tobacco pollen (Nt AHA- Nicotiana H+ ATPase), which shows clear correlation with the flux patterns: NHA is excluded from the apex membrane by mechanisms involving polarized translation (4), actin cytoskeleton and membrane recycling. Nt AHA gets polarized soon after hydration, and its absence is predictive of the germination pore. We will futher discuss the minimal needs for channels to explain all the available evidence, and will present data showing the presence of novel chloride and calcium influx mechanisms.

(1)- Int.J.Dev.Biol. 49:595-614, 2005; Int.J.Dev.Biol. 49:615-632, 2005; (2) BioEssays, 23:86-94, 2001 (3) Int.J.Dev.Biol., doi:10.1387/ijdb.072296em, 2008; Sex.Plant Reprod., doi:10.1007/s00497-008-0076-x <http://dx.doi.org/10.1007/s00497-008-0076-x> , 2008 (4) Plant Cell, 20:614-634, 2008.

EXPRESSION PROFILE OF THE RICE MALE GAMETOPHYTE

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Field grown, disease-free anthers were collected from *Oryza sativa* ssp. *japonica*, cultivar Katy rice plants from Dale Bumpers National Rice Research Center and University of Arkansas Extension Station near Stuttgart, Arkansas, USA for use in characterizing genes expressed in the male gametophyte at anthesis. Triplicate biological samples were collected from three fields and processed separately at all subsequent steps and analyzed using the Affymetrix Rice 57K GeneChip®, a 25-mer oligonucleotide chip providing coverage of most of the rice genome. The Rice Annotation Project (version 5, http://rice.plantbiology.msu.edu/), International Rice Genome Sequencing Project (IRGSP, http://rgp.dna.affrc.go.jp/IRGSP/ and RAP-DB, http://rapdb.dna.affrc.go.jp/) were used as a genome annotation reference, facilitated by the RiceChip site (http://www.ricechip.org/). Affymetrix two-cycle cDNA synthesis kit was used to provide linear amplification of purified isolated RNA of pollen vegetative cells, sperm cells and seedlings. RMA, GCRMA, PLM, dChip and GCOS were used for comparative normalization of results. NCBI Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/, platform GPL2025) data from this microarray provided an additional source of data used to compare other sporophyte tissue sources. All results met quality control criteria and triplicate samples showed high consistency as indicated by Pearson coefficients of correlation exceeding 0.990 for pollen, 0.983 for sperm and 0.992 for seedlings (sporophytic control). Of experimental probe sets, 12,228 (21.4%) reported present or marginal in pollen, 16,120 (28.1%) in sperm and 22,565 (39.4%) in seedlings. Normalized for unique sequence motifs (an estimate of genes), total microarray coverage was 34,282 unique sequences, with 626 sequences (1.83%) unique for the pollen vegetative cell, 1668 (4.87%) unique for sperm cells, 5947 (17.35%) unique for seedlings, and 5537 (16.15%) found in all cells (thus representing true "housekeeping genes". Of shared sequences, those occurring in pollen and sperm were 750 (2.19%), pollen and seedling 1188 (3.47%), and sperm and seedling 2777 (8.10%). Transcriptome data for pollen was complemented by data from proteomic (Dai et al 2006a,b, Imin et al. 2004, Kerim et al. 2003) and MPSS (http://mpss.udel.edu/rice/) studies and reveals a complex and distinctive pattern of expression. Pearson's coefficient of correlation revealed $r=0.177965\pm0.007329$ for pollen versus seedlings, $r=0.249251\pm0.007159$ for pollen versus sperm cells and $r=0.239164\pm0.008182$, reflecting strong divergence in their transcriptional complements. On PCA analysis, pollen and sperm cells diverged strongly from sporophytic tissues, from each other and also from other available GEO datasets. This reflects a common theme with the pollen and sperm transcriptome of *Arabidopsis*, which is the only other species described at this level. The current study represents the first transcriptome of a monocot gametophyte or crop species.

CELL FATE DETERMINATION AND BASAL BODY FORMATION IN *MARSILEA* SPERMATIDS

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Spermatogenesis in the endosporic male gametophyte of the fern *Marsilea vestita* is a rapid process that occurs in the absence of new transcription. When the dry microspore is immersed in water, the single cell undergoes nine rapid division cycles to produce 32 spermatids and 7 sterile cells. We are interested in mechanisms that control cell fate in the gametophyte. Spermatids undergo profound morphogenesis to become spirally shaped, multiciliated gametes. The *de novo* formation of basal bodies (in a particle known as a blepharoplast) is a hallmark of spermatid specification. We previously showed that exon junction complex core components play key roles in defining cell fate by controlling patterns of centrin translation and consequent blepharoplast formation. We are now studying MvU620, which encodes a novel protein with a conserved RNA Recognition Motif from the RRM-1 super family. RNAi silencing of MvU620 suppressed basal body assembly and blocked cytoskeletal formation, with reduced levels of detectable α-tubulin in the cells. We are also studying the role of spermidine in gametogenesis. Spermidine is produced by spermidine synthase (SPDS) and moved into spermatids by a transporter protein (SPDT). Spermidine levels rise initially in jacket cells and later in spermatids. RNAi and drug treatments were employed to lower spermidine levels. Silencing of SPDS or SPDT caused developmental arrest before completion of the last division cycle. The nuclei elongated but did not condense, basal body positioning was altered and the microtubule ribbon was in disarray. After addition of a SPDS inhibitor, spermatid nuclei remained round, centrin failed to localize into basal bodies and the microtubule cytoskeleton failed to form. Spermidine is essential for specific events in early development and later during spermatid morphogenesis. Supported by NSF grant MCB-0720486.

POSTER TALK **P6-02**

FUNCTIONAL ANALYSIS OF THE *ARABIDOPSIS THALIANA* NECTARY TRANSCRIPTOME

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Many flowering plants attract pollinators by offering a reward of floral nectar. Remarkably, the molecular events involved in the development of nectaries (the organs that produce nectar), as well as the synthesis and secretion of nectar, are poorly understood. Indeed, no genes have been shown to directly affect the *de novo* production or quality of floral nectar. To address this gap in knowledge, we have used Affymetrix microarrays to follow global changes in gene expression in Arabidopsis nectaries at two developmental time points (pre-secretory and secretory nectaries), and we have also produced 10,000 ESTs from *Brassica rapa* (oilseed rape) nectary cDNA libraries. From this data we have identified over 300 genes that are expressed >5-fold higher in nectaries than in any other tissues examined, with a significant subset being upregulated at specific floral developmental stages. Several non-nectary-specific pathways, such as auxin metabolism and signaling, are also highly upregulated in nectaries. It is hypothesized that these highly expressed, nectary-specific genes and pathways are required for nectary development and/or function. Putative functions of these genes range from transcription factors to sugar transport and modifying enzymes. Preliminary results indicate that a number of candidate gene mutants display altered nectary and nectar phenotypes.

COMPARATIVE TRANSCRIPTOMICS OF ARABIDOPSIS THALIANA SPERM CELLS

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Despite the central role the sperm cells play during double fertilization in angiosperms we are only beginning to understand in which way they participate in this process. Sperm cells are passively transported to the embryo sac by the pollen tube, but preferential fertilization and delayed sexual fusion to achieve cell cycle synchrony indicate that a complex program of interactions between the fusion partners must exist. A further layer of complexity is added by epigenetic control of imprinting of paternal and maternal genes leading to mono-allelic expression. Genome-wide studies of the genetic basis underlying these observations were especially desirable for the model plant *Arabidopsis thaliana*, but were impeded because no method to isolate sufficient quantities of pure Arabidopsis sperm cells was available.

We have addressed this problem with a novel protocol based on Fluorescence-activated cell sorting, subsequently allowing us to apply GeneChip analysis to the transcriptome of Arabidopsis sperm cells. With 5829 different transcripts detected the mRNA pool of the male gametes shows a remarkable diversity, and it is clearly distinguishable from the transcriptomes of pollen and representative sporophytic tissues. Functional classification of genes with enriched expression in sperm cells revealed that DNA repair, ubiquitin-mediated proteolysis and cell cycle progression are over-represented classes. Furthermore, analysis of small RNA and DNA methylation pathways suggests that distinct mechanisms might be involved in regulating the epigenetic state of the paternal genome. Our study now enables us to address the role of the candidate genes identified directly in Arabidopsis.

EPIGENETIC TRANSPOSABLE ELEMENT REACTIVATION IN *WILD-TYPE* POLLEN

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In plants, the activity of transposable elements (TEs) are regulated by overlapping gene silencing mechanisms including RNAi, small RNA-mediated chromatin modifications, and transcriptional gene silencing. We have used a combination of bioinformatic and molecular techniques in the model plant *Arabidopsis thaliana* to identify a particular developmental tissue location and time where many of the genes responsible for silencing TEs are down regulated, resulting in TE reactivation. In mature pollen tissue, many different types of autonomous retrotransposons, DNA transposons and helitrons become transcriptionally active. We have shown that at least one DNA transposon actively transposes. Additionally, we have sequenced a small RNA library from mature pollen and found a similar shift in TE small RNA accumulation as in a chromatin-remodeling mutant deficient in TE silencing. Pollen is a gametophytic (haploid) tissue that contains three nuclei: a vegetative nucleus that controls pollen development, and two passenger sperm cells. Using GUS reporter lines and genetic analysis, we have determined that the vegetative nucleus is the location of TE activation in pollen. We are currently investigating how TE activation in the vegetative nucleus influences the silencing of TEs in the sperm cells, potentially resetting the epigenetic state of silenced TEs prior to fertilization. The activation of some animal TEs occurs in the cells adjacent to the germ cells, analogous to vegetative nucleus TE expression in plant pollen. Even though plants lack the piRNAs that animals use to target TEs for silencing in germ cells, there may be a common mechanism of reprogramming TE silencing in the male germ cells conserved between plants and animals.

Poster Presentations - Session 1

Pollen Development and Tube Growth

P1-03

LOSS OF MIKC*-TYPE MADS-DOMAIN PROTEINS CAUSES POLLEN GERMINATION AND TUBE GROWTH DEFECTS IN ARABIDOPSIS.

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Members of a divergent clade of MADS-domain transcription factors, called MIKC* proteins, have recently been shown to contribute to pollen function in Arabidopsis. We used a genetic approach to characterize the roles of six predicted MIKC* heterodimers during pollen development. Pollen competition assays with double, triple, and quadruple mutants have shown that AGL30-AGL104, AGL30-AGL66, and AGL65-AGL104 are functional complexes *in vivo*. In order to disrupt all MIKC* complexes simultaneously, we generated an *agl66 agl104* double mutant using strong loss-of-function alleles. The double mutant pollen is viable and morphologically normal, but germination on stigmatic surfaces is delayed and tube growth is impaired. As a result, double mutant pollen is unable to compete against wild type or single mutant pollen, and fertility is severely reduced. Additional pollen competition experiments were used to assess the relative contribution of each MIKC* heterodimer in pollen. We also examined MIKC* expression levels in MIKC* mutant backgrounds and found that the genes are subject to feedback regulation. Other MADS-box genes, such as pollen-enriched AGL18, appear to be direct targets of MIKC* activity. We are currently using higher-order mutants to investigate these and other factors that could be involved in a MADS-based regulatory network during gametophyte development. Supported by the UW-Madison Graduate School.

P1-04

A COLLECTION OF DS INSERTIONAL MUTANTS AFFECTING MALE GAMETOPHYTE DEVELOPMENT AND FUNCTION IN *ARABIDOPSIS THALIANA*

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Functional analyses of the *Arabidopsis* genome will require an intensive analysis of the gametophytic generation, since about 10% of the genes are estimated to be expressed in the male gametophyte and about 9% in the female gametophyte. In this study we present the genetic and molecular characterization of 67 Ds insertion lines showing reduced male gametophytic transmission. Based on genetic and cytological analysis and *in vitro* germination assays, the phenotypes were classified into five groups according to the developmental processes affected; these ranged from defects in early stages of gametogenesis to defects affecting post-pollination stages such as pollen germination, pollen tube growth, polarity or guidance, or pollen tube-embryo sac interactions or fertilization. Genomic sequences flanking both sides of the Ds element were recovered for 43 lines; for 18 the Ds elements were inserted in or close to coding regions, while 10 were located in intergenic/unannotated regions of the genome. For the remaining 15 lines, chromosomal rearrangements might be associated with the transposition event. The isolated mutants affect the expression of genes with putative functions in diverse Gene Ontology (GO) categories, and specify new functions for several unannotated or unknown proteins.

P1-05

ROLES OF LILY STIGMA/STYLE CYSTEINE-RICH ADHESIN (SCA) AND ARABIDOPSIS LIPID TRANSFER PROTEIN (LTP) IN PLANT POLLINATION/FERTILIZATION

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Lily pollen tubes grow adhering to an extracellular matrix (ECM), produced by the transmitting tract epidermis in a hollow style, delivering two sperm cells to the ovule for successful fertilization. SCA, a lily LTP, is involved in the pollen tube adhesion event via an ionic interaction with low-esterified pectin from the stylar ECM. In an attempt to understand the mechanism of action, we studied *in vitro* pollen tube adhesion activity and structures of two partially separated SCA isoforms (SCA1: 9370 Da, SCA3: 9484 Da). Of the two, SCA1 displays a higher activity in the *in vitro* pollen tube adhesion assay. One amino acid change, from Gly26 in SCA1 to Arg26 in SCA3, resulted in a significantly reduced volume for the internal hydrophobic cavity in SCA3. This result indicates that SCA1 may interact with a ligand for its function. To expand our understanding of the biological function of SCA-like molecules, we utilized the genetic system of *Arabidopsis thaliana*. Through an Arabidopsis genome-wide search, we identified a SCA-like Arabidopsis LTP group. Using SALK TDNA insertion lines, we found that a mutation in a SCA-like LTP resulted in some sterility, mainly due to abnormal pollen tube morphology and behavior in the pistil. In reciprocal cross-pollinations to wild-type, *in vivo* growth of the mutant pollen tubes was significantly decreased, resulting in decreased seed set. The mutant pistil also showed defects in seed production, even when wild-type pollen was used. This LTP may play a role in both pollen and pistil function in Arabidopsis fertilization.

P1-06

OPTIMIZATION OF *IN VITRO* GROWTH CONDITIONS FOR FROZEN STORED *ARABIDOPSIS THALIANA* POLLEN

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One of the rare weak points of the model plant *Arabidopsis* is the technical problem associated with the germination of its male gametophyte and the generation of the pollen tube *in vitro*. *Arabidopsis* pollen being tricellular it has a notoriously low *in vitro* germination rate compared to species with bicellular pollen. This drawback strongly affects the reproducibility of experiments based on this cellular system. Together with the fact that pollen collection from this species is tedious, these are obstacles for the standard use of *Arabidopsis* pollen for experiments that require high numbers of pollen tubes and for which the germination rates under control conditions need to be highly reproducible. The possibility of freeze storing pollen after bulk collection is a potential way to solve these problems but necessitates methods that ensure continued viability and reproducible ability to germinate.

Our objective was the optimization of germination conditions for *Arabidopsis* pollen that had been freeze stored. We optimized the concentrations of various media components conventionally used for *in vitro* pollen germination. In addition to these components, medium pH and growth temperatures were tested.

Here we summarize the optimized conditions for pollen germination and growth in different media and under different experimental setups. We suggest how to optimally use these methods for different practical experiments ranging from morphological observations of pollen tubes in optical and electron microscopy to their bulk use for molecular and biochemical analyses or for setups for which a specific medium stiffness is critical.

P1-07

ROLES OF ARABIDOPSIS RETINOBLASTOMA HOMOLOGUE (ATRBR1) IN BOTH MEIOSIS AND MITOSIS DURING MALE GAMETOGENESIS

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The protein Retinoblastoma (pRb) was identified as the first tumor suppressor and has been shown to control the G1/ S cell cycle progression. The model plant *Arabidopsis thaliana* contains single retinoblastoma related gene (*RBR1*).

We found that *rbr1* mutations lead to cell over-proliferation. Pollen mitosis I (PM I) is almost normal in *rbr1* mutant as in wild type. The asymmetrical cell division produces the large vegetative cell and the small generative cell. Followed PM I about half of the *rbr1* pollen could undergo an extra mitosis taken place in the vegetative cell. The extra cell usually shows vegetative identity, but occasionally it looks like a generative cell. Interestingly, sometimes it appears in an intermediate cell size accompanied with weak expression of generative cell marker. The extra mitosis happens to the sperm cells as well, producing the pollen grain with four sperm cells. These findings implicate that RBR1 not only represses cell proliferation, but also restricts the plasticity of cell fate determination during pollen development.

More strikingly, we also observed a role of RBR1 in meiosis. At the end of prophase I *rbr1* mutant contains 10 fully condensed chromosomes in contrast to wild type which has five bivalents of homologous chromosomes. At anaphase I unpaired chromosomes segregate randomly. The second meiotic division produces cells with more than four pools of chromosomes. We are still elucidating whether the defect is due to an accelerated meiotic cell cycle in *rbr1* or because RBR1 is involved in meiotic-specific pathways to control recombination and pairing.

P1-08

THE ROLE OF THE ACTIN CYTOSKELETON IN THE ELONGATION AND TROPISM OF THE POLLEN TUBE.

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During the passage of the pollen tube through the stylar tissue, it has to produce an invasive force and to resist external mechanical compression forces against which it needs to grow and maintain its tubular shape. To find its way inside the style, the pollen tube has to be able to perceive and to react to external signals. At several instances it needs to drastically change its growth direction to be able to enter the micropyle and deliver the sperm cells to the egg apparatus. The pollen tube, therefore, represents an excellent model system to investigate tropic responses in individual plant cells.

We are interested in the role the actin cytoskeleton plays in the elongation growth and tropic re-orientation of the pollen tube. Actin is involved in the transport of secretory vesicles and possibly affects cell morphology by direct mechanical interaction. We pharmacologically reduced or inhibited vesicle flux in order to assess how this affects pollen tube elongation and cell wall composition.

The actin cytoskeleton forms a fringe-like structure close to the growth zone of the pollen tube, representing an active polymerization/depolymerization site. To elucidate the role of this cytoskeletal feature for the development and the control of cell shape, we reduced its dynamics using actin cross-linkers. We combined this approach with an in vitro induction of tropic behavior through application of an electrical field.

Our results show that the actin cytoskeleton is a key feature in determining cell shape and the growth direction in the polarly growing pollen tube.

P1-09

COMPARING POLLEN GERMINATION IN *ARABIDOPSIS THALIANA* ECOTYPES AND OTHER BRASSICACEAE

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Pollen grains deliver and release pollen tube cells to the receptive stigma surface. The long-taught model of pollen cell release, or germination, is that stigma fluid enters a desiccated grain through an aperture in the pollen wall, thereby directing the pollen tube to exit through that same aperture. Surprisingly, *Arabidopsis thaliana*'s pollen germination differs from this model; *Arabidopsis* pollen tubes break directly through the exine wall at its point of contact with the stigma surface, regardless of aperture position. We are surveying germination behaviors between *A. thaliana* ecotypes (Col, Ler, BayO, Sha) and across taxa, focusing especially on the Brassicaceae. We find that *A. thaliana* pollen is not alone in its germination behavior. Of 30 Brassicaceae family members studied, eight species germinate by breaking through durable wall, despite nearby apertures. These eight species are distributed across six tribes. Additionally, we find quantitative differences in aperture use between: 1) the break-out species (ranging between 12 and 67% break-out), 2) *A. thaliana* ecotypes (ranging between 41 and 67% break-out), and 3) in vivo and in vitro germination conditions (break-out declines by almost half in vitro). We now believe that the focused rupture of the wall depends biomechanically upon local swelling of a pectin gel beneath the exine, likely together with weakening of the overlying exine structure. We used atomic force microscopy to measure material properties of *A. thaliana*'s exine wall, and are now using SEM and TEM to seek patterns in structure that associate with these variations in function.

P1-10

SIGNALLING AND CELL SPECIFICATION IN THE ANTHER MICROSPORANGIUM OF *ARABIDOPSIS THALIANA*

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The development of germ cells in plants is held to be different from that of animals, relying on positional signals rather than cell lineage. However, acting downstream of *AGAMOUS*, a set of 'germline' genes directs the formation of the sporocytes, and their surrounding ancillary cells through a system involving cell lineage – reminiscent of animal development. Here a single subepidermal archesporial cell divides and differentiates into the sporocytes and parietal tissue layers to generate the concentrically-organised microsporangium [1].

To throw further light on this unusual system of microsporangial development, and in particular to identify factors controlling cell specification within the lineage, we have focused on the role of the LRR receptor kinase *EXCESS MICROSPOROCYTES1* (*EMS1*) in *Arabidopsis thaliana* [2,3]. *ems1* mutants form an excess of sporogenous cells but no tapetal cells. We have expressed *EMS1* under a range of promoters. Study of the phenotypes resulting suggests that EMS1, together with its ligand TPD1 [4], not only determines tapetal specification and early function, but also regulates cell proliferation. Interestingly the number of sporogenous and tapetal cells in the loculus seems to depend on the timing of ectopic EMS1 expression, and that signals between the two cell populations set the final cell numbers in each. Sporangial development thus emerges as regulated by a rigid 'lineage' system, rare in plants, where each cell type is specified by a unique combination of signals, and cell number is controlled by cross-talk between developing tissues. How this unique sporogenous pathway is coordinated with the development of the anther 'envelope' [1] remains a challenge for the future.

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P1-11

EXPRESSION OF A TRANSLATION INHIBITOR IN THE ARABIDOPSIS GENERATIVE CELL PRODUCES SINGLE GERM CELLS THAT PREFERENTIALLY TARGET THE CENTRAL CELL

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The male germ line of flowering plants undergoes two rounds of mitosis after meiosis to produce two sperm within the pollen cytoplasm. These sperm are delivered to an ovule by a pollen tube where one fuses with the egg to form the embryo and the other fuses with the central cell to form the endosperm. A long-standing question about this system, known as double fertilization, is whether the two sperm are developmentally equivalent and equally likely to fuse with either female gamete; or are the two sperm distinct, fusing with specific targets? By expressing the diphtheria toxin A subunit (DTA), a potent translation inhibitor, from the male germ line-specific *HAP2* promoter, we blocked sperm development before the final cell division and produced pollen tubes that carry a single germ cell (SGC) rather than two sperm. SGCs preferentially fuse with the central cell and initiate endosperm-only seed development when they reach an ovule. Using this new tool, we show that *de novo* translation is required for completion of sperm development, that the *HAP2* promoter is very tightly controlled and activated before the second pollen mitosis, and that male germ cells can be forced to take on a preferential target for gamete fusion. These data suggest that the two sperm carried within a single pollen tube may have distinct gene expression profiles that define their targets for gamete fusion.

P1-12

CONTROL OF MEIOTIC RECOMBINATION IN *ARABIDOPSIS*: A COORDINATING ROLE FOR COMPONENTS OF THE SYNAPTONEMAL COMPLEX AND CHROMOSOME AXES.

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Accurate chromosome segregation in meiosis requires the formation genetic crossovers between homologous chromosome pairs. Understanding the factors that control meiotic recombination is of great agronomic and medical significance yet poorly understood. One important feature of meiotic recombination is that it occurs in the context of dramatic changes in chromosome organization. In prophase I protein axes are elaborated along the length of each homolog. The homologues then align and undergo synapsis when they become intimately linked by a proteinaceous structure, the synaptonemal complex (SC). Towards the end of prophase I when recombination is complete the SC breaks down and the first meiotic division occurs. Studies in *Arabidopsis* are uncovering how interplay between the chromosome axes and SC with the recombination machinery is crucial for the regulated crossover formation. We have been focussing on two proteins ASY1, a component of the protein axes and the SC protein AtZYP1, which are essential for chromosome synapsis and normal crossover formation. Recently we demonstrated that ASY1 is required to co-ordinate the loading of AtDMC1, a RecA homolog that works in conjunction with AtRAD51 to mediate strand exchange between non-sister chromatids. Absence of ASY1 results in destabilization of AtDMC1 loading, such that inter-homolog recombination fails. Analysis of AtZYP1-deficient lines has enabled us to demonstrate that contrary to previous proposals the SC is not essential for the imposition of crossover interference. Instead, we have found that AtZYP1 prevents non-homologous recombination and revealed a role for the SC in the prevention of chromosome interlocks during meiotic prophase I.

P1-13

CHEMICAL GENETICS APPROACH TO STUDY ROP PATHWAY THAT REGULATES TIP GROWTH IN ARABIDOPSIS

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ROPs are plant Rho-related GTPases that are important regulators of various cellular processes including cell polarity and tip growth. Facilitated by its regulatory proteins, ROPs cycle between GTP-bound active form and GDP-bound inactive form. In pollen tubes, ROP activation has been shown to affect actin dynamics and cytosolic calcium accumulation. However, the cause-and-effect relationships between activation of ROP pathway and the aforementioned components (actin dynamics and cytosolic calcium) still need to be elucidated. Using chemical genetics approach, we aim to identify and utilize small molecules that target interaction between active ROP and its negative regulator GTPase-activating protein (GAP). Chemical genetics approach is more advantageous than conventional genetics to study ROP pathway because the rapid, reversible, and tunable effect of small molecules can be applied at particular developmental stage or tissue type. Inhibition of ROP and GAP protein interaction by small molecules would result in accumulation of active ROP whose effects can then be monitored spatially and temporally. Yeast-two-hybrid screen was performed to identify small molecules that inhibit ROP – GAP protein interaction and several putative hits were identified from the screen. *In vitro* protein interaction assays were then performed which confirmed the yeast-two-hybrid results. In addition, chemical treatments on pollen tube produced expected tip swelling phenotype. Further studies would be to confirm the chemical effects on inhibition of protein-protein interaction *in vivo*.

P1-14

REGULATION OF THE FIRST ASSYMETRIC DIVISION OF THE ARABIDOPSIS ZYGOTE BY THE YDA SIGNALING PATHWAY.

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The first division of the Arabidopsis zygote is asymmetric and generates two daughter cells with different developmental fates. The smaller apical cell goes on to produce the proembryo, while the larger basal cell forms the mostly extra-embryonic suspensor. We are interested in understanding how this first division of the zygote is regulated. Previously, we have shown that the MAPKK kinase gene *YODA* (*YDA*) promotes extra-embryonic fate in the cells of the basal cell lineage. *yda* loss-of-function mutants lack the suspensor, while hyperactive YDA variants suppress formation of the proembryo. Through forward and reverse genetic approaches, we have identified several genes which may function in the YDA signaling pathway. Mutations in the *SHORT SUSPENSOR* (*SSP*) and *GROUNDED* (*GRD*) genes cause yda-like embryonic phenotypes. The *SSP* gene encodes a receptor-like cytoplasmic kinase which seems to act upstream of YDA in this signaling event. Our data suggests that *SSP* mRNA accumulates in sperm cells of mature pollen and is delivered to the zygote during fertilization where SSP triggers the activation of the YDA MAP kinase cascade. Thus, *SSP* constitutes a pollen-derived temporal cue that links the onset of YDA-dependent signaling to fertilization. The *GRD* gene encodes a putative transcription factor which may be the nuclear target of the YDA signalling pathway. Our results that GRD protein localizes to the nucleus, and that the grd mutation is epistatic to the hypervariant YDA support this model.

P1-15

ENDOCYTOSIS AND RETROGRADE TRANSPORT IN POLLEN TUBES

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Pollen tubes are elongated single cells that grow through the pistil and deliver sperm cells to the ovule. Interactions between the pollen tubes and pistil are essential for successful fertilization, and are mediated by the pistil extracellular matrix (ECM). Internalization of plasma membrane and vigorous vesicle movements are well documented in pollen tubes growing in culture. However, specific uptake mechanisms (endocytosis) of external molecules (cargo) and their subsequent trafficking (retrograde transport) in plants have not been extensively studied due to lack of defined cargo. In *Nicotiana alata*, secreted ECM pistil proteins have an essential role in self-incompatibility, a mechanism for inhibiting growth of self pollen tubes. Previous immunolocalization studies showed that these pistil proteins are taken up by the pollen tube in bulk and delivered to the vacuole. Our goal is to extend these studies and investigate 1) the type of endocytosis accounting for pistil protein uptake; 2) retrograde transport of specific cargo; 4) endocytosis and retrograde transport *in planta.* Thus far, we have observed Lucifer Yellow, a fluid phase endocytosis marker and pistil proteins are endocytosed by the pollen tube grown in the culture and *in planta*. Observations have been made with transgenic tobacco lines expressing compartment markers fused to GFP or a cytosolic RFP in pollen and secreted CyPet in the transmitting tract cells of pistil.

P1-16

TWO C2 DOMAIN-CONTAINING PROTEINS ARE ESSENTIAL FOR POLLEN DEVELOPMENT IN *ARABIDOPSIS THALIANA*.

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Pollen development is a complex process involving a myriad of cellular activities, resulting in the production of a three-celled male gametophyte. Aiming to get a better understanding of the molecular events implicated in pollen development, we began to search for new genes involved in this process in *Arabidopsis thaliana*. We used a novel approach that combines gene expression profiles in different pollen development stages, followed by a simple screening of insertional mutants of candidate genes based on pollen viability. Using this strategy, we were able to discover new pollen-essential genes in Arabidopsis that were named *DEFECTIVE IN POLLEN DEVELOPMENT* (*DPD*). Two of them encode proteins that share a calcium-dependent membrane binding domain (C2 domain): *DPD1* encodes a plant specific protein with a N-terminal C2 domain, whereas *DPD2* encodes an armadillo/β-catenin repeat protein with a C-terminal C2 domain. Nothing is known about the role these proteins play in plants, but based on our preliminary results it appears quite evident that these proteins are playing a fundamental role in pollen development. Furthermore, given the presence of the C2 domain in both proteins, it appears quite appealing that the binding to membrane mediated by this domain may play a role in the biological function of these proteins. We are characterizing the cytological aspects of the phenotype in mutant pollen grains and starting the functional characterization of both proteins. In addition, our method of targeted identification of pollen-essential genes allowed us to identify other five genes that are essential for pollen development.

P1-17

ARABINOGALACTAN PROTEINS 6 AND 11 ARE REQUIRED FOR STAMEN AND POLLEN FUNCTION IN ARABIDOPSIS.

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Arabinogalactan proteins (AGPs) are cell-wall associated, highly complex glycoproteins which are widely distributed within the plant kingdom. AGPs have been linked to pollen and pollen tube development and function. However, AGPs involvement in these processes has been demonstrated only indirectly. Our study reveals the involvement of two highly similar AGPs from *Arabidopsis*, *AGP6* and *AGP11,* in pollen tube growth. Using RNAi lines with reduced *AGP6* and *AGP11* expression levels and lines harboring mutations in the *AGP6* coding region, we show that defects in these genes lead to reduced fertility. Specific expression of *AGP6* and *AGP11* in anthers and pollen, together with the absence of *AGP6* and *AGP11* expression in the gynoecium, suggests that these genes are required for male function in reproduction. Indeed, our results show that mutations in *AGP6* or reduction of *AGP6* and *AGP11* expression by RNAi causes inhibition of pollen tube growth and hampers pollen release. Thus, our study demonstrates that *AGP6* and *AGP11* are required for successful male reproductive function.

P1-18

HAPLESS MUTATIONS AFFECTING SPERM DEVELOPMENT AND THE PATERNAL CONTRIBUTION TO EMBRYOGENESIS

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Very few mutations have been characterized that disrupt sperm development, double fertilization, or the paternal contribution to embryo development. We rescreened a collection of 36 male gametophyte (*hapless*) mutants and found that 22 affected male gametophyte development. Twelve caused pollen grain structural defects, 10 caused loss of the vegetative nucleus or both sperm cells, and 17 resulted in pollen grains that carried a vegetative nucleus and a single generative cell (SGC) rather than two sperm. We asked whether SGCs produced by these mutants were able to fertilize ovules and if so, whether they preferentially fertilized either the egg or the central cell. *hap7-1* SGCs were equally likely to fertilize the egg or central cell; while *hap23* SGCs may preferentially fertilize the egg. *hap7-1* pollen grains frequently develop normally and contain two sperm; however, when these sperm fertilize a wild-type ovule, the resulting embryos have an enlarged suspensor and a lethal loss of apical-basal polarity. This is one of few paternal effect mutations described in *Arabidopsis*. *hap7-1* was generated by a T-DNA insertion in At5g10920, which is predicted to encode an argininosuccinate lyase and is likely expressed ubiquitously. Future experiments will be aimed at understanding the molecular basis of this interesting paternal effect. Our screen indicated that there are likely a large number of genes that contribute to proper male gametophyte development, that some of these also control embryo development, and that some may be involved in determining target preference for the two sperm during double fertilization.

P1-19

TWO CATION/PROTON EXCHANGERS ARE INVOLVED IN POLLEN TUBE GUIDANCE

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Higher plant reproduction depends on accurate delivery of sperms to the ovule by the pollen tube. Guiding signals from the female cells are being identified, though how the pollen senses, interprets and responds to the signals is largely not known. About 15 CHX genes are expressed specifically and preferentially in pollen though their functions are unknown (Sze et al, 2004). A reverse genetics approach was used to determine the function of two related genes CHX-A and CHX-B. Single T-DNA insertion mutants (*aa* or *bb*) showed similar in vitro pollen germination and tube growth to that of wild-type. However, male gene transmission was specifically blocked when both genes are defective. When wild-type pistil was pollinated with a limited number of pollen grains from Aabb plants, seed set was reduced about half of that from AAbb plants, which suggest *ab* pollen is infertile. *ab* pollen tube elongates similarly to *a*B pollen. However, unlike aB pollen, double mutant pollen fails to find the ovule and mainly grows inside the transmitting tissue. CHX-B protein fused to GFP was localized to PVC/vacuole when transiently expressed in mesophyll protoplast. These results demonstrate that CHX-A and CHX-B perform overlapping but essential function in male fertility. To our knowledge, this is the first transporter mutant with a defect in pollen tube guidance. The cellular and molecular bases of this intriguing phenotype are under investigation.

Reference:

Sze H, Padmanaban S, Cellier F et al. (2004) Plant Physiology 136: 2532-2546

P1-20

MITOCHONDRIAL DYNAMICS IN PLANT MALE GAMETOPHYTE STUDIED BY FLUORESCENT LIVE IMAGING

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Live imaging of organelles is a powerful method for studying their dynamic behaviors. Here we attempted to visualize mitochondria in the pollen grain of *Arabidopsis thaliana* that are composed of one vegetative cell (VC) and two sperm cells (SCs). Combination of mitochondria-targeted fluorescent proteins with VC- or SC-specific expression allowed us to observe precise number and dynamic behavior of mitochondria in the respective cell types. Furthermore, live imaging of SC mitochondria during double fertilization confirmed that sperm mitochondria enter into egg and central cells. We also attempted to visualize mutant mitochondria that were elongated due to a defect in mitochondrial division. This mutant phenotype was indeed detectable in VC mitochondria of a heterozygous F1 plant, suggesting active mitochondrial division in male gametophyte. Finally, we performed mutant screening and isolated a putative mitochondrial protein-transport mutant whose phenotype was detectable only in haploid cells. The transgenic materials presented in this study are useful for not only live imaging but also studying mitochondrial functions by mutant analysis.

Reference)

Matsushima, R., Hamamura, Y., Higashiyama, T., Arimura, S.-i., Sodmergen, Tsutsumi, N., Sakamoto, W. Mitochondrial dynamics in plant male gametophyte visualized by fluorescent live imaging.
Plant and Cell Physiology in press

Matsushima, R., Hu, Y., Toyoda, K., Sodmergen, Sakamoto, W.
The model plant *Medicago truncatula* exhibits biparental plastid inheritance
Plant and Cell Physiology 49: 81-91, 2008

P1-21

TWO UDP-GLUCOSE PYROPHOSPHORYLASE, ATUGP1 AND 2, PLAY AN ESSENTIAL AND REDUNDANT ROLE IN PLANT GROWTH AND POLLEN DEVELOPMENT IN *A.THALIANA*

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UDP-glucose pyrophosphorylase (UGPase) is an important enzyme for a synthesis of UDP-glucose, which acts as a precursor for the synthesis of the carbohydrate and cell well components. The *Arabidopsis thaliana* genome contains two putative UGPase genes, *AtUGP1* and *AtUGP2.* Both genes are expressed in roots, siliques, leaves, flower buds, and open flowers. In order to determine the role of UGPase in plant growth and anther development, we approached a reverse genetic study using the T-DNA insertion mutants (*atugp1* and *atugp2*). Despite significant decrease of UGPase activity in *atugp1* and *atugp2* single mutants, none was essential for normal growth. In contrast, *atugp1/atugp2* double mutant displayed dramatic general growth defects and male sterile, suggesting that AtUGP1 and AtUGP2 function redundantly. In the anther of *atugp1/atugp2*, pollen mother cells were normal, but normal callose deposition was not observed in tetrads. Interestingly, vegetative growth of double mutants was restored in the 1.5% sucrose medium. Taken together, these data suggest that the AtUGP1 and AtUGP2 genes are functionally redundant and UGPase activity is required for callose deposition in pollen development and plant growth in Arabidopsis.

P1-22

EXTRACELLULAR ATP REGULATES POLLEN GERMINATION AND POLLEN TUBE GROWTH VIA NITRIC OXIDE

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Pollen tubes release ATP into their extracellular matrix as they grow. Recent reports suggest that extracellular ATP (eATP) may play a role in plant signaling, and induce diverse responses ranging from increased cytosolic calcium to changes in auxin transport. The results reported here focus on potential signaling pathways that may connect the stimulus of eATP to growth changes, using pollen tubes as the test material. Raising the concentration of eATP either by adding it to the medium or by suppressing the expression of ectonucleotidases that remove eATP inhibits both pollen germination and pollen tube growth. The same inhibitory effects are observed by increasing the medium concentration of ATPγS, a poorly-hydrolysable ATP analog, but not of AMPαS. Because pollen tube elongation is also sensitive to nitric oxide (NO), we are investigating whether there is a connection between the two pathways, using chemical and genetic approaches. Thus far we have found that increasing the medium concentration of eATPγS induces increased NO levels in pollen tubes, chemical agonists of the NO signaling pathway lower the threshold of eATPγS that inhibits pollen germination, and an antagonist of the NO signaling pathway blocks the ATPγS-induced inhibition of both pollen germination and pollen tube elongation. These results suggest that increases in eATP are transduced into inhibitory growth changes in pollen via the NO signaling pathway. To further test this hypothesis, we are investigating the eATPγS response of mutants defective in NO production. (Supported by NSF grant to SJR).

P1-23

THE AMBROSIA PROJECT: A CLASSROOM PROJECT FOR CHARACTERIZING A CDNA LIBRARY FROM GIANT RAGWEED POLLEN

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It has been estimated that up to 20% of the population is allergic to Ragweed pollen causing significant reductions in work/school productivity and millions of doctor's office visits. Pollen biology, therefore, is an excellent way to link plant science with medicine. A major source of allergenic pollen is Giant Ragweed (Ambrosia trifida L.), a common weedy plant. In spite of this species' impact on public health there are only six nuclear DNA sequences from A. trifida in the NCBI nucleotide database (as of Sept. 2008). To examine gene expression in Giant Ragweed pollen we constructed a cDNA library using mRNA isolated from dehiscing male flowers. Botany students characterize the library as one of their laboratory exercises. The Ambrosia Project is a collaborative effort by which students randomly select cDNA colonies for plasmid purification, and perform restriction digestion, gel electrophoresis and sequencing. Once sequenced, the students distinguish cDNA from vector and predict the translation product. Students do BLAST searches, learn about the gene product, and speculate on its function in plants. Students have already isolated cDNA clones that are homologous to metallothionein-like genes, ferritin, auxin response factor-2, and a sweet-tasting thaumatin-like protein. Numerous genes for allergenic proteins have also been identified, including lipid transfer proteins and pectate lyase. Students appear to enjoy the prospect of discovering unique genes in this uncharacterized species, and like seeing their names published in the NCBI EST database. At the same time they learn important molecular techniques and concepts that are applicable to other biological disciplines.

P1-24

MOLECULAR STUDIES ON BT-, LD- AND CW-TYPES OF CYTOPLASMIC MALE STERILITY IN RICE

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Cytoplasmic male sterility (CMS) is often associated with an unusual open reading frame (*orf*) found in mitochondrial genomes, and is considered to be caused by genome barriers between nuclei and mitochondria. In many CMS lines, pollen fertility is recovered by a nuclear-encoded gene known as a fertility restorer gene (*Rf*). We have been studying three types of CMS rice: BT-CMS originated from Chinsurah Boro II, LD-CMS from Lead Rice and CW-CMS from Chinese Wild rice. We have shown that the *Rf1* gene for BT-CMS encodes pentatricopeptide repeat (PPR)-containing protein. In the BT-CMS line, the mitochondrial genome of the BT-cytoplasm contains two duplicated copies of the *atp6* gene. It has been reported that a unique sequence (*orf79*) located downstream from one of *atp6* genes causes male sterility. We revealed suppression mechanism of mitochondrial ORF79 accumulation by Rf1 protein. ORF79, however, is not likely to be a cause of LD-CMS. In contrast*, Rf17 for* CW-CMS was revealed to be a gene encoding acyl-carrier protein synthase-like domain. We found that reduced expression allele of *Rf17* gene restored fertility in haploid pollen, while normal expressing allele causes pollen to die in CW-CMS. Comparison of three types of CMS/*Rf* system with a relationship to mitochondrial retrograde signaling will be presented.

P1-25

THE SIGNIFICANCE OF POLLEN TUBE SHAPE FOR UNDERSTANDING DYNAMIC TIP GROWTH

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The growing region at the apical end of pollen tubes has frequently been modeled as a spherical shell, its shape maintained by an unvarying spatial pattern of cell wall expansion rates. Using high resolution wide-field fluorescent microscopy to visualize growing pollen tubes and digital analysis of tip shape, we have found that the apical dome of lily pollen tubes is only occasionally spherical. Typically, the shape of the tip varies rhythmically between an oblate spheroid and a spheroid. Pollen tubes in oscillatory growth show a periodic change in apical shape with the same frequency as changes in growth rate and a shift in phase. Curvature analysis of tip shape in two dimensions revealed not only stable oscillations in shape, but also a more subtle pattern in which the region of reduced curvature was seen to move back and forth across the tip as growth oscillated. Since the driving force for cell expansion, turgor, is high in these cells, and the rigidity of the cell wall is also high, it is unlikely that these shape changes are the result of transient changes in local turgor pressure. Rather, dynamic changes in wall curvature must be the result of dynamic changes in cell wall properties.

P1-26

***RUPTURED POLLEN GRAIN 1*, AN MTN3/SALIVA FAMILY GENE, PLAYS AN IMPORTANT ROLE IN MICROSPOROGENESIS AND POLLEN WALL DEVELOPMENT IN ARABIDOPSIS**

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During microsporogenesis, the plasma membrane of microsporocytes (microspores) plays multiple roles in pollen wall development. Nevertheless, plasma membrane proteins that participate in these processes are still not well known. We have characterized a plasma membrane protein-encoding gene, *Ruptured Pollen Grain 1* (*RPG1*), which is required for exine pattern formation and cell integrity of microspores in *Arabidopsis thaliana*. The *rpg1* mutant exhibits severely reduced male fertility with otherwise normal phenotype. Histological analysis suggested that the microspores are mostly aborted during post-meiotic microsporogenesis in *rpg1*. Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) examination revealed that primexine formation of *rpg1* microspores is aberrant at the tetrad stage, which results in defective sporopollenin deposition on microspores and locule wall. *RPG1* is an MtN3/saliva family gene, which is expressed specifically in microsporocytes and tapetum during male meiosis. RPG1 is integral to the plasma membrane. Furthermore, phylogenetic analysis and a phosphor-mimic experiment demonstrated that the conserved serine residues in the intracellular regions of the protein are critical for RPG1's function.

P1-27

DECIPHERING THE RELATIONSHIP BETWEEN CELL MECHANICS AND POLLEN TUBE GROWTH DURING OSCILLATORY GROWTH

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The pollen tube is a perfect experimental model to study plant cell growth and morphogenesis as its growth rate is extremely fast and its expansion is unidirectional. Just as in other plant cells, the mechanical properties of the pollen tube rely to a significant degree on the cell wall architecture. Plant cell growth results from the interplay between cell wall mechanical properties and the hydrostatic pressure that act as a driving force. The nature, structure and spatial distribution of the polysaccharide polymers in the cell wall are assumed to define the plant cell's ability to grow and are thus responsible for the cylindrical shape of the pollen tube. Intriguingly, the pollen tube growth rate typically is not steady but it rather oscillates in with a regular, species-dependent frequency. Numerous intracellular processes have been shown to oscillate together with the growth rate albeit not necessarily in phase with it. In order to decipher the causal relationship between cell wall mechanics and pollen tube growth, we assessed the temporal changes in mechanical properties of the pollen tube cell wall in the growing region using micro-indentation. We identified the temporal correlation between cell wall softening and growth rate using Fourier and cross-correlation analyses. Our results are consistent with our previously proposed mechanical model of the oscillatory growth as being the equivalent of a mechanical harmonic oscillator.

P1-28

SR45, A SPLICING FACTOR, IS REQUIRED FOR NORMAL POLLEN DEVELOPMENT.

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Pollen development is crucial for reproduction in flowering plants. In a previously identified splicing factor mutant, *sr45-1*, we found that production of both pollen and seeds was significantly reduced. In addition to several other phenotypes, we observed that the stamens were shorter and anthers were less full, both of which could contribute to reduced pollination. A more interesting phenotype was revealed by a close look at pollen grains. Instead of having three nuclei, mutant pollen have only one vegetative nucleus and one sperm nucleus, indicating that either no nuclear division occurred in the generative nucleus in mutant pollen, or one of the sperm nuclei disappeared after the division. SR45 is one of 19 SR protein splicing factors in *Arabidopsis*. In order to explore the possibility of cross-regulation, we examined the expression pattern of a distinct SR protein, *SR1*, in both wild type and *sr45-1*, using a *SR1-GFP*-fusion gene whose expression is dependent on both normal transcription and normal splicing. GFP fluorescence was found in all three nuclei in wild type pollen with the strongest signal in the vegetative nucleus. However, in *sr45-1* mutant pollen, the GFP signal was much weaker and was detected in only the vegetative nucleus. These results suggest that SR45 may regulate pollen development by altering the splicing pattern of other genes, including *SR1*. More gene targets are still under investigation.

Poster Presentations- Session 2

Female Gametophyte Session

P2-03

DISTINCT EXPRESSION PATTERNS OF TWO TYPE II ARACS IN ARABIDOPSIS

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RAC/ROP GTPases are molecular switches that shuttle between active GTP-bound and inactive GDP-bound states affecting plant growth and developmental signaling. There are eleven Rac/Rop genes, Arac 1-11(ROP1-11), in Arabidopsis and they share more than 70% amino acid identity with each other. The high sequence homology among the Arac family members has led to the suggestion that these proteins might be functionally redundant. Arac7/ROP9 and Arac10/ROP11 are closely related and belong to what are classified as type II Aracs – they are further classified into group 1 and group 2 Aracs, respectively, within type II. The data illustrated here shows that Arac7 has a pattern of expression clearly distinct from that of Arac10. Using the transgenic expression of Arac7 promoter fused with GUS, we see expression of Arac7 throughout the developmental stages of embryos, in the early stages of lateral root formation and in the root tips. On the other hand, Arac 10 has a broad vegetative expression pattern. In particular contrast with Arac7's promoter activity, Arac10 promoter drives high expression in the guard cells and in reproductive parts, namely stigma and pollen, where Arac7 is inactive. Furthermore, the activity of the Arac7 promoter is stimulated by auxin and repressed by ABA, hinting at a role for Arac7 in these two hormone signaling pathways. Functionally, it would be interesting to examine the role of Arac10 in pollen-pistil interacting pathways and Arac7 in embryo and early seedling development.

P2-04

A NOVEL TOBACCO STIGMA/STYLE-SPECIFIC GENE, SCI1, IS A COMPONENT OF THE SIGNAL TRANSDUCTION PATHWAY CONTROLLING CELL PROLIFERATION/DIFFERENTIATION.

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The success of plant reproduction depends on the appropriate development of the reproductive organs that involve specific regulatory networks. We have undertaken the characterization of a gene that encodes a small lysine-rich protein with both putative nuclear localization signal and cyclin interaction domain, plus 21 predicted phosphorylation sites. The protein-GFP fusion was targeted to the interchromatin region, inside the nucleus, and showed specificity to some nuclear bodies. Real time RT-PCR and *in situ* hybridization experiments revealed the developmental regulation of the mRNA that is present exclusively in the specialized tissues of stigmas/styles. Transgenic RNAi and overexpression (OE) plants resulted in stigmas with remarkably enlarged and reduced areas, respectively, as a result of the disparity in cell number. These results characterize this gene product as a negative cell cycle regulator *in planta*, which we denominated SCI1 (stigma cell-cycle inhibitor 1). Furthermore, the disparity in cell division affected the differentiation timing of the papillar cells, showing that its differentiation is coupled to stigma cell division, consistent with a role of SCI1 in controlling cell proliferation/differentiation. Moreover, SCI1 influenced the transcript levels of the NtARF8, NtAux/IAA13 and NtAux/IAA19 auxin-related genes, as determined by real time RT-PCR in stigmas/styles of the transgenic plants. We propose that SCI1 is a developmentally regulated stigma/style-specific gene that acts as a component of the signal transduction pathway controlling cell proliferation/differentiation and may have interconnections with the auxin pathway.

P2-05

DUAL ROLES FOR THE RECEPTOR KINASE CLV1 IN REGULATION OF FRUIT ORGAN NUMBER

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In species such as tomatoes and peppers, increases in fruit size are directly correlated with increases in organ number. Previous studies of floral meristems of Arabidopsis mutants with increased numbers of floral organs indicated that the extra organs are produced due to the presence of extra cells. For example, mutation of individual components of the CLAVATA meristem maintenance pathway increases the number of cells in the floral meristem and also produces extra fruit organs[1]. Through the characterization of a new allelic series of mutants in the receptor kinase *CLAVATA1* (*CLV1*), we have identified a second signaling mechanism that regulates fruit organ number by restricting cell proliferation in developing fruit. This developmental process is temporally distinct from the pathway functioning in floral meristems, acts specifically in developing fruit and also requires CLV1. Finally, we speculate that loss of this fruit-specific mechanism leads to the ectopic activation of the transcription factor network known to regulate fruit organ specification, resulting in the production of fruit with extra organs. Supported by NSF IBN-0347675.

1. Clark, S., Running, M., and E. Meyerowitz (1993) *Development* 119: 397-418.

P2-06

***SEUSS-LIKE* AND *SEUSS-MODIFIER* LOCI ENCODE NOVEL FUNCTIONS REQUIRED FOR OVULE DEVELOPMENT IN *ARABIDOPSIS THALIANA*.**

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Mutations in the *Arabidopsis* gene *SEUSS* condition a variety of phenotypes, including floral organ identity transformations and defects in ovule development. *SEUSS* encodes a transcriptional adaptor protein that complexes with LEUNIG and represses *AGAMOUS* transcription during floral organ identity specification. During ovule development, however, the mechanisms of *SEU*SS action are less well characterized.

SEUSS shares extensive sequence similarity with three *Arabidopsis SEUSS-LIKE* genes (*SLK1*, *SLK2* and *SLK3*). In order to better understand the developmental functions of *SEUSS* and *SEUSS-LIKE* genes we: 1) analyzed double mutant combinations of *seuss* and *seuss-like* mutations and 2) carried out a screen for genetic enhancers of the *seuss* gynoecial and ovule phenotypes.

While single mutant *slk1*, *slk2* or *slk3* plants do not display obvious phenotypic abnormalities, mutations in *slk1* or *slk2* dramatically enhance the *seu* mutant phenotype This data suggests a degree of overlapping function is shared between *SEU* and *SLK* genes. The *seu slk1* double mutants display enhanced floral, gynoecial and ovule defects including enhanced disruptions of integument and female gametophyte development. Additionally, our screen for *SEUSS-MODIFIER* mutants uncovered several loci that enhance the ovule defects of the *seu* single mutant. We have isolated two of these loci using a map-based cloning approach. One encodes a previously known regulator of ovule development (*STRUBBELIG*/*SCRAMBLED*) while the second encodes a cytochrome P450 gene required for brassinosteroid synthesis. Our work demonstrates previously unknown roles for the *SEUSS-LIKE* genes during ovule development and has identified a novel regulator of *Arabidopsis* ovule development with a role in brassinosteroid synthesis.

P2-07

THE WYRD MUTANT IN ARABIDOPSIS IS AFFECTED IN FEMALE GAMETOPHYTE DEVELOPMENT.

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We identified the wyrd mutant affected in reproductive function by EMS mutagenesis in Arabidopsis. The seed set in the wyrd mutant was reduced and the missing seeds consisted of both infertile ovules and aborted seeds, indicating that fertilization and seed development are affected by the mutation. Transmission of the mutation through both gametes was reduced. Test-crosses revealed its gametophytic nature: pollination of the mutant with wild-type pollen did not rescue the female sterility of wyrd. Morphological analysis showed that the wyrd embryo sacs are affected in central cell development. In the majority of the mutant ovules, polar nuclei failed to fuse and did not form a diploid mature nucleus; they often acquired a different size. Most of the wyrd central cells did not become fertilized upon pollination, although the egg cells occasionally formed a zygote-like structure. However, some of the fertilized wyrd central cells can develop an endosperm arrested at different stages and forming nuclei of irregular shape and size. It is likely that cell specification within the wyrd embryo sac is not established properly, leading to defects in polar nuclei fusion and improperproliferation of endosperm nuclei.
We identified the WYRD gene on chromosome 5 using mapping approach. It codes for expressed protein with a conserved domain indicating its possible involvement in cell cycle. Functional analysis of the WYRD gene and its product will provide new clues to understand specification of embryo-sac cells and will shed light on development of the female gametophyte and fertilization process.

P2-08

NOVEL TRANSCRIPTION FACTORS CAN TRIGGER A DEVELOPMENTAL PATHWAY RELATED TO EGG CELL-IDENTITY IN *ARABIDOPSIS THALIANA*

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A typical plant life cycle comprises the alternation between a gamete-producing gametophytic and a spore-producing sporophytic generation with the evolutionary trend towards gametophyte reduction. The angiosperm gametophyte consists of four distinct cell types, including two essential gametes, the egg and central cell that give rise to embryo and endosperm upon fertilisation, respectively.

Here, we describe a class of a new, plant specific transcription factors (TF), represented by the RKD factors of *Arabidopsis thaliana.* Consistent with a function as TFs, RKD-GFP fusion gene products have been localised in the cell nucleus. RT-PCR, *in situ* hybridisation and the expression of promoter::GUS reporter gene fusions indicate that RKD factors encoding genes are expressed in the egg apparatus, consisting of egg cell and two synergids. Ectopic expression of RKD as well as RKD-GFP fusion products in sporophytic tissue leads to cell proliferation and the expression of gametophytic and egg cell marker genes. The data suggest that RKD factors trigger a given sporophytic cell to enter a developmental pathway related to egg cell-identity. RKD factors are related to MINUS DOMINANCE (MID) of the green algae *Chlamydomonas reinhardtii*. MID is necessary and sufficient for gamete differentiation, indicating a high phylogenetic conservation of the function.

Ongoing work aims to elucidate the molecular mechanisms of RKD functions and includes the identification of binding *cis*-motifs and the search for interacting factors.

P2-09

TARGETED DEGRADATION OF A CYCLIN-DEPENDENT-KINASE INHIBITOR ICK4/KRP6 BY THE RING TYPE E3 LIGASES IS ESSENTIAL FOR MITOTIC CELL CYCLE PROGRESSION DURING *ARABIDOPSIS* GAMETOGENESIS

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Following meiosis, the plant gametophytes develop through two or three rounds of mitosis. Although the ontogeny of gametophyte development has been defined in *Arabidopsis*, the molecular mechanisms regulating the mitotic cell cycle progression are not well understood. We have been interested in dissecting the roles of RING-finger E3 ligase-involved protein degradation in gametophyte development in *Arabidopsis*. Here we report that two redundant RING-finger E3 ligases RHF1a and RHF2a play an important role in *Arabidopsis* gametogenesis. The *rhf1a rhf2a* double mutants are defective in the formation of both male and female gametophytes. The defects were caused by the arrest at interphase of the mitotic cell cycle at the microspore stage of pollen development and at female gametophyte stage 1 (FG1) of embryo sac development. We demonstrate that RHF1a directly interacts with and targets a CDK inhibitor ICK4/KRP6 for proteasome-mediated degradation. Inactivation of the two *RHF* genes leads to the accumulation of ICK4/KRP6, and reduction of *ICK4*/*KRP6* expression largely rescues the gametophytic defects in *rhf1a rhf2a* double mutants, indicating that ICK4/KRP6 is a substrate of RHF E3 ligases. Interestingly, *in situ* hybridization showed that *ICK4*/*KRP6* was predominantly expressed in sporophytes during meiosis. Our findings indicate that RHF1a/2a-mediated degradation of the meiosis-accumulated ICK4/KRP6 is an essential step to ensure the progression of subsequent mitoses to form gametophytes in *Arabidopsis*.

P2-10

YEAST 1-HYBRID: A TOOL TO UNDERSTAND EMBRYO SAC CELL DIFFERENTIATION

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The life cycle of plants alternates between a haploid gametophyte and a diploid sporophyte generation. With respect to animals, a unique feature of the plant life cycle is represented by the existence of a multicellular haploid generation referred to as the gametophyte, which major function is to produce haploid gametes. In flowering plants, the gametophytes are comprised of very few cells embedded within the sexual organs of the flower. The female gametophyte (embryo sac or megagametophyte) develops within the ovule, which is protected by the ovary. Among angiosperms, the female gametophyte has a variety of forms. The most common polygonum type embryo sac consists of seven cells and four different cell types: three antipodal cells, two synergid cells, one egg cell, and one central cell. These seven cells are of clonal origin: they are all derivative of the haploid functional megaspore through a process named megagametogenesis: Megagametogensis involves three nuclear division cycles, followed by nuclei migration and cell differentation. The mechanisms that underlie the formation of the four cell types forming the mature embryo sac are still unknown.

Our goal is to identify key regulatory transcription factors involved in embryo sac cell differentiation. For this purpose we are performing yeast one hybrid screenings using promoter fragments of genes which are specifically transcribed and translated in embryo sac cells. Interesting promoters were chopped in fragments of roughly 200 bases length and offered to a normalised *Arabidopsis thaliana* cDNA library. Sequences analysis from the first screenings allowed to identify interesting candidate regulators.

P2-11

ANALYSIS OF CHROMATIN CHANGES ASSOCIATED WHIT THE CONTROL OF REPRODUCTIVE DEVELOPMENT IN SEXUAL AND APOMICTIC PLANTS

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Progression through the sexual plant reproductive process implies successive developmental transitions including somatic-to-germ cells (sporogenesis) and spores-to-gametophytes (gametogenesis). Each transition requires temporal and spatial regulation of transcriptional programs. Apomixis is an alteration of the sexual pathway that bypasses both meiosis and double fertilization, giving rise to clones. Molecular mechanisms controlling apomixis are unknown but some evidences suggest that apomixis is associated with the heterochronic expression of sexual reproductive programs, and therefore eventually on modifications of the control of transcriptional transitions. Several studies have demonstrated the crucial role of chromatin-based regulation in developmental transitions. Several lines of evidences also suggest that transitions during reproductive development are epigenetically controlled through dynamic changes in chromatin structure. In this study we compared the role of chromatin remodeling complexes in the differentiation between sexual and apomictic pathways. Using sexual maize and maize-Tripsacum apomictic hybrids, we analyzed key developmental steps of sexual plant reproduction including megasporogenesis, megagametogenesis and early embryogenesis. Expression data for a large number of chromatin-modifying proteins suggest specific differences between both reproductive pathways. Based on cytological and molecular expression studies, we have selected a small number of key factors for detailed functional analysis, in order to assess their role in the expression of the apomictic trait.

P2-12

LOOKING FOR GENES INVOLVED IN MALE FLOWER CARPEL ABORTION IN THE DIOECIOUS *OPUNTIA STENOPETALA*

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Unisexual flower development is a process where separate male or female flowers are developed either on the same plant (monoecious) or on separate plants (dioecious). Most dioecious plants have an hermaphroditic stage early in flower development, followed by a differential abortion or arrest of a sex organ. *Opuntia stenopetala* is a dioecious species, carpel and stamen differentiation is initiated in all their flowers, later in development the inappropriate reproductive organ is arrested resulting in male or female flowers. Male flowers present a phenotype without stigma and incomplete ovule development. Genetics and molecular processes leading to dioecy are poorly understood. In this study, we look for genes associated to mechanisms involved in carpel arrest in male flowers of *O.stenopetala*, specially those related with loss of style and stigma tissues. Our approach is based on *Arabidopsis* mutants affected in carpel development. *STYLISH1* (*STY1*), *TOUSLED* (*TSL*) and *CRABS CLAW* (*CRC*) mutants shows a significant loss of carpel tissues developing reduced amounts of stylar and stigmatic tissues. *STY* is probably involved in auxin biosynthesis regulation, *TSL* is linked to cell cycle progression and *CRC* controls polarity of carpel margins. We identified from *O.stenopetala* partial cDNAs, orthologs of *STY* (*OstSTY*), *CRC* (*OstCRC*) and *TSL* (*OstTSL*). Phylogenetic analyses suggest that we identified orthologs for *STY* and *TSL* genes. Expression analysis shows that both *OstSTY* and *OstTSL* mRNAs accumulates preferentially in female carpel and *OstCRC* is detected only in male carpel which suggest that these genes play a role in female sterility in this species.

P2-13

THE MAIZE *STUNTER1* MATERNAL EFFECT MUTATION AFFECTS MALE AND FEMALE GAMETOPHYTES

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stunter1 is a maternal effect mutant that displays defective maize kernels. The small, stunted kernels exhibit a reduced but normal endosperm with a relatively normal embryo. In an attempt to identify the genetic lesion and better characterize the *stt1* mutant, we are using map-based cloning and conducting analyses of the male and female gametophytes and the developing seed. The lesion is located in a 5 cM region on the long arm of Chromosome 2. The mutant displays incomplete and variable penetrance of the kernel phenotype and reduced transmission of the mutation through the male and female parents. In addition, the *stt1* mutation causes significant changes in the sizes of both the male and female gametophytes. The *stt1* pollen grains and embryo sacs are smaller than wild type. The reduction in size of *stt1* embryo sacs is largely due to differences in the central cell, which is approximately half the size in the mutant embryo sacs compared to wild type. Additionally, the antipodal cells of mutant embryo sacs appear larger, less cytoplasmically dense, and possibly fewer in number than wild type. The results strongly suggest that *stt1* is a maternal effect mutant and that the morphology of the mutant embryo sacs prior to fertilization influences endosperm development, ultimately leading to the production of miniature kernels from mutant embryo sacs.

P2-14

EPIGENETIC DIMORPHISM BETWEEN THE TWO FEMALE GAMETES IN THE *ARABIDOPSIS* OVULE CORRELATES WITH DIFFERENTIAL TRANSCRIPTIONAL REQUIREMENTS IN THE EMBRYO AND THE ENDOSPERM

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In Angiosperms, female gametogenesis results in two gametes, the egg cell and the central cell. A double fertilization event produces the zygote and the embryo-supporting endosperm, respectively. Using antibodies against histone 3 lysine 9 dimethylation, we show that the female gametes in *Arabidopsis* are epigenetically differentiated, and that these differences persist following fertilization. We also observed that the endosperm reprograms histone 3 methylation patterns immediately after fertilization, while the egg and the zygote retain comparable patterns. This indicates transgenerational inheritance in the zygote of at least part of the epigenetic information encoded in the egg cell. Furthermore we show by monitoring RNA Polymerase II elongation that this epigenetic dimorphism correlates with transcriptional differences between a relatively quiescent zygote and a transcriptionally active endosperm. Depriving the embryo and the endosperm of transcription following fertilization is deleterious to endosperm development, but does not block zygotic divisions. This suggests that the zygote might rely extensively on maternal products deposited in the female gametes, and may explain why genomic imprinting is found exclusively in the endosperm, and absent in the embryo.

P2-15

ACTIN RELATED PROTEIN 6 (ARP6) PLAYS A CRITICAL ROLE IN MEIOSIS DURING PRODUCTION OF FEMALE BUT NOT MALE GAMETES IN ARABIDOPSIS THALIANA

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We explored the basis of low seed set in Arabidopsis mutant actin-related protein 6 (ARP6). ARP6 is a component of chromatin remodeling complex protein that regulates gene expression (Deal et al 2005, Plant cell). Reciprocal crosses involving two *arp6* mutant alleles and wild type (WT) revealed that *arp6* defects are strictly restricted to female sporophytic tissues. Consistent with this, male gametophyte development and function were normal in both *arp6* mutant alleles.

To understand the nature of female sporophytic defects in *arp6* mutants, Lat52:GUS pollen was grown on WT or *arp6* pistils and stained for Gus activity. In the *arp6* pistils, although pollen tube germination and growth were comparable to WT, only 54.28% of ovules were targeted by pollen tubes compared to WT (86.90%). To identify which *arp6* sporophytic tissue caused the pollen tube targeting defects, we performed *in vitro* pollen tube guidance assay involving several combinations of WT and *arp6* pistil and ovules. Results from these experiments revealed that *arp6* ovules are defective in pollen tube attraction. Using callose staining of cell plate formation during female meiosis, we identified that meiosis progression is abnormal in *arp6* ovules and that the defects begin to manifest during the end stages of meiosis I. Examination of female meiosis in *arp6* using chromosome spreads revealed defects in chromosome organization at the diplotene stage with chromosomes showing partial desynapsis and loss of structure. Although ARP6 is expressed in a variety of reproductive tissues, our results indicate that it plays a critical role during female meiosis.

P2-16

FUNCTIONAL ANALYSIS OF THE *ARABIDOPSIS* EGG CELL SPECIFIC PROTEIN ATEC1 DURING FERTILIZATION

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The establishment and maintenance of the four different cell types of the female gametophyte of higher plants as well as the pollen tube attraction and reception, the sperm cell release and the fusion of the gametes is likely to involve extensive mechanisms of cell-cell communication.

In order to identify key regulators in gamete communication we are analyzing a gene family from *Arabidopsis* with homology to an egg cell specific transcript from wheat (Sprunck *et al*., 2005). *In situ* hybridization and promoter-reporter gene analyses revealed that the *Arabidopsis AtEC1* (*Arabidopsis thaliana* Egg Cell 1) gene family of five members has an egg cell-specific expression pattern.

Based on subcellular localization studies we conclude that AtEC1 accumulates exclusively in the mature egg cell and that its secretion is triggered during fertilization. Though quintuple mutants develop phenotypically normal embryo sacs and pollen tube reception appears not to be affected, approximately 50% of the egg cells fail to become fertilized. Occasionally, *ec1* ovules are targeted by more than one pollen tube.

Screening a yeast two-hybrid pollen cDNA library we identified a B' regulatory subunit of the Phosphatase 2A to interact with AtEC1, indicating that the numerous predicted phosphorylation sites at the C-terminus of AtEC1 might be a substrate for the Protein Phosphatase 2A (PP2A), provided by the arriving pollen tube.

Here we will present our recent findings about the functional role of AtEC1 in egg cell signaling during fertilization. Current work involves heterologous expression and purification of AtEC1 for *in vitro* binding assays and the identification of its putative receptor.

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P2-17

REORGNIZATION OF OF SHOOT APICAL MERISTEM IS IS ESSENTIAL FOR SOMATIC EMBRYO INDUCTION IN ARABIDOPSIS

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Somatic embryogenesis is an important pathway for regenerating entire plants and as a potential model for studying the regulation of gene expression required for the developmental events in plant embryogenesis. During somatic embryo formation, the shoot apical meristem might occur. In this study, reorganization of shoot apical meristem was examined during somatic embryo formation of Arabidopsis. In the liquid medium containing 2,4-D for 14 day-culture, the discs-like embryonic calli were formed. To induce somatic embryo formation, the embryonic calli were transferred to the liquid medium by removal of auxin for 8-day culture. Expression of several genes involved in zygotic embryogenesis were induced during somatic embryo induction, suggesting that these genes play important roles in this process. CLV3 and WUS genes are required for stem cell formation in shoot apical meristem. Expression patterns of WUS and CLV3 indicate that both genes in non-overlapping domains are induced during somatic embryo regeneration, and the organizing center formation is earlier than both stem cells and proembryos. Further analysis shows that WUS activation is necessary for somatic embryo induction and it functions the up-stream of genes involved in embryogenesis, such as LEC1, LEC2, CUC2 and STM. Moreover, somatic embryo formation is correlated with auxin polar transport which is involved in PIN1-dependent transport. In addition, the induction of WUS expression requires the suitable level of auxin. Our results suggest that somatic embryo induction require the reorganization of shoot apical meristem, which correlates with the PIN1-dependent auxin transport.

P2-18

HIGHLIGHTS ON ADVENTIVE EMBRYONY AND ENDOSPERM DEVELOPMENT IN *CITRUS MADURENSIS* LOUR.

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Adventive embryony, a phenomenon with tremendous potential for plant breeding is observed in *Citrus madurensis*. In this species, the ovule is anatropous, bitegmic and crassinucellate. There is a distinct gradation in dimensions of the cells and their nuclei at different regions of the nucellus. Embryonal initials differentiate in the nucellus in response to pollination. Only the initials formed in the layers adjacent to the micropylar half of the embryo sac develop into embryos. There is no uniformity in the size or shape of the adventive embryo initial cells. The AEIC's undergo irregular divisions and many push their way into the embryo sac. Once inside the embryo sac, further divisions follow a more or less regular pattern.Subcellular structures in the initial cells point to high metabolic activity. *C.madurensis* differs from other *Citrus* cultivars in its ability to produce embryos without the stimulus of fertilization, in localized differentiation of embryos in the micropylar region of the nucellus, in the peculiar distribution of cell organelles in the embryonal initials and proembryos. It also shows presence of cytolysosomes in the nucellar initials.

Zygote, if formed, divides rarely to develop into a proembryo. It is significant that the endosperm degenerates in embryo sacs where zygotic embryo forms, probably on account of competition for crucial substances for growth or due to the inhibiting action of the zygotic proembryo. Embryoid like structure observed at the chalazal end of the endosperm, projecting into the cavity formed by the degenerating hypostase deserves further attention.

P2-19

CHARACTERIZATION OF AN *ARABIDOPSIS* MUTANT DEFECTIVE IN ATTRACTING POLLEN TUBE INTO OVULE.

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A pollen tube navigates past several different cells in the pistil before it enters the ovule to reach the embryo sac. The signals that mediate pollen tube entry into the ovule remain poorly characterized. Arabidopsis mutant *fem137* was obtained by screening a T-DNA insertion collection for plants with reduced seed containing siliques. *fem137*/+ plants have significantly reduced seed set (30-40 seeds per silique) compared to wild-type (~ 60 seeds per silique). To explore the basis of reduced seed set, we pollinated *fem137*/+ pistils with LAT52: GUS pollen and stained for GUS activity. In the *fem13*7/+ pistils, although pollen tube germination and growth in transmitting tract was normal, nearly half of the ovules were not targeted by the pollen tube and remained unfertilized. These results indicated that *fem137* ovules were defective in attracting pollen tubes. Confocal Laser Scanning Microscopy and expression of embryo sac cell-specific markers were used to monitor female gametophyte (FG) developmental defects. These analyses revealed that nuclei migration and cell-fate specification in *fem137* FGs were comparable to wild type; however *fem137* embryo sacs were noticeably reduced in size relative to wild type embryo sacs. To further dissect the role of *FEM137* in FG development, we sought to identify the gene(s) disrupted in *fem137*. Since the kanamycin marker on the T-DNA does not co-segregate with the reduced seed phenotype, we are using map-based cloning procedure to identify the gene(s) defined by *fem137* mutation. Progress on the cloning and characterization of *fem137* will be presented
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Poster Presentations - Session 3

Endosperm and Imprinting Session

P3-03

TRANSCRIPTIONAL REGULATION OF VP1 EXPRESSION IN MAIZE

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Seed maturation and dormancy are important for stand establishment, yield, seed quality and longevity in crops. Several plant hormones are key regulators of seed maturation and the dormancy-germination transition. During the maturation phase of seed development, abscisic acid (ABA) regulates the expression of genes, including a transcription factor network, which promotes maturation and represses seed germination. The Viviparous1 (vp1) gene, encoding a B3 domain-containing transcription factor, is an important mediator between the seed maturation and germination programs in maize and is a key regulator in the response to ABA. Using cultured embryos, we previously found that ABA and osmotic potential could activate Vp1 expression and inhibit germination (Cao et al., 2007, Plant Physiol 143:720). Here we show that hypoxia can also reversibly inhibit maize embryo germination. Furthermore, Vp1 has a role in both ABA and hypoxia mediated inhibition of maize embryo germination. We are currently testing the role of ABA in anaerobiosis-induced germination inhibition and the role of Vp1 in these processes. To further understand the regulation of seed maturation, we are examining the transcriptional regulation of Vp1. A 958-base Vp1 promoter fragment that confers proper expression to a GUS reporter was identified. Five oligonucleotides of the promoter region have been identified that specifically bind embryo nuclear proteins in electrophoretic mobility shift assays. Using yeast one-hybrid screens, we have identified two candidates, S13 and M97. S13 and M97 bind to the CE1L and MLE elements of the VP1 promoter respectively. S13 is targeted to the nucleus and is a Zn2+ binding protein. Functional assays to understand the role of these proteins in the transcriptional regulation of VP1 are currently ongoing.

P3-04

POLYCOMB GENES CONTROLLING ENDOSPERM DEVELOPMENT IN RICE

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Genes of the Polycomb group family (PcG) have been implicated in the maternal control of endosperm development in the model species, Arabidopsis thaliana. Wild type PcG proteins assemble in a chromatin remodeling complex and repress transcriptional activity of target genes. Genetic studies in Arabidopsis have identified four PcG genes, MEA, FIS2, FIE and MSI1 that are required for normal endosperm proliferation and patterning. In maize, FIE and three orthologs of MEA, Mez1, Mez2, and Mez3 have been identified.

Although no functions are known for these genes in maize, some maize PcG genes are known to be expressed from only one parental allele during endosperm development while the other allele is silenced typically through methylation. We are investigating the potential functions of candidate orthologs of the PcG genes in seed development in rice. Sequence database searches have identified rice homologs of the Arabidopsis FIS2, MSI1, and maize Mez and FIE2 genes. Preliminary expression analysis suggests the existence of seed specific PcG genes in rice. Furthermore, phenotypic analysis of an EZ1 insertion line shows a disruption in endosperm development following fertilization. These data indicate that PcG genes might play an essential role in endosperm patterning in cereals.

P3-05

THE STUDY Of MECHANISM Of SEED SIZE CONTROL IN *ARABIDOPSIS*

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In flowering plants, the seed is produced by a double fertilization event, and is composed of the endosperm and the embryo, surrounded by maternal integuments. The endosperm provides nutrients to the embryo, and also plays a central role in the coordination of seed size determination. The mutants of *IKU* pathway genes, *IKU2* encodes a leucine rich repeat (*LRR*) kinase receptor, and *MINI3* encodes a transcription factor of the plant specific WRKY family, produce small seeds and mimic the phenotype of maternal genome excess or paternal genome de-methylation. By transcriptome analysis, comparing gene expression in seeds from wild type, *iku1* and *iku2* mutants, we found that nine of ten transcript factors, which expression are changed in *iku* mutants, have high epigenetic marker in their locus, this give the cues they may play the role in linking seed size control and epigenetic pathway. *Chengyu*, another endosperm development arrest mutant, produces seeds even smaller than *haiku* mutant seeds. Genetic evidences show chengyu does not belong to *IKU* pathway. By positional cloning, we found that the *chengyu* phenotype may result from a high conserved proline to leucine mutation in a ribosomal protein large unit gene. In animals some ribosomal proteins play a role in size control, but the detailed mechanisms remain unknown. We have thus identified in *Arabidopsis* a pathway that is likely conserved between plants and animals.

P3-06

11S GLOBULINS DURING OLIVE (*Olea europaea* L.) SEED EMBRYOGENESIS and GERMINATION

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Seed storage proteins (SSPs) are the main sources of carbon, nitrogen and sulphur in seeds during embryo development and growth. The storage and further mobilization of SSPs are highly specific processes, in which specialized cellular compartments are involved. Protein bodies (PBs) represent the generally accepted subcellular location for such proteins within the reserve tissues (embrionary cells and endosperm in the seeds). The olive tree (*Olea europaea* L.) is one of the most important Mediterranean crops. Studies concerning the structure and the organization of the different tissues of the mature seed (endosperm and embryo) and their evolution throughout seed development and germination are scarce in spite of the remarkable agronomical interest of this species. The predominant use of vegetative reproduction methods in olive cultivation may account for this lack of information regarding the sexual reproductive side of olive tree.
Antibodies to purified 11S-type globulins, the mayor protein forms contained in the mature olive seed [1, 2, 3], were used with the aim of performing a histological and ultra-structural study of seed embryogenesis and germination in this plant. The behaviour of these storage proteins (their accumulation in PBs during development and maturation and their mobilization and degradation during in vitro germination) during zygotic embryogenesis and during in vitro germination of the olive seed were analyzed by using immunocytochemical approaches at light, transmission and scanning electron microscopy.

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P3-07

DISSECTION OF SEED SIZE CONTROL IN *ARABIDOPSIS THALIANA*

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The seeds of flowering plants are complex organs, composed of three different genetic systems: The two zygotic products of double fertilization, the diploid embryo and the typically triploid endosperm are surrounded by the maternally derived tissues of the seed integuments. To ensure its functional integrity as one organ, seed development and growth has to be tightly coordinated between these different components. But which components are influencing the final seed size, and to what degree?

Organ growth is predominantly determined by two factors, cell number and cell size. Today, very few mutants have been characterized that display an altered seed size and a molecular mechanistic understanding of seed size control is still missing. Here, we chose to manipulate cell proliferation as the downstream target of organ size regulation. We focus on the Cyclin-Dependent Kinase CDKA;1, the central cell cycle regulator of Arabidopsis and homolog of the yeast cdc2/CDC28 kinases by using either previously generated *cdka;1* mutants or expression of KRP-type CDK inhibitors. Our approach relies on a tissue specific modulation of cell cycle activities in the different seed compartments, i.e. the zygotic embryo and endosperm versus the maternal integuments.

Currently, chimeric seeds resulting from crosses of different genotypes and CRE-lox derived heterologous recombination events are being analyzed and the final outcome of these experiments will be presented here.

P3-08

GENETIC REGULATION OF MAIZE ALEURONE DEVELOPMENT

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Cereal endosperm is one of the most important plant tissues for humankind. Different cell types have different functions and compositions. Understanding the genetic control of cell differentiation offers the potential to manipulate seed characteristics through developmental engineering. Maize endosperm is comprised of four major cell types: starchy endosperm, aleurone, transfer cells and embryo surrounding region cells. Several groups have been focusing on aleurone differentiation and identified several genes involved in aleurone cell fate specification. These include *cr4*, *dek1* and *sal1*, which are proposed to compose part of a signaling system. Here we report on a new mutant, *thick aleurone**, that functions as a negative regulator of aleurone cell fate; *thk** mutants have aleurone layers averaging 4-6 cell thick instead of the normal single cell layer. Surprisingly, *thk** appears epistatic to *dek1* suggesting that *Dek1+* is a negative regulator of *Thk*+. Double mutant studies are consistent with a model where the cell fate specification genes function upstream of *naked endosperm**, a gene proposed to be involved in aleurone cell differentiation and a potential target of regulation by the cell fate signaling system.

P3-09

SHB1 REGULATES SEED DEVELOPMENT THROUGH MINI3 AND IKU2 SIGNALING IN *ARABIDOPSIS*

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Double fertilization in angiosperms leads to the formation of a diploid embryo and a triploid endosperm. Seed development in *Arabidopsis* then undergoes an initial phase of active endosperm proliferation followed by a second phase in which embryo grows at the expense of the endosperm and the mature seed contains only a single layer of endosperm cells. As the mature seed size is largely attained during the initial phase, the seed size is coordinately determined by the growth of the maternal ovule, endosperm, and embryo. Here, we identify SHB1 (SHORT HYPOCOTYL UNDER BLUE 1) as a new and positive regulator of *Arabidopsis* seed development through both cell size and cell number control. SHB1 contains SPX and EXS domains homologous to SYG1 protein family. *shb1-D*, a gain-of-function allele, increases seed size and *shb1*, a loss-of-function allele, reduces seed size. The *shb1* mutations are transmitted zygotically and through the endosperm genomes. The increase in *shb1-D* seed size is associated with the timing of endosperm cellurization, enlargement of chalazal endosperm, and embryo development. The *shb1* mutations also regulate the expression of two other genes that affect endosperm development and seed size, *MINI3* and *IKU2*, a WRKY transcription factor gene and an LRR receptor kinase gene. *SHB1* is expressed in embryo, chalazal endosperm, and peripheral endosperm. Therefore, a signal generated by SHB1 in the early phase from embryo and endosperm promotes a large seed cavity and a greater endosperm growth to fill the cavity. Subsequently in the second phase, SHB1 enhances the embryo cell proliferation and expansion through a yet unknown pathway independent of IKU2.

Poster Presentations - Session 4

Compatible and Incompatible Pollinations Session

P4-04

EVALUATING *NATRXH* ROLE IN POLLEN REJECTION IN *NICOTIANA* AND UNRAVELING THE SECRETION PATHWAY OF THIS NON-ORTHODOX SECRETION PROTEIN

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NaTrxh encodes a thioredoxin *h* (Trx h) whose transcript is more abundant in self-incompatible (SI) *Nicotiana* species. NaTrxh is secreted to the extracellular matrix (ECM) of the stylar transmitting tissue in *N. alata*. *In vitro* assays show that NaTrxh interacts with 120K and S-RNases, reducing the latter. To evaluate its role in SI, we are silencing *NaTrxh* in transgenic *Nicotiana* plants.

Because of the unexpected NaTrxh localization in the ECM, we are evaluating if there is a responsible sequence for its secretion. NaTrxh possesses an N-terminus extension, typical for the subgroup 2 of Trx h, we tested if this functions as a signal peptide. Our data indicate that residues between Ala-17 and Pro-27, called Nβ domain, are essential for NaTrxh secretion. *In silico* tertiary structure modeling shows that the Nβ domain is exposed and that it could be necessary for NaTrxh stability and/or protein-protein interactions. Interestingly, when an NaTrxh mutant without the Nβ domain is overexpressed as a glutathione S-transferase fusion protein in *E. coli*, it is not properly folded, suggesting that this domain is not only involved in NaTrxh secretion, but it is involved in protein stability as well.

Since the Nβ domain is not an orthodox signal peptide, we are testing whether NaTrxh is secreted through the endoplasmic reticulum (ER)/Golgi apparatus route by co-expressing ER and Golgi fluorescent protein markers with NaTrxh:GFP or different NaTrxh mutants (with N-terminus deletions)

in *N. benthamiana* leaves, by transient expression assays, and in stable transgenic *N. tabacum* lines. *DGAPAIN207406*; *PAIP629015*.

P4-05

GENETIC CONTROL OF REPRODUCTIVE TRACT DEVELOPMENT

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In Angiosperms, the female gametophyte develops deep within the tissues of the carpel. This presents a barrier to the transportation of the male sperm cell to achieve fertilization of the egg cell. To overcome this problem the female reproductive tract plays a critical role in promoting pollen tube growth through the carpel to unfertilized ovules within the ovary. A specialized tissue in the center of the style and septum called the transmitting tract functions to facilitate pollen tube growth throughout the ovary in an apical to basal direction. This is accomplished, at least in part, through secretion of an extensive extracellular matrix (ECM) rich in acidic polysaccharides, and through a program of developmentally controlled cell death. We have begun using molecular and genetic techniques to identify regulatory genes necessary for the specification of the reproductive tract in *Arabidopsis*. This has resulted in the discovery of the *HECATE* genes, bHLH transcription factors, which are important for formation of its primary tissues. In addition, we have also identifed the *NO TRANSMITTING TRACT* (*NTT*) gene, which has shed light on the importance of the transmitting tract for pollen tube movement. Abnormal transmitting tract development in *ntt* mutants prevents pollen tubes from reaching the basal end of the carpel, and results in fruit that have seed only at their apical end. Neither ECM formation nor cell death occurs in the transmitting tract region of *ntt* mutants, and profound aberrations in pollen tube movement are observed.

P4-06

ROLE OF THE *SLF* GENE IN SELF-COMPATIBILITY IN *PETUNIA HYBRIDA*

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Self-incompatibility (SI) is crucial to the success of angiosperms and involves the recognition and rejection of self-pollen or genetically similar pollen. SI is often genetically controlled by a single *S*-locus and involves a specific interaction between the pistil and pollen. *Petunia hybrida* exhibits *S*-RNase-based gametophytic SI, in which *S*-RNases encoded by the *S*-locus are essential for rejecting pollen. *S*-RNases are produced by the transmitting tract of the pistil, whereas pollen produces a distinct pollen-S factor. The pollen-S factor has been identified as an F-box gene, named SLF in the Solanaceae. This suggests that SLF may interact with the "non-self" *S*-RNases and send them down a proteolytic degradation pathway, thereby preventing their activity.

Two homozygous stocks of *Petunia hybrida* (S_oS_o; self-compatible and S_bS_b; self-incompatible) were investigated in terms of phenotype and genotype. Previous experiments showed that these stocks share the same *S*-RNase allele (P. Wright and T. Robbins – unpublished). More recent data indicate that the self-compatibility of S_oS_o is not caused by the altered sequences of either the *S*-RNase or *SLF* gene, nor is it due to significantly altered transcriptional activity of these *S*-locus components. One possibility is that the difference between those stocks lies in the presence of some modifier genes. However these would have to be *S*-linked modifiers, as self-compatibility is tightly linked with the S_o haplotype in segregation studies. Independent studies have shown that self-compatibility in the Solanaceae can result from *SLF* duplications and that possibility is currently being investigated for the S_o haplotype.

P4-07

Dissection of Functional Domains of the S-Locus-F-Box Protein of *Petunia inflata* in Interactions with S-RNase

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Petunia inflata S-Locus F-box (*PiSLF*) was shown to encode the male determinant of S-RNase-based self-incompatibility based on the finding that its S_2 allele, $PiSLF_2$, when expressed in transgenic plants, caused breakdown of SI function in S_1 and S_3 pollen (i.e., heteroallelic pollen), but not in S_2 pollen (e.g., homoallelic pollen). This finding is consistent with the phenomenon of competitive interaction observed in the Solanaceae. Here, we have further shown that two additional alleles, $PiSLF_1$ and $PiSLF_3$, also cause specific breakdown of SI function in pollen when introduced into S_2S_3 transgenic plants. Previous *in vitro* binding results showed that a PiSLF interacted with its non-self S-RNases more strongly than with its self S-RNase. To determine the biochemical basis of this differential interaction, we have carried out *in vitro* binding assays using various truncated forms of $PiSLF_2$, as well as chimeric proteins between $PiSLF_1$ and $PiSLF_2$, and between $PiSLF_2$ and PiSLFLb-S_2 (a PiSLF-like protein). The results have led us to propose that PiSLF contains three functional domains, with FD2 responsible for strong interactions between PiSLF and S-RNase, and FD1 and FD3 negatively regulating the interactions between FD2 and S-RNase during self-interactions. To test the validity of these *in vitro* results, we will express chimeric proteins, b-2-b (with FD1 and FD3 of PiSLFLb-S_2 and FD2 of $PiSLF_2$), 2-b-2, 2-3-2 (with FD1 and FD3 of $PiSLF_2$ and FD2 of $PiSLF_3$), 1-2-1 and 2-1-2, in S_2S_3 transgenic plants to examine their effects on SI function of transgenic pollen. The results obtained so far are presented.

P4-08

NASTEP, A STIGMATIC KUNITZ PROTEINASE INHIBITOR-LIKE PLAYS A ROLE IN POLLEN REJECTION IN NICOTIANA

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The pistil is a gatekeeper that evolved in many species to recognize and reject the self-pollen avoiding endogamy and encouraging cross-pollination. Recognition is a complex process and specific factors are needed. Pollen rejection is genetically controlled by the locus *S.* In Solanaceae, the *S* locus encodes in the pistil the S-RNase and the male determinant SLF/SFB (*S*-locus F box protein). Both are essential but not enough for pollen rejection, because unlinked genes to S-locus are also required. We identified a stigma-specific protein from *N. alata*, NaStEP (*N. alata* Stigma Expressed Protein), which is homologous to Kunitz-type proteinase inhibitors. Immunohistochemical and protein-blot analyses revealed that NaStEP is only detectable in stigmas of SI species *N. alata*, *N. forgetiana* and *N. bonariensis*, but not in the SC species *N. tabacum*, *N. plumbaginifolia*, *N. benthamiana*, *N. longiflora* and *N. glauca* studied. NaStEP contains the vacuolar targeting sequence NPIVL, and immunocytochemistry experiments showed vacuolar localization in unpollinated stigmas. After self-pollination or pollination with pollen from the SC *Nicotiana* species, NaStEP was found in the stigmatic exudate. Synthesis of this protein was strongly induced in *N. alata* following incompatible pollination with *N tabacum* pollen. Transfer of NaStEP to the stigmatic exudate was accompanied by perforation of the stigmatic cell wall, which appeared to release the vacuolar contents to the apoplastic space. Once in the exudate, NaStEP is associated with pollen tubes. When NaStEP was silenced in *Nicotiana* transgenic plants, self-incompatibility was disrupted, indicating that *NaStEP* is part of the pollen rejection pathway in *Nicotiana*.

P4-09

STUDY OF PRE- AND POST-FERTILIZATION CHANGES IN WHEAT-MAIZE CROSSES.

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The present study aimed to investigate interactions between pollen tubes and stigma or style in terms of certain parameters like pollen tube growth, pollen germination and abnormal pollen tube development along with compositional changes in ovary during fertilization (post- fertilization events) to quantify the success of wheat-maize crossing. The experimental material consisted of eighteen F_1s derived from the crosses between nine cold susceptible (female) and two cold tolerant varieties (male) respectively. *Z. Mays* 'Pragati' was chosen for intergeneric crossing. Subsequent to pollination, spikes were collected in a chronological sequence for pollen tube studies using normal light and fluorescence microscopy. Microtome preparations of pistils were stained using Coomassie Brilliant Blue and Periodic Acid Shiff's staining to monitor histological and compositional (protein and carbohydrate) changes.

Mean percent pollen germination and mean pollen tube growth were found to be maximum in (Druchamp × UP2425) × Pragati cross (62.48%, 174.66 μm) with lowest percent abnormal pollen tubes. Embryo Formation Frequency (EFF) showed highly positive correlation with percent pollen germination (0.986) and pollen tube length (0.965). Swelling and coiling of pollen tubes accounted for the largest number of aberrations and low EFF. Histological studies indicated aberrant secretions and a complete absence of insoluble carbohydrates on the entire extra-ovarian area and the obturator region, thus preventing pollen tube growth. The correlation between parameters used here and EFF provides an important criterion for selecting beneficial crosses of cold-tolerant wheat-maize. Two crosses viz. Druchamp with UP2425 and UP2590 provide exciting possibilities for further plant-

breeding investigations.

P4-10

FUNCTIONAL STUDIES OF S-RNASE-BASED SELF-INCOMPATIBILITY IN *PETUNIA INFLATA*

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In *Petunia inflata*, *S-RNase* and *S-locus F-box* (*PiSLF*) control the pistil and pollen specificity, respectively, in self-incompatibility. We have shown that PiSLF may be a component of an E3 ubiquitin ligase complex, which contains PiCUL1-G and PiSBP1 (a RING-HC protein), and that a PiSLF interacts with its non-self S-RNases more strongly than with its self S-RNase. We propose that a PiSLF mediates ubiquitination/degradation of its non-self S-RNases in the cytoplasm of a compatible pollen tube, but allows its self S-RNase to inhibit growth of incompatible tubes. To understand the interactions between PiSLF and S-RNase, we carried out comparative studies of six *S*-linked pollen-expressed *PiSLF-like* genes and *PiSLF*. An *in vitro* binding assay showed that none of these PiSLF-like proteins interacted with S-RNases to any significant extent, and an *in vivo* functional study showed that three of these *PiSLF-like* genes examined did not function in SI. We have identified three PiSLF-specific regions, and their possible roles in allele-specific interaction with S-RNases have been identified from studies of various truncated and chimeric forms of PiSLF. We next showed that PiSBP1, along with E1 and E2, mediated ubiquitination of S-RNase *in vitro*. We are testing a hypothesis that PiSBP1 is also responsible for basal degradation of all S-RNases *in vivo*. Lastly, since native S-RNase expressed in pollen does not affect its SI function, we are testing a hypothesis that glycosylation is required for sequestration of S-RNase in pollen and that the active form of S-RNase in the cytoplasm is the deglycosylated form.

P4-11

CALCIUM SENSING BY CALCINEURIN B-LIKE 10 DURING FLOWER ORGAN MATURATION

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Calcium is a component of signal transduction pathways that allow plants to respond to numerous endogenous and environmental signals in order to grow, develop and reproduce. Changes in intracellular calcium are perceived by calcium binding proteins whose diverse tissue expression patterns, differential affinity for calcium and interaction with different target proteins allows for specificity during calcium signaling. Calcineurin B-Like 10 (CBL10) is a calcium binding protein that mediates flower organ maturation in Arabidopsis. Successful fertilization during flowering requires the coordinated development of stamens and pistils. Stamens must elongate and dehisce to release pollen onto the stigma while the pistil prepares to receive the pollen and promote growth and targeting of the female gametophyte. *cbl10* flowers are infertile with shortened stamens and undehisced anthers. When pollen is manually placed on *cbl10* pistils, pollen tube growth is arrested preventing fertilization. Several plant hormones have roles in stamen and pistil development. We are determining the hormonal pathway in which CBL10 might play a role by applying hormones to *cbl10* flowers to rescue the *cbl10* phenotype and through genetic analysis with hormone signaling mutants. We are also measuring expression levels of genes important for stamen and pistil development in the *cbl10* mutant.

P4-12

STRUCTURAL MODULES FOR RECEPTOR DIMERIZATION IN THE S-LOCUS RECEPTOR KINASE EXTRACELLULAR DOMAIN

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The highly polymorphic *S*-locus receptor kinase (SRK) is the stigma determinant of specificity in the self-incompatibility response of the Brassicaceae. SRK spans the plasma membrane of stigma epidermal cells, and it is activated in an allele-specific manner on binding of its extracellular region (eSRK) to its cognate pollen
coat-localized *S*-locus cysteine-rich (SCR) ligand. SRK, like several other receptor kinases, forms dimers in the absence of ligand. To identify domains in SRK that mediate ligand-independent dimerization, we assayed eSRK for self-interaction in yeast. We show that SRK dimerization is mediated by two regions in eSRK, primarily
by a C-terminal region inferred by homology modeling/fold recognition techniques to assume a PAN_APPLE-like structure, and secondarily by a region containing a signature sequence of the *S*-domain gene family, which might assume an EGF-like structure. We also show that eSRK exhibits a marked preference for homodimerization over heterodimerization with other eSRK variants and that this preference is mediated by a small, highly variable region within the PAN_APPLE domain. Thus, the extensive polymorphism exhibited by the eSRK not only determines differential affinity toward the SCR ligand, as has been assumed thus far, but
also underlies a previously unrecognized allelic specificity in SRK dimerization. We propose that preference for SRK homodimerization explains the codominance exhibited by a majority of SRKs in the typically heterozygous stigmas of self-incompatible plants, whereas an increased propensity for heterodimerization combined with reduced affinity of heterodimers for cognate SCRs might

underlie the dominant–recessive or mutual weakening relationships exhibited by some SRK allelic pairs.

P4-13

FINE MAPPING OF A POLLEN UNILATERAL INCOMPATIBILITY LOCUS IN TOMATO

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In the Solanaceae, unilateral incompatibility (UI) is a stylar pollen rejection phenomenon that most often occurs when self-compatible species are used as pollen parents in crosses with related self-incompatible species (the 'SI x SC rule'). The wild tomato relative *Solanum lycopersicoides* is SI and rejects pollen of cultivated tomato, *S. lycopersicum*, by UI, as do pistils of hybrids between the two; pollen of *S. pennellii* can overcome this barrier. Our previous research identified three QTLs, located on chromosomes 1, 6 and 10, from *pennellii* that are required to overcome UI on diploid or sesquidiploid *lycopersicum* × *lycopersicoides* hybrids. The chromosome 1 factor maps to the same position as the *S* locus, suggesting it may be involved in pollen UI. The chromosome 6 gene, herein referred to as *ui6.1*, acts as a gametophytic factor in a two gene system involving the chrom. 1 QTL. Preliminary results show *ui6.1* is located on the short arm of chromosome 6. Fine mapping was initially impeded by suppressed recombination between *pennellii* and tomato chromosomes in this region. Previous studies demonstrated that recombination frequency is positively correlated with the length of the alien segment. To exploit this relationship, recombinants were isolated from progeny of heterozygous substitution lines containing a long (80cM) segment derived from *pennellii*. So far, three BC populations of 3618 individuals have been genotyped. Recombination frequency in female gametes was much higher than in male gametes, and fortuitously relatively normal in the region of our target locus. In this fashion, the *ui6.1* factor was mapped to a 0.10 cM region with one crossover on one side and two crossovers on the other side. Physical mapping of this gene using BAC and cosmid libraries is in progress.

P4-14

STUDYING PROTEIN-PROTEIN INTERACTIONS IN RNASE-BASED SELF-INCOMPATIBILITY IN *PETUNIA HYBRIDA* AND *PRUNUS AVIUM* USING THE YEAST TWO-HYBRID SYSTEM

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Self-incompatibility (SI) in the Solanaceae and the Rosaceae is controlled gametophytically by a single locus, *S*, with multiple alleles. Two genes at the *S* locus determine the incompatibility response of style and pollen respectively. One encodes stylar ribonucleases (*S*-RNases), the other pollen-expressed F-box proteins (SLF or SFB).
Despite many advances in our understanding of RNase-based SI, much is still unknown about the mechanism. Biochemical and genetic studies indicate that additional stylar and pollen factors are involved. The precise role of the pollen-expressed F-box gene SLF/SFB is unclear. F-box proteins form part of E3 ubiquitin ligase complexes, providing target specificity, suggesting a role for ubiquitin-mediated protein degradation via the 26S proteasome. It has been proposed that SLF/SFB targets S-RNases for degradation. However, our work on pollen-part mutants of cherry suggests that SLF/SFB provides specificity to the inactivation of S-RNases carried out by a general inactivation mechanism in pollen tubes, by protecting 'self' S-RNases from degradation. It is also unclear at this stage whether the mechanism of SI in the Rosaceae and Solanaceae is the same.

We are currently using a yeast two-hybrid screening approach to identify proteins from style and pollen that interact with SLF in *Petunia hybrida* and with SFB in cherry (in parallel). This should help clarify the role of SLF/SFB in SI in members of two distantly related families (Solanaceae and Rosaceae respectively). Progress towards the identification of additional factors will be presented at the meeting.

P4-15

GENETIC ANALYSIS OF A INTRA-SPECIES UNILATERAL INCOMPATIBILITY IN *BRASSICA RAPA*.

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Plants have evolved many systems to prevent irrelevant fertilization. Among them, incompatibility is well-organized system, in which pollen germination or pollen tube growth is inhibited in pistils. Self-incompatibility (SI), rejecting self-pollen, promotes outbreeding in flowering plants. On the contrary, inter-species incompatibility, preventing gene flow among species to restrict outbreeding, usually occurs unilaterally, and is known as unilateral incompatibility (UI). In Brassicaceae, little is known about molecular mechanism of UI, although the *S*-locus genes have been characterized to know how to recognize the self-pollen in the SI system. In the present study, we characterized novel UI observed between the same species, *Brassica rapa*; pollen of Turkish SI lines was specifically rejected in pistil of Japanese commercial SI variety Osome. Incompatible phenotype of this intra-species UI was closely resembled with those of SI. The segregation analysis revealed that this UI recognition is regulated by single pollen and stigma factor each other, furthermore, both of this UI factors were not linked to the *S*-locus.

P4-16

GENETIC MAPPING OF MALE MEDIATED NONRANDOM MATING LOCI IN *ARABIDOPSIS THALIANA*

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In many flowering plants in diverse taxa, both self-compatible and self-incompatible, when pollen of mixed genetic lineages compete for fertilization, the progeny from the competition differ in proportion from the initial pollen deposition. This phenomenon is called nonrandom mating. Nonrandom mating can be due to male mediated competition or female mediated cryptic choice. We have constructed a Kanamycin-marker scoring system to efficiently assay nonrandom mating in *Arabidopsis thaliana*. Using this system, in combination with pollen from recombinant inbred lines constructed between Columbia and Landsberg accessions, we are mapping the genetic loci responsible for male mediated competition.

P4-17

IDENTIFICATION OF PROTEIN KINASES THAT MEDIATE THE SELF-INCOMPATIBILITY REACTION IN *PAPAVER RHOEAS*.

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Self-incompatibility (SI) is the major mechanism used by flowering plants to prevent self-pollination and thus avoid inbreeding. It is a genetically controlled mechanism that encodes a highly specific recognition system that inhibits the growth of incompatible pollen, whereas compatible pollen from an unrelated plant of the same species is able to grow and effect fertilization. In *Papaver rhoeas* inhibition of incompatible pollen is mediated by a signal transduction pathway that activates a complex network of intracellular events including Ca^{2+}-dependent phosphorylation of p26, an inorganic pyrophosphatase required for pollen tube growth, depolymerisation of the pollen actin cytoskeleton and ultimately programmed cell-death (PCD).

An important aim of work is to identify and characterize components of the signalling network that mediate the SI response. Recent studies have revealed that p56 a mitogen-activated protein kinase (MAPK) is involved in activation of PCD during SI. MAPKs have been shown to be important signalling components in a range of cellular responses in eukaryotes. In *Arabidopsis thaliana* there are 20 MAPKs with roles in cell division, abiotic stress response, wounding response and hormone signalling.

Using a combination of bioinformatics and proteomics data it has been possible to identify and clone a candidate p56 gene from *Papaver* pollen. The putative p56 gene has homology to *AtMPK9* and is a member of the TDY class of MAPKs. It has been designated *PrMAPK9b*. An antibody raised against recombinant PrMAPK9b protein is being used to confirm that this gene/protein does indeed correspond to p56.

P4-18

IDENTIFICATION AND FUNCTIONAL ANALYSIS OF THE POLLEN DETERMINANT OF SELF-INCOMPATIBILITY IN PAPAVER RHOEAS

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Plants have evolved self-incompatibility (SI) systems to allow self-recognition and prevent self-fertilization. In *Papaver rhoeas* the pistil component encoded by the *S* locus is a small secreted protein ligand (S protein). The SI response is mediated by a Ca^{2+}-dependent signalling network triggered by interaction of the pistil S proteins with incompatible pollen. This results in depolymerization of the actin and microtubule cytoskeleton, phosphorylation of target proteins and PCD-mediated death of incompatible pollen.

Identification of the pollen *S* locus determinant has been a long-term goal. Here we present data describing the isolation and functional characterization of three alleles of the *P. rhoeas pollen S receptor* (*PrpS*) gene. The evidence suggests that *PrpS* is the pollen S determinant in *Papaver*. *PrpS* is a highly polymorphic gene that is physically linked to the stigma *S* gene and is specifically expressed in pollen. PrpS encodes a ~20 kDa protein with three predicted transmembrane domains, and has no homology to proteins in existing databases. PrpS is associated with the plasma-membrane, suggesting that it is likely to be a transmembrane receptor. Use of *PrpS* anti-sense oligonucleotides in the SI bioassay have provided good evidence that PrpS is functionally involved in SI. Experiments are currently investigating if PrpS binds the pistil S protein, in order to provide firm evidence for our long-standing hypothesis that Papaver SI is triggered by a receptor-ligand interaction.

Poster Presentations - Session 5

Evolution of Reproductive Development

P5-03

CROSS-KINGDOM TRANSCRIPTOME ANALYSIS OF MALE GAMETES TO UNRAVEL THE GENETIC BASIS OF EVOLUTIONARY CONSERVED FUNCTIONS

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In angiosperms, after meiosis the haploid gametophytes go through two or three rounds of mitosis to produce the gametes. Two sperm cells travel quiescently within the growing pollen tube towards the embryo sac, wherein double fertilization occurs by fusion with the egg cell and central cell. Recent advances in Arabidopsis start now to unravel the complexity of this hallmark of angiosperm biology, but both cellular and molecular mechanisms remain rather unknown.

The accumulation of a diverse set of mRNAs in male gametes has been observed in maize, Plumbago and lily generative cells, but the progress remained slow because not always a homolog or direct comparison could be established with Arabidopsis. We have developed a method to isolate Arabidopsis sperm cells by Fluorescence-Activated Cell Sorting, thus obtaining enough biological material for a microarray-based expression study. Numerous candidates were identified and their putative role in fertilization can now be tested directly in Arabidopsis.

Sexual reproduction has undoubtedly been the key to the success of eukaryotes, thus understanding the function and distribution of proteins involved in fertilization through a range of organisms should certainly shed light on the adaptive changes that occurred in their evolution. To address this question we have added the transcriptomes of human and Drosophila sperm to our plant data set. Comparative analyses of the three transcriptomes not only promise to reveal a possible common genetic basis of core functions

shared across kingdoms, but by inverse inference, which functional modules underlie functions specific to plant sperm cell.

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P6-03

GEX1 FORMS HOMODIMERS AND HAS MULTIPLE ROLES BEFORE AND AFTER FERTILIZATION

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Double fertilization occurs when the two sperm cells, carried by the pollen tube, are released in a synergid cell of the embryo sac and then fertilize the egg and the central cell to generate the zygote and primary endosperm cell, respectively. Proteins on the surfaces of the sperm, egg, central and synergid cells might be important for guidance, recognition or fusion of the gametes during fertilization. Here we present functional analyses of a plasma membrane-localized protein in Arabidopsis, GEX1, which is expressed in the sperm cells and in the egg cell and interacts with itself to form homodimers. Plants with a T-DNA insertion in the 3' end of the *GEX1* coding region had 25% reduced seed set. These zygotes arrested at 2 or 4 cells while the endosperm developed correctly, indicating a role for GEX1 during the first zygotic divisions. Down-regulation of *GEX1* using an antisense construct showed that GEX1 in the female gametophyte has a role in micropylar pollen tube guidance, and that in the male gametophyte, GEX1 is required for pollen tube arrest in a synergid cell. Because the phenotypes of the T-DNA insertion line and the antisense lines are different, we suggest that GEX1 has multiple roles before and after fertilization.

P6-04

MIS-REGULATION OF A NAT-SIRNA PAIR IN SPERM CELLS RESULTS IN SINGLE FERTILIZATIONS.

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We noticed that plants with T-DNA insertions in a gene we call *T10* had reduced seed set (50-80% in homozygotes). Reciprocal crosses with WT indicated the male gametophyte was affected, although pollen of homozygous t10 plants appeared normal and could germinate, suggesting a defect in fertilization. *T10* is in reverse orientation to the adjacent gene that we call *M.* The 3'UTR of *T10* protrudes ~80 bp into the last exon of *M*, creating a transcript overlap. We hypothesized that *T10* and *M* generate a pair of natural antisense small interfering RNAs (nat-siRNA) and that the transcript of *T10* down-regulates *M* transcripts in sperm cells. Q-PCR with flowers confirmed that the T10 transcript was almost absent from the t10 mutant, while *M* transcripts were increased. Promoter-reporter lines confirmed that *T10* is expressed specifically in sperm cells while *M* is expressed in both the vegetative and sperm cells. Co-expressing T10 and a GFP-M fusion in leaves resulted in down-regulation of GFP, supporting our hypothesis. Moreover, plants in which *M* was overexpressed in sperm had phenotypes similar to t10 mutants. DIC imaging of the undeveloped seeds 2-4 days after pollination showed that in the t10 mutants single fertilizations are prevalent (~40% of either embryo or endosperm) compared to their incidence in other known mutants. These results suggest that proper regulation of *M* in sperm cells is important for fertilization and supports the idea that sperm randomly fertilize the egg or central cell.