Arabidopsis DELLA mutants are defective in male meiotic cytokinesis

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Environmental factors influence plant development and in particular the reproductive system is sensitive to extreme temperature conditions causing reduction in fertility. Previously we reported that a short period of cold stress evokes the formation of diploid pollen by interfering with the organization of the radial microtubule array at the telophase II tetrad stage, and consequently leading to incomplete or irregular cytokinesis. A subpopulation of binuclear and polynuclear microspores then forms which eventually develop into diploid or polyploidy pollen grains. However, the involved molecular regulators and signaling pathways are still unknown. Here, we show that GA signaling plays a role in post-meiotic cytokinesis. By cytological and genetic approaches, we found that Arabidopsis GA signaling mutants show post-meiotic cytokinesis defect in male gametes meiocytes similar to those observed in cold-treated wild type Columbia-0 plants. Moreover, male sporogenesis in the GA mutants is not overly sensitive to cold suggesting that cold-induced defects in male meiotic cytokinesis stress may be mediated by GA signaling. In conclusion, we propose that GA signaling contributes to the establishment of post-meiotic cytokinesis in male meiosis and potentially functions in cold-induced diploid male gametes formation.

Pattern formation on pollen surface: Aperture number and positions are determined through a ploidy-sensitive mechanism

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Pollen presents a powerful model for the controlled formation and deposition of extracellular structures. Pollen grains are surrounded by a complex extracellular structure, pollen wall exine, which assembles into intricate 3D patterns exhibiting enormous morphological diversity across species, yet very conserved within a species. What genetic and developmental programs control formation of the precise patterns on pollen surface is essentially unknown. We will present data on the formation of one specific patterning element on pollen surface – pollen apertures. Apertures are the characteristic openings in exine that are species-specific in their number, morphology, and location – for example, Arabidopsis pollen has three equidistant longitudinal apertures. Existence of apertures indicates that, in a given species, certain areas on the pollen surface differ from the rest of the surface and that these areas can broadcast their differences to the exine deposition machinery. Eighty years ago (Wodehouse, 1935), it was proposed that aperture number and placement might be determined by the geometry of tetrads of microspores, the precursors of pollen grains arising via meiotic cytokinesis, and by the number of last-contact points between sister microspores at the end of cytokinesis. By using Arabidopsis mutants with abnormal numbers of apertures and/or abnormal conformations of meiotic products, we finally tested this model. We conclude that, contrary to the Wodehouse model, the last-contact points do not serve as determinants of aperture number and the correct geometric conformation of a tetrad is neither necessary nor sufficient to generate a correct number of apertures. Instead, the number of apertures is specified via a ploidy-sensitive mechanism, which functions upstream of the aperture factor INP1 and guides INP1 to its highly specific positions on microspore surface to define areas where apertures will form.

Identifying the wall-degrading enzymes responsible for microspore release from the pollen tetrad in Arabidopsis thaliana

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Dissolution of the pollen tetrad walls after meiosis, and subsequent release of the haploid microspores, is an essential step in pollen development. The thick tetrad wall consists of callose (β-1,3-glucan), with a primary cell wall of cellulose, hemicellulose and pectin surrounding the whole structure. Despite the importance of microspore release, the wall-degrading enzymes involved have yet to be identified in Arabidopsis thaliana.

In this study 18 endo-β-1,3-glucanase genes were identified in A. thaliana that are expressed in the young bud at microspore release. Single and multiple-gene T-DNA insertion knockout lines showed no obvious phenotypic effect, indicating likely gene redundancy. GFP-tagging of the two most specifically expressed genes (A6 and A6F) showed that A6 is present in the locule at microspore release, whereas A6F is not. Early ectopic expression of A6 and A6F resulted in defects in pollen development indicating that both proteins possess enzymatic activity. However, early dissolution of the tetrad was not achieved in these lines suggesting that A6 and A6F may not be able to effectively target the callose wall or that other factors are required.

Although endo-β-1,3-glucanases are likely to be most important for callose degradation, there are exo-β-1,3-glucanase encoding genes that are expressed at microspore release. Two GH3 exo-β-1,3-glucosidases were found to be highly expressed at microspore release and so may play a role alongside endo-β-1,3-glucanases in tetrad dissolution. These and other data will be discussed to provide insight into our current understanding of this essential developmental process.

Anther and pollen development in Arabidopsis thaliana: evidence supporting the cluster model of anther wall layer development

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Anther and pollen development, being easily accessible organs within the flower, are well-characterized processes in many plant species. However, the progression of certain pre-meiotic developmental stages or the function of some tissues are still controversial and under debate. Using different whole-mount clearing and staining techniques for bright field, fluorescence, and confocal microscopy, we report a thorough and detailed description of all stages of anther and pollen development in the model species Arabidopsis thaliana. A special emphasis is given to the evidence in support of the cluster model of archesporial and anther wall layer development, as recently suggested for Zea mays, against the popular lineage model. An in depth analysis of callose dynamics within different anther tissues and how it might help to unravel the still unknown function of the middle layer is also included. The potential of these techniques, either for a quick screening of all developmental stages or for a detailed characterization of a specific process, after an environmentally or genetically induced developmental aberrations is also illustrated.

MALE MEIOCYTE DEATH2 Encoding an F-box Protein is Required for Male Meiocyte Development and meiotic progression in Rice

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F-box proteins constitute a large superfamily in plant genomes. F-box proteins play important roles in controlling many biological processes, but their function in male meiocyte development remains unclear. A novel F-box gene in rice, Male Meiocyte Death2 (MMD2), discovered in this study have an essential role in meiocyte development and meiotic progression. MMD2 belongs to FBX subfamily and exists only in angiosperms. MMD2 acts predominantly during leptotene to pachytene of meiosis prophase I in male meiocytes. In the absence of MMD2, telomere bouquet formation and homologous chromosome pairing and synapsis were disrupted. The mutant meiocytes were arrested at pachytene stage and directed to apoptosis at later stage. In addition, a persistent γH2AX foci and the absence of COM1 and RAD51C signals were detected in the mutant meiocytes, indicating that MMD2 is not required for double-strand break (DSB) formation but is crucial for the DSB end-processing and repair. Further analyses showed that MMD2 could physically interact with the rice Skp1-like Protein1 (OSK1), indicating that MMD2 functions as the component of a SCF E3 ligase to regulate the meiotic progression in rice. This study is the first report the involvement of the F-box protein in meiosis in plants and will contribute to elucidation the specific roles of posttranslational modification mediated by the ubiquitin proteasome system in meiosis.

Differential epigenetic specification of parental centromeres leads to genome elimination in Arabidopsis

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Centromeres are chromosomal loci that attach to spindle microtubules through kinetochores during cell division and are essential for chromosomal inheritance. CENH3 is a centromere specific histone H3 variant essential for functional centromere specification. Arabidopsis (2n=10) cenh3 mutant plants (-/-) can be rescued with a variant CenH3 transgene (GFP-tailswap), which also marks the functional centromere. Crossing the GFP-tailswap complemented plant to wild-type male leads to complete elimination of chromosomes contributed by the mutant parent and results in haploid progeny exclusively carrying the wild-type genome. Here we present the cytological observations of cellular events leading to genome elimination in hybrid embryos of Arabidopsis. All the nuclei in 2-4 cell stage embryos resulting from the genome elimination cross displayed five bright GFP-tailswap signals which are often accompanied with 1 to 8 faint GFP-tailswap signals. Those 5 centromeres displaying bright GFP-tailswap signals assembled efficient kinetochores and also loaded with wild type CENH3 expressed from the male parent. Upon crossing with wild-type tetraploid male (2n=20), similar stage embryos exhibited 10 bright GFP-tailswap signals in each nucleus and thus revealing the paternal and wild type origin of those centromeres displaying bright GFP-tailswap signals. We conclude that during centromeric specification in the parent, the variant CENH3 creates an altered epigenetic environment, which in the hybrid embryos leads to functional impairment and eventual chromosomal loss.

Control of Anther Cell Differentiation by the EMS1 Receptor Kinase-linked Signaling Complex in Arabidopsis

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In flowering plants, successful sexual reproduction depends on the specification of distinct reproductive cells that conduct meiosis to form gametes and associated somatic cells that provide nutrition and developmental cues to ensure successful gamete production. Anther, the male reproductive organ in a flower, typically has four lobes (microsporangia). Within each of these lobes in a mature anther, the central reproductive microsporocytes (or pollen mother cells) are surrounded by four concentrically organized somatic cell layers: epidermis, endothecium, middle layer, and tapetum. Microsporocytes give rise to pollen via meiosis, while somatic cells, particularly the tapetum, are required for the normal development and release of pollen. Our previous studies showed that the EMS1 leucine-rich repeat receptor-like kinase (LRR-RLK) plays an essential role in somatic and reproductive cell differentiation during anther development. Recent research in my lab for the first time demonstrates that TPD1 is a novel small secreted cysteine-rich protein ligand for EMS1. The TPD1-EMS1 signaling initially promotes the periclinal division of secondary parietal cells to form a monolayer of tapetal precursor cells and then determines the fate of functional tapetal cells. We also identified the EMS1 co-receptor SERK1/2 and its downstream player CA1. EMS1 and SERK1/2 functions in the same genetic pathway. Bimolecular fluorescence complementation (BiFC) and Förster resonance energy transfer (FRET) analyses demonstrate that TPD1 interacts with EMS1 in vivo. Trans-phosphorylation between EMS1 and SERK1/2 enhances the EMS1 kinase activity. In addition, we determined phosphorylation sites of EMS1 and their biological significance. Our yeast two-hybrid screening identified the EMS1 downstream player CA1. Besides several lines of evidence supports the biochemical interaction between EMS1 and CA1, our results show that loss-of-function mutants of CA1 are defective in tapetal cell differentiation, while overexpression of CA1 results in the formation of additional tapetal cells. A model will be discussed to explain how the TPD1-EMS1/SERK1/2-CA1 signaling pathway controls anther cell differentiation. Collectively, our results highlight a novel mechanism by which signals from reproductive cells determine somatic cell fate in plant sexual reproduction.