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EXPERT VIEW

Molecular mechanisms of pollen aperture formation in Arabidopsis and rice

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Abstract

Apertures are specialized regions on the pollen surface that receive little to no exine deposition, forming distinct structures important for pollen function. Aperture number, shape, and positions vary widely across species, resulting in diverse, species-specific patterns that make apertures fascinating from both cell biological and evolutionary perspectives. Aperture formation requires developing pollen to establish polarity and define specific regions of the plasma membrane as aperture domains. In the decade or so since the discovery of the first aperture factor, INAPERTURATE POLLEN1 (INP1), pollen apertures have become a powerful model for investigating how cells form distinct plasma membrane domains. Recent studies in Arabidopsis and rice, two species with contrasting aperture patterns, have identified key molecular players that regulate aperture domain specification and development. In this review, we summarize these advances and discuss directions for future studies on the molecular mechanisms controlling aperture formation.

Keywords: Aperture, aperture factor, callose wall, cell polarity, exine, microspore, pattern formation, plasma membrane domain, pollen.

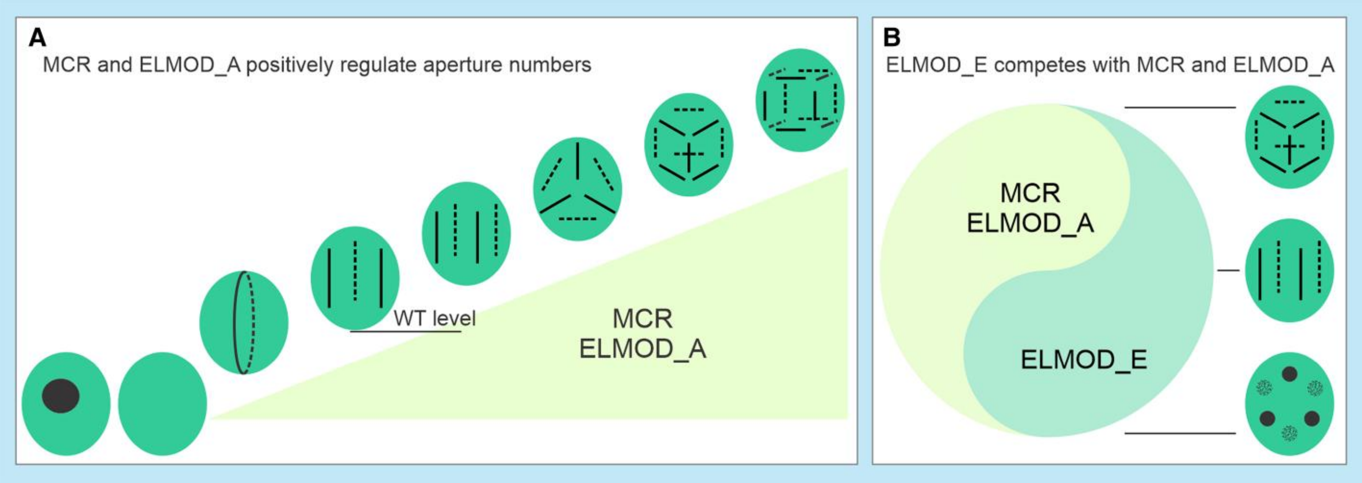
Introduction

Pollen grains, the male gametophytes of flowering plants, are enclosed in a specialized cell wall, the exine. Certain regions of the pollen surface receive little or no exine deposition, developing into pollen apertures (Zhou and Dobritsa, 2019). Apertures serve as portals for pollen tube emergence and water flux, and provide structural flexibility for pollen volume changes during desiccation and hydration (Heslop-Harrison, 1979a; Katifori *et al.*, 2010; Vieira and Feijó, 2016; Wang and Dobritsa, 2018).

Apertures vary in their numbers, positions and morphologies, creating striking patterns on the pollen surface (Furness and Rudall, 2004). While usually conserved within a species, aperture patterns can differ dramatically between species, suggesting that the mechanisms underlying their formation are robust, yet evolutionarily flexible (Zhou and Dobritsa, 2019). The two most typical aperture patterns are seen in the model dicot *Arabidopsis thaliana* and the model monocot rice (*Oryza sativa*) (Walker *et al.*, 1975; Ressayre *et al.*, 2002). Arabidopsis pollen develops three equidistant, longitudinal furrows centered at the pollen equator (tricolpate pattern), while rice pollen has a single circular pore located at the distal pole (monoporate pattern).

Aperture patterns are established during microsporogenesis. The first signs appear at the tetrad stage of pollen development,

Box 1. Key recent developments in understanding aperture pattern formation



Zhou *et al.* (2021) demonstrated that three members of the ancient ELMOD protein family influence pollen aperture patterning in Arabidopsis. (A) MCR and ELMOD_A act synergistically to promote the formation of furrow-shaped apertures, with the number of developing aperture domains being sensitive to the levels of MCR and ELMODA. Wild-type (WT) levels of MCR and ELMOD_A produce three apertures, whereas higher or lower levels result in pollen with more or fewer apertures, respectively. (B) When overexpressed, ELMOD_E alters aperture number, shape, and position, resulting in the formation of supernumerary round apertures. ELMOD_E competes with MCR and ELMOD_A, potentially for the same interactors.

when four microspores—the products of male meiosis—are enclosed in a common callose wall. At this stage, the microspores form distinct plasma membrane (PM) domains at the future aperture sites. These domains lack PM undulations and stay tightly appressed to the callose wall, preventing primexine, the exine precursor, from forming between the PM and the callose wall (Dobritsa *et al.*, 2018; Zhang *et al.*, 2020). How these domains differ from the rest of the PM, and what mechanisms determine their number, positions, and morphology in different species, are intriguing questions at the intersection of cell biology and evolutionary biology. The discovery in Arabidopsis, about a decade ago, of the first aperture factor, INAPERTURATE POLLEN1 (INP1), which specifically aggregates at the aperture domains (Dobritsa and Coerper, 2012), established pollen apertures as a model for studying how cells develop polarity and form distinct PM domains (Zhou and Dobritsa, 2019). In this review, we highlight recent advances in uncovering the molecular mechanisms of pollen aperture formation in Arabidopsis and rice.

Members of the ELMOD protein family control aperture patterns

Although the striking patterns of pollen apertures have long fascinated palynologists, evolutionary biologists, and plant physiologists, how these patterns are established is still largely unknown. Recently, however, three members of the ancient ENGULFMENT AND CELL MOTILITY DOMAIN (ELMOD) protein family were identified as key regulators of aperture patterning (Zhou *et al.*, 2021). A genetic screen in Arabidopsis uncovered a mutant, *macaron* (*mcr*), producing pollen with a single ring-like furrow that divides pollen into two symmetrical halves (Plourde *et al.*, 2019). This aperture develops through a fusion of two longitudinal domains positioned on opposite sides of the pollen grain (Zhou *et al.*, 2021). Thus, loss of MCR reduces the number of aperture domains from three to two, and changes their positions, without affecting domain shape or orientation.

Notably, the reduction in aperture number caused by the *mcr* mutation occurs across different ploidy levels (Plourde *et al.*, 2019). Ploidy is known to influence aperture numbers, potentially by altering gene expression through changes in gene dosage (Reeder *et al.*, 2016; Plourde *et al.*, 2019). Additionally, Arabidopsis plants with elevated *MCR* levels produce pollen with more than three apertures—up to 12 in some cases—with aperture number correlating with expression level of *MCR* (Zhou *et al.*, 2021) (Box 1A). These findings suggest that *MCR* expression must be tightly regulated in wild-type Arabidopsis to ensure the formation of exactly three apertures, and that ploidy-dependent variations in aperture number could be due to changes in the dosage of *MCR* and related genes.

MCR (also known as ELMOD_B) belongs to the ancient ELMOD protein family, which includes six members in

Arabidopsis: ELMOD_A through ELMOD_F (East *et al.*, 2012). ELMOD proteins are widespread in the plant kingdom, and in angiosperms, cluster into four distinct clades: A/B, C, E, and F, named after the corresponding Arabidopsis proteins (Zhou *et al.*, 2021). Most angiosperms have at least two genes within the A/B clade, suggesting strong evolutionary pressure to maintain multiple copies, and highlighting the functional importance of this clade. Indeed, Arabidopsis ELMOD_A functions partially redundantly with MCR: although its single loss-of-function mutants have normal apertures, the *mcr elmod_a* double mutant produces one defective round aperture or loses apertures entirely (Zhou *et al.*, 2021). Furthermore, overexpression of *ELMOD_A* produces pollen with four to six furrows. MCR and ELMOD_A, therefore, act synergistically to promote the formation of furrow-shaped apertures, with MCR playing a more prominent role (Box 1A).

Another member of the ELMOD protein family, ELMOD_E, exerts broad influence on aperture patterning when overexpressed, affecting aperture number, shape, and position (Zhou *et al.*, 2021). Instead of the three longitudinal furrows, overexpression of *ELMOD_E* induces multiple round apertures. High MCR levels can suppress ELMOD_E activity and vice versa, suggesting that these proteins may compete for interactors (Box 1B). The diverse effects of ELMOD proteins on aperture patterning, along with their dosage sensitivity, raise the hypothesis that interspecific differences in aperture patterns may be caused by modulation of ELMODs, their effectors, or their upstream regulators. Identifying the regulators and interactors of ELMOD proteins will be essential to understand how aperture patterns are established.

ELMOD proteins are found across all eukaryotic supergroups, supporting their ancient origin (East *et al.*, 2012). Mammalian ELMODs are implicated in diverse processes, including cell division, microtubule dynamics, ciliogenesis, mitochondrial fusion, vesicle trafficking, and PM domain formation (Ivanova *et al.*, 2014; Suzuki *et al.*, 2015; Turn *et al.*, 2020, 2021; Liu *et al.*, 2023). They act as non-canonical GTPase-activating proteins (GAPs) for the ADP-ribosylation factor (Arf) family of GTPases, including both Arf and Arf-like (Arl) proteins (Bowzard *et al.*, 2007; Ivanova *et al.*, 2014; Turn *et al.*, 2020, 2022).

The biochemical function of plant ELMODs remains unknown. However, a yeast two-hybrid screen identified barley ELMOD_C as an interactor of a Rho-of-Plants GTPase-activating protein (ROP-GAP) (Hoefle and Hückelhoven, 2014). ROP GTPases are major regulators of cell polarity and domain formation in plants (Yang and Lavagi, 2012; Feiguelman *et al.*, 2018; Denninger, 2024), so it remains to be seen whether the ELMODs involved in aperture formation regulate Arf or ROP GTPases, or participate in some other mechanisms. Investigating the biochemical activity and interaction networks of Arabidopsis ELMODs will be key to understanding how they contribute to PM domain specification and aperture patterning.

Other factors controlling aperture patterns in Arabidopsis

The aperture domain-associated protein kinase D6 PROTEIN KINASE-LIKE3 (D6PKL3), which acts downstream of MCR, and interacts with phosphoinositides enriched at the aperture domains, also appears to influence aperture numbers (Lee *et al.*, 2018). In transgenic plants overexpressing *D6PKL3*, 5–25% of pollen grains develop more than three apertures, suggesting that *D6PKL3* may have some effect on aperture patterning.

In the *doughnut* (*dnt*) mutant, pollen develops two round, hole-like apertures relocated from the pollen equator to the poles (Plourde *et al.*, 2019). This striking phenotype indicates that the *DNT* gene, whose identity has not yet been reported, regulates all aspects of aperture patterning, including aperture number, shape, and positions. Characterizing the *DNT* gene and investigating the relationships between ELMODs, D6PKL3, and DNT will provide important insights into the establishment of aperture patterns.

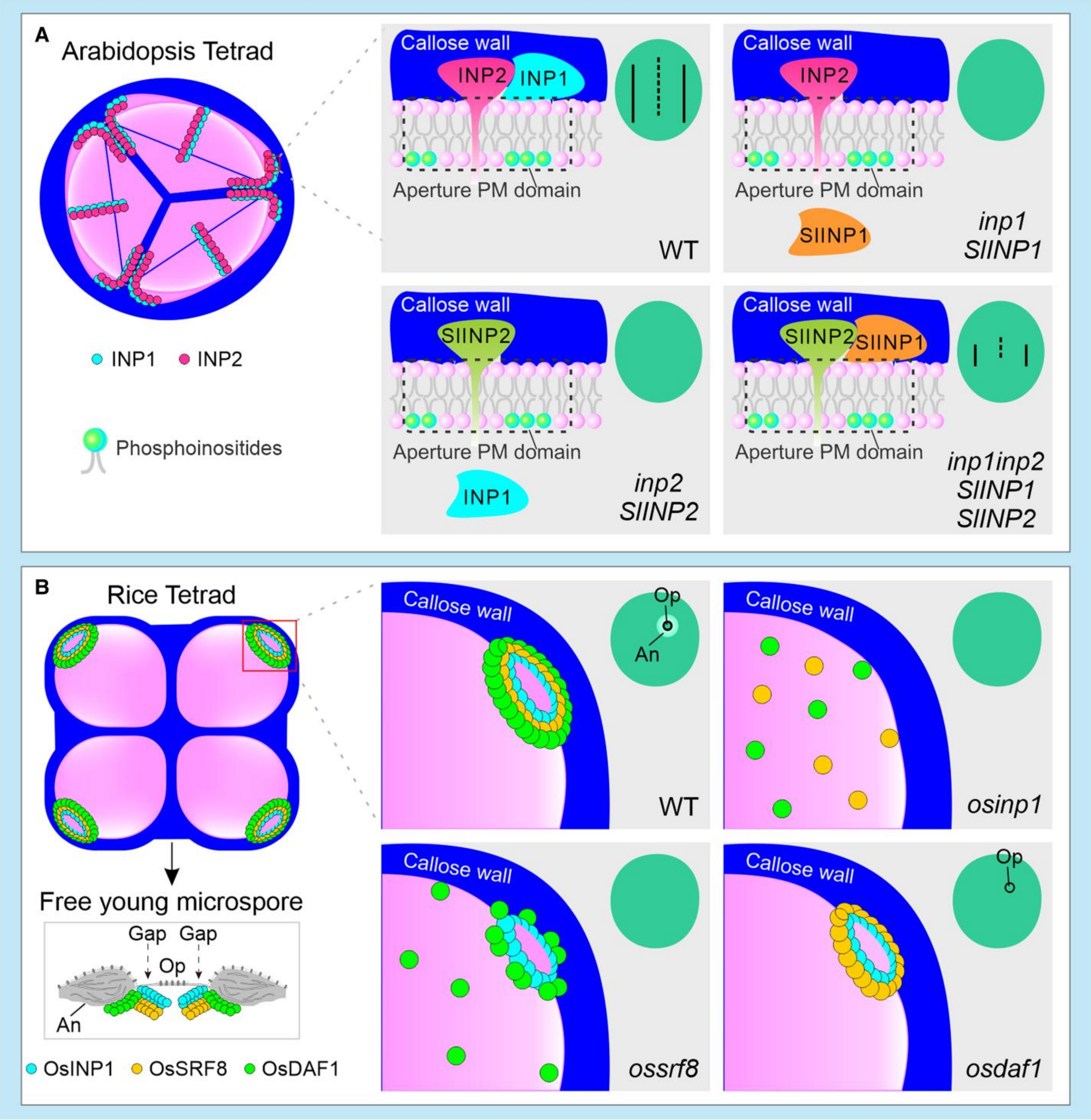
INP1 and its interacting partners are aperture executors

The first identified aperture factor, INAPERTURATE POLLEN1 (INP1), is essential for executing steps leading to pollen aperture formation. Its disruption results in a complete loss of apertures (Dobritsa *et al.*, 2011; Dobritsa and Coerper, 2012). INP1 contains a single recognizable domain—the plant-specific DELAY OF GERMINATION1 (DOG1) domain—whose function remains unknown (Marchler-Bauer *et al.*, 2017). INP1 acts downstream of ELMODs, DNT, and, likely, D6PKL3 (Lee *et al.*, 2018; Plourde *et al.*, 2019; Zhou *et al.*, 2021), and is proposed to assemble at the extracellular side of the aperture domains, marking positions of future apertures and facilitating, directly or indirectly, close contact of these PM domains with the callose wall, which is required for proper INP1 localization (Dobritsa *et al.*, 2018).

Despite significant sequence divergence, the role of INP1 as a core aperture factor is conserved across angiosperms. Disruption of INP1 homologs leads to aperture loss in diverse species such as maize (*Zea mays*), rice, and California poppy (*Eschscholzia californica*) (Li *et al.*, 2018; Zhang *et al.*, 2020; Mazuecos-Aguilera *et al.*, 2021). In dioecious grapes (several *Vitis* species and *Muscadinia rotundifolia*), *INP1* is part of the sex-determining region in the genome, and carries a conserved loss-of-function mutation in female haplotypes, which is believed to cause aperture loss and pollen sterility in female plants (Massonnet *et al.*, 2020, 2025). However, *INP1* homologs from distantly related species cannot substitute for Arabidopsis *INP1*, suggesting that these rapidly evolving proteins may require species-specific partners (Li *et al.*, 2018).

Recently, another aperture program executor, INP2, was identified as an interacting partner of INP1 (Lee *et al.*, 2021).

Box 2. Key recent developments in understanding how aperture domains are converted into apertures



(A) Lee *et al.* (2021) discovered that the DOG1-domain proteins INP1 and INP2 interact in a species-specific manner to execute aperture formation. Recruitment of INP1 to the aperture domains and successful aperture formation occur only in Arabidopsis microspores expressing INP1–INP2 pairs from the same species, but not in cross-species combinations. (Top left) In wild-type (WT) Arabidopsis, INP1 and INP2 interact, presumably at the aperture domains, resulting in the formation of apertures (shown in the oval on the right). (Top right) In the Arabidopsis *inp1* mutant, transgenic tomato INP1 (SlINP1) fails to interact with Arabidopsis INP2 and does not accumulate at the aperture domains, resulting in the absence of

Box 2. Continued.

apertures. (Bottom left) In the Arabidopsis *inp2* mutant, transgenic tomato INP2 (SlINP2) likewise fails to interact with Arabidopsis INP1, also preventing aperture formation. (Bottom right) When SlINP1 and SlINP2 are co-expressed in Arabidopsis double mutants, SlINP1 is recruited to the aperture domains and apertures are partially restored.

(B) Zhang *et al.* (2020) demonstrated that rice OsINP1, like its Arabidopsis homolog, is essential for aperture formation. They also identified the aperture decorator OsDAF1, whose disruption leads to the loss of annulus, and showed that OsDAF1 is recruited to the aperture PM domain via interaction with OsINP1. Chen *et al.* (2024) and Zhang *et al.* (2024) demonstrated that OsSRF8 is another aperture executor that acts downstream of OsINP1 and directly interacts with it at the aperture PM domain. This complex subsequently recruits OsDAF1 to this domain to promote annulus formation. (Top left) In WT microspores, OsINP1, OsSRF8, and OsDAF1 localize to the single ring-like domain, leading to the formation of a pore-like aperture with annulus (An) and operculum (Op). (Top right) In the *osinp1* mutant, the aperture fails to develop, and OsSRF8 and OsDAF1 do not assemble at the aperture domain. (Bottom left) In the *ossrf8* mutant, the aperture fails to develop; OsINP1 still localizes normally to the aperture domain, while OsDAF1 localization is reduced. (Bottom right) In the absence of OsDAF1, the annulus fails to form, even though both OsINP1 and OsSRF8 still assemble at the aperture domain.

Although not closely related to INP1, INP2 also contains a DOG1 domain. Like INP1, INP2 is essential for aperture formation, and exhibits similar expression patterns and parallel trends of evolutionary divergence. INP1 and INP2 interact when both arise from the same species (Arabidopsis or tomato), but no interaction occurs between INP1 and INP2 from different species. Strikingly, tomato SlINP1 and SlINP2 can rescue Arabidopsis mutants only when both are expressed, providing strong support to the idea that these proteins interact and function as species-specific partners (Lee *et al.*, 2021) (Box 2A).

The mechanism of INP1–INP2 cooperation in aperture formation remains unclear, especially since INP2 proved to be challenging to visualize, and the biochemical function of these proteins is unknown. Since INP1 appears to accumulate on the extracellular side of the aperture domains, and requires INP2 for localization, one possibility is that INP1 interacts with the extracellular portion of INP2. The N-terminal transmembrane domain of INP2 can act as a PM anchor, to form a protein complex that assembles at the outside of the aperture domains and facilitates contact with the callose wall (Box 2A). Notably, apertures restored by co-expressing tomato INP1 and INP2 in Arabidopsis were shorter than those in wild-type Arabidopsis or tomato (Lee *et al.*, 2021), suggesting that additional species-specific factors may be required for full functionality of these proteins.

In rice, OsINP1 also functions as an aperture executor, despite sharing only 35% sequence identity with its Arabidopsis homolog (Zhang *et al.*, 2020). Its expression pattern, localization, and the loss-of-aperture phenotype in null mutants closely resemble those of Arabidopsis INP1, supporting a conserved role (Box 2B). A putative rice homolog of INP2 has also been identified, but unlike Arabidopsis INP2, it lacks the predicted N-terminal transmembrane domain (Lee *et al.*, 2021). It remains to be determined whether OsINP2 participates in aperture formation and acts as a species-specific partner of OsINP1.

A newly discovered OsINP1 interactor, STRUBBELIG-RECEPTOR FAMILY 8 (OsSRF8), also called APERTURE MISSING1 (OsAM1), has further expanded the network of rice aperture proteins (Chen *et al.*, 2024; Zhang *et al.*, 2024). Like OsINP1, OsSRF8 accumulates at the distal poles of tetrad-stage microspores, marking future aperture sites. Loss of OsSRF8 leads to the complete absence of apertures (Box 2B). OsSRF8 belongs to the leucine-rich repeat receptor-like kinase superfamily, and its extracellular domain ~~portion~~ interacts with OsINP1. Importantly, members of the STRUBBELIG family, including OsSRF8, lack the conserved residues necessary for kinase activity, and are thought to function as membrane-spanning scaffold proteins, rather than active kinases (Chevalier *et al.*, 2005; Eyüboglu *et al.*, 2007; Vaddepalli *et al.*, 2011; Chen *et al.*, 2024; Zhang *et al.*, 2024).

Genetic evidence suggests that OsSRF8 acts downstream of OsINP1: OsINP1 localizes normally to the aperture domain in *ossrf8/osam1* mutants, but OsSRF8 fails to localize correctly in *osinp1* mutants (Chen *et al.*, 2024; Zhang *et al.*, 2024), suggesting that OsINP1 recruits OsSRF8 to the aperture domain (Box 2B). How these proteins cooperate to execute pollen aperture formation remains unknown. Notably, two other STRUBBELIG-receptor family members, STRUBBELIG (SUB) and SRF3 from Arabidopsis, have been implicated in callose accumulation and the regulation of callose synthases (Chaudhary *et al.*, 2020; Platre *et al.*, 2022). Therefore, it is worth investigating whether OsSRF8 also associates with the callose wall, either through direct binding to callose/callose-associated proteins, or by modulating callose biosynthesis or deposition. This could help explain how the

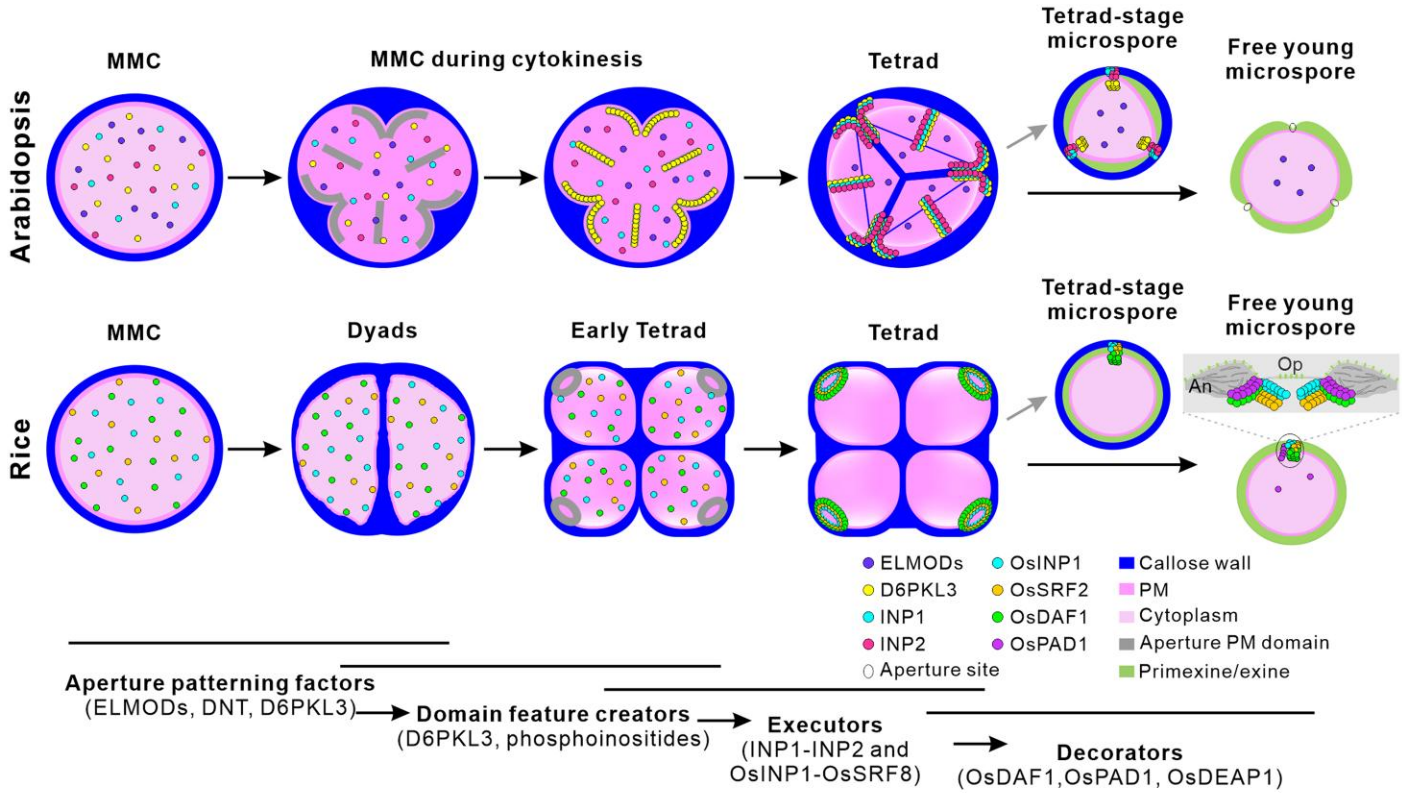


Fig. 1. Proposed working model of the aperture formation pathway. In microspore mother cells (MMCs), aperture pattering factors (such as Arabidopsis MCR, ELMOD_A, ELMOD_E, DNT, and D6PKL3), aperture executors (INP1–INP2 in Arabidopsis, OsINP1–OsSRF8 in rice), and rice aperture decorator OsDAF1 begin to be expressed. Guided by as-yet-unknown spatiotemporal cues, patterning factors directly or indirectly define polarity sites and specify certain PM regions as aperture domains, thereby determining the aperture patterns of mature pollen. These domains subsequently recruit domain feature creators, like D6PKL3 and phosphoinositides, which help establish distinct membrane properties that, in turn, attract aperture executors. At the tetrad stage, the executors INP1 and INP2 in Arabidopsis, and OsINP1 and OsSRF8 in rice, form complexes that assemble on the extracellular surface of the aperture PM domains, and create a structural bridge tethering these PM regions to the overlying callose wall. This tethering prevents primexine formation and exine deposition at the aperture domains, allowing apertures to form. In rice, apertures possess additional specialized structures, including the annulus (An), operculum (Op), fibrillar-granular layer, and Zwischenkörper layer. The executors OsINP1and OsSRF8 recruit the aperture decorators, OsDAF1 and OsPAD1, which contribute to the formation of these structural features.

OsSRF8–OsINP1 complex prevents primexine formation and exine deposition at aperture sites. Whether this interaction is species-specific is another intriguing question for future research.

Aperture decorators in rice

Compared with Arabidopsis, pollen apertures of rice and other grasses are more complex and exhibit unique structural features, including the operculum, annulus, fibrillar-granular layer, and Zwischenkörper layer (Christensen and Horner Jr., 1974; El-Ghazaly and Jensen, 1986; Banks, 2003; Zhang *et al.*, 2020). The operculum is a circular region of exine in the center of the aperture, bordered by the annulus—a ring of thickened, elevated exine. Just beneath the operculum lie two structurally distinct layers, which provide support to the operculum: the fibrillar-granular layer and the Zwischenkörper layer (El-Ghazaly and Jensen, 1986). The annulus and the operculum begin developing after the release of young microspores from tetrads, followed by the formation of the fibrillar-granular layer and the Zwischenkörper layer (El-Ghazaly and Jensen, 1986; Zhang *et al.*, 2020). However, the molecular mechanisms underlying the formation of these specialized features of apertures remain poorly understood. Below, we describe three recently discovered molecular components, which we call aperture decorators, involved in the formation of these features in rice.

The first identified aperture decorator is DEFECTIVE IN APERTURE FORMATION1 (OsDAF1), a lectin receptor-like kinase, also known as OsLecRK-S.7, OsLecRK5, and ABNORMAL POLLEN1 (AP1) (Peng *et al.*, 2020; Wang *et al.*, 2020; Zhang *et al.*, 2020; He *et al.*, 2021). Loss of OsDAF1 results in pores lacking the annulus. Like the aperture executors OsINP1 and OsSRF8, OsDAF1 is initially distributed diffusely in the cytoplasm and uniformly in the PM, but during the late tetrad stage, it assembles at the PM into a small ring that marks the future aperture site (Zhang *et al.*, 2020). The similar localization of OsDAF1, OsINP1, and OsSRF8 suggests they may function together. Indeed, OsDAF1 physically interacts with both OsINP1 and OsSRF8. Notably, the correct localization of OsDAF1 depends strongly on OsINP1 and, to a lesser extent, on OsSRF8, while neither executor depends on OsDAF1 for their localization. This indicates that the aperture decorator OsDAF1 acts downstream of OsINP1 and OsSRF8, and these proteins may recruit OsDAF1 to aperture sites to promote annulus formation (Chen *et al.*, 2024; Zhang *et al.*, 2024) (Box 2B).

However, the interactions among OsDAF1, OsSRF8, and OsINP1 are somewhat complex (Zhang *et al.*, 2020, 2024; Chen *et al.*, 2024). Like OsSRF8, OsDAF1 contains an extracellular domain, a single transmembrane domain, and an intracellular kinase domain. As expected, the extracellular region of OsSRF8 interacts with OsINP1, while its intracellular kinase domain interacts with that of OsDAF1. Surprisingly, OsINP1 also interacts directly with the intracellular kinase domain of OsDAF1, raising questions about whether OsINP1 localizes to the extracellular or intracellular side of the PM. In Arabidopsis, INP1 has been shown to associate with both the PM and callose wall in plasmolyzed tetrads, supporting the view of its extracellular localization (Dobritsa *et al.*, 2018). However, in rice, OsINP1 may interact transiently with the intracellular domain of OsDAF1 during its trafficking. Alternatively, OsINP1 may possess an unrecognized transmembrane domain, allowing it to span the membrane and interact with both extracellular and intracellular partners.

Interestingly, OsDAF1—under the names of OsLecRK5 and AP1—was also shown to interact with, and phosphorylate the UDP-glucose pyrophosphorylases OsUGP1 and OsUGP2 (Wang *et al.*, 2020; He *et al.*, 2021), enzymes involved in the biosynthesis of several cell wall polysaccharides, including callose (Chen *et al.*, 2007). In Arabidopsis, the presence of the callose wall is essential for aperture formation and proper localization of INP1 (Dobritsa *et al.*, 2018). In several other species, aperture sites correlate with the positions of so-called callose plugs or additional callose deposits formed by microspores at the tetrad stage (Prieu *et al.*, 2017). This raises the intriguing possibility that annulus formation in rice may require the synthesis of additional callose, potentially triggered by OsDAF1-mediated signaling.

Loss of the annulus is also seen in rice mutants lacking DEFECTIVE EXINE AND APERTURE PATTERNING1 (DEAP1), a fasciclin-like arabinogalactan (FLA) protein (Zhou *et al.*, 2022). DEAP1 is a PM-associated protein with a C-terminal glycosylphosphatidylinositol (GPI)-anchor essential for attaching the protein to the extracellular side of the PM. Although localization of DEAP1 to the aperture site has not yet been demonstrated, several FLAs are known to mediate PM–cell wall interactions (Seifert, 2018), raising the possibility that DEAP1 facilitates connections between aperture domains and the callose wall during annulus formation. DEAP1 does not interact directly with either OsDAF1 or OsINP1, yet it was found to interact with at least two rice homologs of the Arabidopsis aperture factor D6PKL3 (OsD6PKL3.6 and OsD6PKL3.7). As Arabidopsis D6PKL3 is believed to reside on the cytoplasmic side of aperture domains (Lee *et al.*, 2018), this finding raises the possibility that rice D6PKL3 homologs may differ from their Arabidopsis counterpart in their subcellular localizations and functions. Alternatively, OsD6PKL3s, while still localized intracellularly, may help facilitate the journey of DEAP1 to its position at the aperture domain.

POLLEN APERTURE DEFECT 1 (OsPAD1), a non-specific lipid transfer protein (nsLTP), also known as OsLTP47, is another recently identified aperture decorator in rice (Chen *et al.*, 2022; Wang *et al.*, 2024). OsPAD1 interacts with OsINP1 and phosphoinositides (Wang *et al.*, 2024). These interactions likely facilitate its recruitment to the aperture domain and/or its retainment at this site. In *ospad1* null mutants, the annulus, fibrillar-granular layer, and Zwischenkörper layer are all absent, and the operculum becomes detached, leading to leakage of cellular contents through the aperture. However, how this nsLTP contributes to the formation of these aperture structures is presently unknown.

Conclusions and future directions

Recent discoveries of molecular players involved in pollen aperture formation in Arabidopsis and rice have significantly advanced our understanding of the underlying mechanisms, and led to the development of the working model for this process (Fig. 1). At first, aperture patterning factors, such as ELMODs, DNT, D6PKL3 and, potentially, their rice homologs, receive as-yet-unknown spatiotemporal cues that define microspore polarity. These patterning factors then directly or indirectly specify certain PM sites as aperture domains, therefore establishing the aperture pattern of mature pollen. The aperture domains become refined through the recruitment of D6PKL3 and phosphoinositides, which in turn attract aperture executors to these sites. The aperture executors, which include INP1–INP2 in Arabidopsis and OsINP1–OsSRF8 in rice, assemble on the extracellular side of the aperture PM domains, creating a structural bridge which tethers these PM sites to the callose wall. This connection restricts primexine formation and exine deposition at the aperture domains, thereby allowing apertures to develop. In rice, the executors OsINP1and OsSRF8 also recruit aperture decorators, such as OsDAF1 and OsPAD1, which contribute to the formation of specialized structural features of the apertures.

Despite these advances, many questions remain. Important unknowns are the nature of the initial cues that aperture patterning factors receive, and the mechanisms through which patterning factors establish distinct aperture PM domains. For example, ELMOD proteins display diffuse cytoplasmic localization and nuclear enrichment (Zhou *et al.*, 2021), yet somehow promote the formation of PM domains. How exactly they do this is a mystery. Whether patterning factors have conserved functions in species with different aperture patterns, is another open question.

Furthermore, although many aperture executors and decorators localize to the aperture PM domains, the mechanism that guides them to these sites is unknown. Since some of these proteins are positioned on the extracellular side of the PM, their potential interaction with the callose wall or its associated proteins merits further investigation. Proximity labeling, which has recently allowed successful protein interactome studies in other challenging plant systems (Mair *et al.*, 2019; Li *et al.*, 2024; Tang *et al.*, 2024), may help overcome the difficulties of working with aperture factors, which tend to be expressed at low levels, in a small number of cells, and for a short period of time. Capturing the interactors of known aperture factors could significantly advance our understanding of the molecular mechanisms through which aperture domains are created.

Aperture patterns vary widely across species but are highly conserved within species, raising the possibility that these traits are not only under developmental control, but subject to selective pressures during plant evolution (Heslop-Harrison, 1979b; Furness and Rudall, 2004). A particularly intriguing, though challenging, direction for future research will be to determine whether different aperture patterns serve as adaptations to specific reproductive strategies or ecological conditions. Such studies could provide important insights into the evolutionary logic underlying the diversification of pollen aperture patterns.

Author contributions

YZ and AAD conceived and wrote the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Data availability

No data is available for this review.

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