Peptide Signaling Regulated by CCG Transcription Complex during Pollen Tube Guidance in Arabidopsis

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In flowering plants, signals generated by the female gametophyte are involved in guiding the pollen tube to the embryo sac exactly. Previous studies showed that the synergid-secreted cysteine-rich polypeptides LURE attract pollen tube in Torenia fournieri and Arabidopsis thaliana. Furthermore, the egg apparatus-expressed diffusible peptide EGG APPARATUS1 was identified as the pollen tube attractant in Zea mays. Although these progresses provide novel insights of the pollen tube guidance, very little is known about how the gametic cells within the embryo sac coordinately regulate the signaling. Previously, we found that CENTRAL CELL GUIDANCE (CCG), a central cell expressed transcription factor is required for pollen tube attraction. Recently, we showed that CCG, together with a novel protein CCG BINDING PROTEIN1 (CBP1), recruits the mediator complex and the RNA PolIII transcription machinery to transcription factors within the central cell to directly or indirectly regulate the expression of genes that involved in pollen tube guidance. However, the regulation mechanisms still remain to be solved.

To further understand how the CCG complex functions, transcriptome analysis using RNA-seq was performed. Through this approach, we identified a family of secreted peptides. Preliminary data suggest that might they play a role in pollen tube guidance. Further analysis of their function is being carried out.
Forces Generated by Polar Cell Growth within a 3D Matrix

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We started to develop an assay to investigate how mechanical and environmental signals influence pollen tube behavior in a 3D matrix. By using automated time lapse imaging of pollen tubes growing through media of varying stiffness we aim to investigate the forces generated by the growing cell and to study how the surrounding matrix influences cell growth. Various growth parameters (velocity, direction, pollen tube dimensions) will be analyzed using the imaging analysis tool “ClickPoints”. Software solutions to investigate further parameters (cytoskeleton and vesicle dynamics and cell wall size) are planned. Our new assay system allows us to study the cellular growth behavior by imitating the resistance cells usually face during growth through tissue or soil. Ultimately we will apply this method to screen for mutant phenotypes in polar growing cell types.
Arabidopsis pollen tube growth and adhesion using an enzymatic approach

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During sexual reproduction of flowering plants, pollen grains land on the stigma, rehydrate and produce pollen tubes that grow through the female transmitting tract tissue by perceiving different signals promoting their adhesion and guidance. This allows a correct delivery of the male gametes to the ovule and ensures the proper fertilization.

Our study aims in understanding the pollen tube adhesion process. An in vitro adhesion bioassay was developed in 96 well-plates based on the work developed on lily pollen tubes. Several cell wall fractions sequentially extracted from Arabidopsis thaliana pistils were tested and only the pectin-enriched fraction allowed pollen tube adhesion. In order to check if the pectin structure able to induce adhesion was specific of the female part or could be substituted with other pectin-enriched fraction from other origins, similar extractions were performed on leaves and commercially available lemon pectins with different degree of methylesterification (DM) were tested. The data indicated that they were also able to induce high rates of pollen tube adhesion suggesting the presence of the similar adhesive structure in all the extracts. According to the monosaccharide composition of all the extracts, it suggested that homogalacturonan (HG) may be an important structural feature because the commercial lemon pectins were mostly composed of this domain. In order to determine the minimal structure required for pollen tube adhesion, an enzyme treatment was performed on the pectin-enriched extracts. When the pectin-enriched fraction from Arabidopsis leaves were treated with an endo-polygalacturonase (PGase), enzyme that cleaves α-(1-4) glycosidic bonds of the HG backbone, adhesion was totally inhibited. In contrast, adhesion assays with PGase-treated lemon pectins with a DM of 85%, which are less susceptible to PGase cleavage, allowed partial adhesion. These results suggest that HG is the most important structure for promoting the pollen tube adhesion.
Live-cell analysis of sperm cell positioning during double fertilization in Arabidopsis thaliana

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Double fertilization is a unique reproductive system of angiosperms, in which two male gametes, sperm cells, fertilize two female gametes, an egg cell and a central cell, respectively. During double fertilization, two sperm cells are released into narrow region between the egg and central cell and fuse with the female gametes strictly one-on-one. In animals, polyspermy block, which is the mechanism to avoid multiple sperm entry into the egg, is well known. Although polyspermy block in the egg cell is expected to be responsible for successful double fertilization of angiosperms (Scott et al., 2008), it is still unclear how one-on-one fusion is achieved during gamete communication. We previously showed sperm cell dynamics including a stationary phase at the narrow region between two female gametes by live-cell imaging with high-speed confocal laser scanning microscopy in Arabidopsis thaliana (Hamamura et al., 2011). In this study, we performed disruption of single female gametophytic cell within the ovule by femtosecond pulse laser of multi-photon microscopy and analyzed fertilization process of two sperm cells released into the laser-disrupted ovule. In the egg-disrupted ovule, we frequently observed that one of the two sperm cells fertilized the central cell and the other remained unfertilized, indicating that the central cell had the mechanism of polyspermy block. In addition, we performed live-cell imaging using tetraspore mutant, which possesses multiple sperm cells in one pollen tube, to analyze fertilization process when excess sperm cells are released into the ovule. We also examined correlation between positioning of sperm cells in the female gametophyte and the pattern of fertilization. Our results suggested that both the egg and central cell have mechanism to fertilize only one sperm cell just after release of sperm cells and that sperm cell release into proper position could ensure one-to-one sperm cell distribution for successful double fertilization.
Continuous convergence of actin meshwork for nuclear migration in rice zygote during karyogamy

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Transmission of genetic material from parents to next generation is a fertilization-dependent event. Fertilization comprises two sequential gametic processes: plasmogamy and karyogamy. Karyogamy is completed with migration and fusion of gametic nuclei in the fused gamete. In animals, microtubules organized by centrosomes control a female/male pronucleus migration. In contrast, the nuclear migration in angiosperms is controlled by actin filaments (AFs), however, the mechanisms are unclear.

In this study, we observed the spatial and temporal movement of AFs and sperm nucleus in the rice zygote which is produced by in vitro fertilization system. Quantitative analyses for dynamics of AFs showed that the actin meshwork convergences toward the egg nucleus. AFs appeared to interact with a portion of the sperm nuclear membrane, and the halt of the convergences of actin meshwork caused the arrest of nuclear migration. These results suggest that the sperm nuclear membrane and the AFs physically interact with each other during karyogamy, and that the sperm nucleus migrates through the convergence of the actin meshwork in fused egg cells. AF dynamics such as continuous convergence in plant female gametes for delivering organelles has not been reported in plant somatic cells or in animal cells, suggesting that female gametes in angiosperms possess a characteristic cytoskeleton system for organelle trafficking.
Development of polyspermic rice zygotes produced in vitro and possible contribution of polyspermy to polyploid formation

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In most animals and fucoid algae, polyspermy-derived extra centrioles lead aberrant nuclear and cell division, resulting in zygotic or early embryonic lethality. To ensure producing diploid zygotes, animals have developed two kinds of polyspermy block responses at the plasmagamy and karyogamy steps. In angiosperms, polyspermy block functions in the egg cell and the central cell to promote faithful double fertilization, although the mechanism of polyspermy block remains unclear. In contrast to the case in animals and fucoid algae, polyspermic zygotes formed in angiosperms are not expected to die because angiosperms lack centrosomes. However, there have been no reports on the developmental profiles of polyspermic zygotes at cellular level in angiosperms. In this study, we produced polyspermic rice zygotes by electric fusion of an egg cell with two sperm cells, and monitored their developmental profiles. Two sperm nuclei and an egg nucleus fused into a zygotic nucleus, and the triploid zygote divided into a two-celled embryo via mitotic division with a typical bipolar microtubule spindle. The two-celled proembryos further developed and regenerated into triploid plants. These findings suggest that polyspermic plant zygotes have the potential to form triploid embryos.

In natural and cultivated plants, polyploidization is a common phenomenon. Triploid plants are considered as the intermediate stage in the formation of stable tetraploid plants. As for the mechanism of triploid formation, it is generally accepted that an unreduced gamete fuses with a reduced gamete. In addition to this mechanism, the possibility of polyspermy has been proposed for maize and wheat. However, the polyspermic pathway has been regarded as an uncommon mechanism of polyploid formation, partly because it is difficult to reproduce the polyspermy situation in zygotes and to analyze the developmental profiles of polyspermic zygotes. The present study showing normal development of polyspermic rice zygotes may support the possibility of triploid formation via polyspermy.
Measuring the mechanical properties on of the pollen tube cell wall - the microscopic 3-point bending test

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The mechanism of pollen tube growth has been modeled multiple times. However, because of the lack of absolute values for its mechanical properties, these models rely on a series of assumptions and educated guesses. A crucial mechanical property of the pollen tube is the mechanical stiffness or Young's modulus of its cell wall. Measuring this property in situ is technically challenging, in particular when the cell is alive and growing. We used microfluidics to quantify the stiffness of the pollen tube cell wall by designing a microscopic 3-point bending test. Pollen grains are trapped and positioned within a microfluidic chip, the growing pollen tube guided into a microchannel and then exposed to a bending force created through directed fluid loading. The flexural rigidity of the pollen tube and the Young's modulus of the cell wall are estimated through finite element modeling of the observed fluid-structure interaction. For one of our model species, the Camellia pollen tube, we determined an average value for the Young's modulus of 350 MPa - similar to teflon. This value is in agreement with the result of an independent method based on cellular shrinkage after plasmolysis and with the mechanical properties of in vitro reconstituted cellulose-callose material.
A Protein S-Acyl Transferase (PAT) is essential for both male and female fertility in Arabidopsis thaliana

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S-acylation, commonly known as S-palmitoylation, is a reversible posttranslational lipid modification in which fatty acid, usually palmitic acid, covalently attaches to specific cysteine residues of proteins. Palmitoylation enhances the hydrophobicity of proteins and contributes to their membrane association. It plays roles in protein trafficking and signaling. A family of Protein S-acyl Transferases (PATs) is responsible for this reaction. PATs are multi-pass transmembrane proteins that possess a catalytic Asp-His-His-Cys (DHHC) cystine rich domain. In Arabidopsis there are at least 24 such PATs, four of which have been characterised, revealing their important roles in growth, development, stress responses and early senescence. Here, we reported the characterisation of another novel PAT and the diverse roles it plays in Arabidopsis sexual reproduction. Loss-of-function mutation by T-DNA insertion in this PAT results in failure of seed production in the Arabidopsis mutant. Further studies confirmed that the sterility of the mutant is caused by defects in both male and female sporophytes and gametophytes. This highlights the importance of protein lipid modification by S-palmitoylation in the development and specification of male and female sporophytes and gametophytes.
MLO function in plant reproduction: comparing their potential roles in signaling and development

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Mildew resistance locus o (MLO) proteins are a plant-specific family of seven-span transmembrane proteins with conserved calmodulin binding domains in their C-terminal cytoplasmic tails. There are 15 MLOs in the model species Arabidopsis thaliana with representatives of five out of the seven major clades identified across plant species. MLOs have been implicated in a number of physiological processes including: pathogen susceptibility, root thigmomorphogenesis, and pollen tube (PT) reception. MLO proteins have been found to be mostly redundant in their ascribed functions within their identified clades (i.e. Clade V functions in susceptibility and Clade I functions in root touch response). Due to this, higher order mutants are typically required for phenotypic analysis of MLO function. NORTIA (NTA) is the MLO protein involved in the regulation of PT reception. In the nortia (nta-1) single mutant, there are reception defects in ~30% of ovules. NTA is a part of Clade III along with MLO10, MLO8, MLO5, and MLO9 (listed from most similar to most divergent compared to NTA). Several approaches were taken to evaluate the functional redundancy of Clade III. FRET-based protein interaction assays were used to determine the conservation of MLO calmodulin-binding activity and the formation of homo-oligomers across Clade III. Ectopic expression of MLO10 and MLO8 in the synergid cells of nta-1 plants demonstrated that MLO10 is capable of functioning in PT reception while MLO8 is not, indicating that the other members of this clade may not act redundantly with NTA. In this study, GUS fusion constructs for MLO10 and MLO8, under control of their native promoters, were analyzed to determine their expression patterns within developing ovules. Neither MLO10 nor MLO8 showed expression in synergid cells. With no characterized insertion lines available for either MLO10 or MLO8, targeted mutagenesis using a CRISPR-Cas system was used to generate knockout mutants and identify potential roles that either of these MLOs may play in ovule development or signaling during reproduction. Early analysis of mlo10 and mlo8 mutants revealed decreased fertilization rates in both with similar defects occurring during embryo sac development.
Trade-offs between infection and fertility in Arabidopsis thaliana

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Pollination and pollen tube reception are very important processes during plant reproduction. They involve a series of intercellular communication pathways between male and female tissues. Pollen lands on the stigma and is recognized by the female stigma cells. Following this recognition, pollen tubes (PTs) germinate and grow through the transmitting tract of the pistil, gaining competency to respond to attractive signals from the female gametophyte. After the PT receives the signal, it locates and enters the ovule and will release two sperm cells to complete double fertilization. In the model plant Arabidopsis thaliana, this intercellular signaling pathway uses NORTIA (NTA), a Mildew resistance locus o (MLO) family protein, FERONIA (FER), a CrRLK1L-type receptor-like kinase, LORELEI (LRE), a GPI-anchored protein, and other as yet to be identified proteins. Both FER and other members of the MLO gene family are involved in the infection process of the fungal pathogen, powdery mildew. In recent years, plant-pathogen research labs have screened hundreds of naturally occurring Arabidopsis accessions for variable responses to pathogen infection. Different accessions of Arabidopsis display various susceptibility phenotypes when infected with different pathogens. Although they already identified accessions with increased susceptibility and increased resistance and many of these accessions display constitutive defense responses, it is not known if there is a trade-off between disease resistance and fertility. We acquired 180 accessions from the 1001 genomes project to conduct a large scale screen by counting the number of fertilized vs. unfertilized ovules in developing siliques. So far, the results show that around 21% of the accessions with increased resistance to PM have reduced fertility. These low fertility accessions display a range of fertility defects in both the male and female sides of reproduction. Female developmental defects include ovules with abnormal embryo sac development, and male developmental defects include large percentages of ovules with no PT attracted or some displayed an nta-like PT overgrowth phenotype. For future study, we will use genome wide associate studies on our data to determine if there are trade-offs between disease susceptibility and fertility in Arabidopsis and to identify new candidate genes that could be involved in these pathways.
Distinct signatures in ion dynamics underlie growth regimes in Arabidopsis pollen tubes

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Apical growth and ion dynamics are closely related processes in pollen tubes (PT), since sustained growth rates rely on the presence of ion gradients at the tip (especially H+ and Ca2+), while the dissipation of such gradients leads to PT growth arrest. Oscillations in both growth rate and ion dynamics have been reported in PTs, occurring in synchrony with different cellular processes, which show the same frequency as growth in distinct phase relationships. However, the biological functions of such oscillations remain elusive. Here, the effects of ion dynamics in growth were evaluated by quantitatively characterizing their oscillatory signatures in different growth regimes in Arabidopsis thaliana PTs (Col-0) and mutants for specific ion transporters, showing phenotypic effects in ion fluxes, growth, polarity and guidance. Accurate non-invasive measurements of growth, extracellular ion fluxes and intracellular ion dynamics, together with diverse computational tools, revealed distinct oscillatory signatures according to the elongation rate. Growth arrest is accompanied by a high frequency of spikes in extracellular fluxes (H+, Ca2+, Cl-) and intracellular concentrations (H+, Ca2+), with a drop on the baseline level. Sustained growth, on the other hand, occurs in an elevated baseline, while oscillations show a lower frequency/amplitude, or none at all. Mutants for different ion transporters showed changes in oscillatory signature compatible with the observed reduction in growth and ion fluxes, providing additional clues to fertilization phenotypes with potentially dramatic fitness effects. Additional studies show intraspecific and interspecific differences in frequency and amplitude of ion dynamics in distinct growth regimes, supporting that growth modulation can be achieved by tuning ion flux oscillations. These results allow developing new hypotheses about the roles of ion-based oscillations in the process of fertilization, particularly pollen tube growth, polarity establishment, guidance and intercellular interactions.
Search and analysis of genes involved in sexual reproduction based on development of novel CRISPR/Cas9 vectors

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An embryo sac contains two synergid cells, an egg cell and a central cell, and these cells play cell-specific roles in pollen tube guidance, pollen tube reception and double fertilization. Identification of cell-specific genes would contribute to elucidation of molecular mechanisms of sexual plant reproduction. However, transcriptome analyses revealed that gametophytic genes exhibited high redundancy in both Torenia and Arabidopsis. Therefore, efficient multiple knock out is critical to investigate function of these genes.

CRISPR/Cas9 system is a powerful tool for genome editing. Only two components, Cas9 protein and single guide RNA (sgRNA), can induce DNA double strand break (DSB) in a sequence-specific manner. Since the DSB results in a few base pairs insertion or deletion and it disrupts target gene function, CRISPR/Cas9 system enables us to produce knock out organisms.

Here we show a newly developed CRISPR/Cas9 vector that can induce mutations with a high efficiency in Arabidopsis thaliana. We have constructed a CRISPR/Cas9 vector carrying an early embryo stage promoter-driven Cas9 and confirmed that mutations induced by this vector could be transmitted to the next generation. Furthermore, we obtained null mutants in some targeted genes in T1 generation. Now we are working on multiple knock out experiments focused on synergid cell specific genes that might be involved in male (pollen tubes) - female (synergid cells) interactions.
A membrane proteomics approach to identify gamete-expressed cell surface proteins involved in double fertilization

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The delivery of two functional sperm cells into the female gametophyte and their fusion with two female reproductive cells (egg cell and central cell) is a characteristic feature of flowering plants, called double fertilization. The molecular mechanisms of gamete recognition, adhesion and fusion are largely unknown. So far, only three proteins are reported in Arabidopsis thaliana to act on the gamete surfaces to accomplish double fertilization: the two essential sperm cell plasma membrane proteins GCS1/HAP2 (GENERATIVE CELL SPECIFIC1/HAPLESS 2) and GEX2 (GAMETE-EXPRESSED 2) are required for gamete adhesion and fusion during fertilization, while the family of small cysteine-rich EC1 (EGG CELL 1) proteins is secreted by the egg cell upon sperm cell delivery to rapidly activate the sperm cells, indicated by a shift of GCS1/HAP2 from the sperm endomembrane system to the plasma membrane.

We aim to learn more about the membrane protein composition of flowering plant gametes. To assess the complexity of the sperm membrane proteome and to identify cell surface proteins potentially involved in male-female gamete interactions we established a method to isolate large quantities of maize sperm cells and perform membrane protein extractions followed by high-throughput proteomics. Selected candidates from these mass spectrometry datasets are currently investigated in maize and putative orthologs are studied in Arabidopsis regarding their expression pattern and function during double fertilization. Isolated maize sperm cells are furthermore used to study the molecular mechanisms involved in sperm cell activation and to identify EC1 interacting proteins on the sperm cell surface.
NAC transcription factors redundantly control ovule senescence and receptivity in Arabidopsis

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Successful pollination and fertilization is a crucial, yet vulnerable step during the sexual reproduction of flowering plants. In lastingly unpollinated flowers, senescence is initiated in the female tissues, leading to an irrevocable loss of seed set potential. Our lab focuses on the elucidation of the molecular mechanisms that control the duration of floral receptivity at the level of the stigma and the ovules. Arabidopsis ovules degenerate about four days after anthesis. Precisely when ovule receptivity is lost remains difficult to determine as stigma degeneration precedes ovule degeneration. We performed tissue-specific time course RNAseq experiments covering the critical interval during which floral receptivity drops. Next to core marker genes of programmed cell death (PCD), several transcription factors were among the earliest differentially expressed genes, highlighting the relevance of transcriptional control during age-induced floral organ senescence and PCD. The underlying transcriptional network was dissected by reverse genetics approaches giving rise to mutants with dramatically extended ovule longevity. By understanding the mechanisms that control floral longevity, this trait can be modulated to optimize the window for successful seed set in crops, as well as to prolong the life time of ornamental flowers.
Evolutionary and Functional Analysis of LORELEI, a GPI-Anchored Membrane Protein Involved in Pollen Tube Reception in Arabidopsis thaliana

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During angiosperm reproduction, once a pollen tube enters an ovule, it arrests growth in the synergid cell of the female gametophyte and lyses to release sperm cells for double fertilization. In Arabidopsis thaliana, mutations in LORELEI (LRE), a glycosylphosphatidylinositol (GPI) anchored membrane protein, disrupt pollen tube growth arrest in the synergid cell and consequently the pollen tube coils within the female gametophyte. Furthermore, this “coiling” behavior is seen even when two closely related species are crossed to each other, suggesting that pollen tube reception is a pre-zygotic barrier in interspecific crosses. LRE and its most closely related paralog, LORELEI-LIKE GPI-Anchored Membrane Protein 1 (LLG1), are the result of a whole genome duplication. Previously, we demonstrated LLG1 is not involved in pollen tube reception. Instead, it functions in vegetative tissues, where LRE is not expressed (Li et al., 2015). To understand the evolution LRE and LLG1, post gene duplication in Brassicaceae, we tested for positive selection along the branches leading to the LRE and LLG1 clades using the Branch Sites Model Test in PAML. We performed a Model C Clade Test in PAML to verify differences in selective pressure between LRE and LLG1 post gene duplication. We found that LRE experienced relaxed selection post gene duplication, consistent with its hypothesized role in reinforcing pollen tube reception as a pre-zygotic barrier in interspecific crosses within Brassicaceae. Future work will identify the role of the amino acid residues identified in PAML analysis in gene-specific functions and in conferring species specificity.
Maternal haploids are preferentially induced by CENH3-tailswap transgenic complementation in maize

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Doubled haploid plants are invaluable breeding tools but many important crop species are recalcitrant to available haploid induction techniques. To test if haploid inducer lines can be engineered into crops, CENH3-/- and CENH3:RNAi lines were complemented by AcGREEN-tailswap-CENH3 or AcGREEN-CENH3 transgenes. Haploid induction rates were determined following testcrosses to wild-type plants independently controlling for inducer parent sex and transgene zygosity. CENH3 fusion proteins were localized to centromeres and did not cause vegetative defects or male sterility. CENH3:RNAi lines did not demonstrate consistent knockdown and rarely produced haploids. In contrast, many of the complemented CENH3-/- lines produced haploids at low frequencies. The rate of gynogenic haploid induction reached a maximum of 3.6% in several hemizygous individuals when backcrossed as males. These results demonstrate that CENH3-tailswap transgenes can be used to engineer in vivo haploid induction systems into maize plants.
A novel glycosyltransferase related to animal protein O-fucosyltransferases is involved in pollen tube guidance in Arabidopsis

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Fertilization is an essential event in the plant life cycle, and involves the fusion of male and female gametes to produce a new embryo. Male gametes are housed in pollen grains and are translocated to the egg cell through a specialized pollen tube, which must rapidly elongate down the female transmitting tract to successfully locate unfertilized ovules for fertilization to commence. T-DNA insertional mutations in a glycosyltransferase-like gene (At3g05320) compromised self-fertilization in Arabidopsis. Phylogenetic analyses indicate that this gene shares sequence similarity to animal protein O-fucosyltransferases, which attach fucose residues to Ser/Thr residues of target proteins. Therefore, we named this protein Arabidopsis O-fucosyltransferase 1 (AtOFT1). Mutations in oft1 resulted in reduced silique elongation, reduced seed set, and an uneven distribution of fertilized seeds within the carpel. Segregation distortion assays indicated that this fertilization defect was due to incompetent pollen, but pollen tube germination assays revealed that oft1 mutant pollen was viable, based on the observation that oft1 mutant pollen germinated and produced pollen tubes with similar lengths to wild-type controls. However, analine blue staining of oft1 pollen tubes in dissected carpels revealed that oft1 mutant pollen tubes consistently grew past unfertilized ovules, suggesting that oft1 mutants are defective in pollen tube guidance. These observations suggest that the putative OFT1 glycosyltransferase may catalyze a protein glycosylation events that is essential for pollen tube guidance and fertilization.
Ultrastructural analysis of Arabidopsis thaliana ovules

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Arabidopsis thaliana has polygonum type embryo sacs composed of seven cells; three antipodal cells, the egg cell, the central cell, and two synergid cells. The synergids are found in the micropylar region near the entrance to the female gametophyte. These support cells are important for pollen tube attraction, repulsion, arresting the growth of the pollen tube once it has reached the synergid cell, and pollen tube rupture. The purpose of this project is to fully characterize the ultrastructure of Colombia Wild type Arabidopsis embryo sacs, in preparation for future ultrastructural studies of several mutant lines such as LORELEI and FERONIA. Ovules were emasculated at Arabidopsis floral stage 12, and were fixed 12-24 hours later. Standard TEM methods were followed to embed individual ovules in a modified Spurr’s resin. From our studies we have been able to assemble ultrastructural montages of entire female gametophytes. Several of the ovules examined showed signs of cellular degeneration, confirming that unpollinated ovules may only be viable for a discrete amount of time. There are distinct cell walls around the antipodal cells, however the cell membranes separating the egg cell from the central cell and the synergid cells does not always seem to be continuous. Although there are basic descriptions of Arabidopsis ovule ultrastructure, this study will be able to provide a greater understanding of the embryos sac ultrastructure just prior to pollination.
Is HAP2(GCS1) the only DUO1-regulated gene required for gamete fusion?

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The mechanisms required for gamete plasma membrane fusion, a critical event in all sexually reproducing organisms, have not been defined in any species. HAP2(GCS1) is a candidate gamete fusion protein that is broadly conserved in eukaryotes and has been shown to be essential for gamete fusion in several species including Arabidopsis. It is not yet known what molecules function with sperm-expressed HAP2(GCS1) to mediate gamete fusion in flowering plants. We set out to determine whether other sperm-expressed genes are required for gamete fusion by asking whether expression of HAP2(GCS1) is sufficient to restore fertility to duo1 mutant sperm. duo1 mutant pollen produce a single sperm-like cell that is incapable of fusing with either the egg cell or the central cell. DUO1 is a critical regulator of sperm identity and is required for the expression of HAP2(GCS1) and several other sperm-expressed genes. We find that expression of HAP2(GCS1) in duo1 sperm initiates development of seeds containing either an embryo or a central cell. However, these events are rare, suggesting either that HAP2(GCS1) is not sufficient to mediate efficient gamete fusion or that duo1 mutant sperm are incapable of initiating development following gamete fusion. To distinguish between these two possibilities, we are developing sperm nuclear markers to quantitatively determine the frequency of single gamete fusion events, and we are testing whether the ploidy of duo1 sperm limits their fertility following fusion. Our goal is to define the genes that enable sperm to fuse with the egg and central cell and successfully promote seed formation.
Triploidy and its effect on Arabidopsis reproduction

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In plants, auto- and alloploidy is considered to mainly arise from the fusion of unreduced gametes or somatic doubling. Somatic doubling can result in an instant tetraploidization of a diploid plant. By contrast, polyploidization through unreduced gametes or following interploidy crosses often involve a triploid bridge. The term triploid bridge refers to the presence of a triploid plant that serves as a transition towards a more stable tetraploid stage (Mason & Pires, 2015; Ramsey & Schemske, 1998). Here we provide insights into some of the unique morphological characteristics of triploid plants and discuss its effect in the reproduction of Arabidopsis thaliana.
LORELEI Function in Pollen Tube Reception at the Interface of the Synergid Cell and the Pollen Tube Requires the Modified Eight-Cysteine Motif and FERONIA Receptor-Like Kinase

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In angiosperms, pollen tube reception by the female gametophyte is required for sperm release and double fertilization. In Arabidopsis lorelei (lre) mutants, pollen tube reception fails in most female gametophytes, which thus remain unfertilized. LORELEI(LRE) encodes a putative glycoosphatidylinositol (GPI)-anchored surface protein with a modified eight-cysteine motif (M8CM). LRE fused to cYFP (LRE-cYFP) remains functional and localizes in the synergid plasma membrane-rich filiform apparatus, the first point of contact between the pollen tube and the female gametophyte. Structure-function analysis using LRE-cYFP showed that LRE function in pollen tube reception requires the M8CM, but not the GPI anchor addition domains. Consistent with this, LRE-cYFP-TM, where GPI anchor addition domains was replaced with a single-pass transmembrane domain, fully complemented pollen tube reception defect in lre-7 female gametophytes. Ectopically expressed and delivered LRE-cYFP from pollen tubes to the synergid cell surface could non-cell autonomously complement the pollen tube reception defect in lre female gametophytes, only if expressing FERONIA. These results indicated that LRE and FERONIA jointly have a synergid cell surface-specific function in pollen tube reception, at the interface of the synergid and the pollen tube. Our study established a model to investigate GPI anchoring of plant proteins, which remain poorly characterized.