MEMBRANE DOMAINS

Building portals in pollen

Pollen apertures are the manifestation of distinct plasma membrane domains on the pollen surface. A new study discovered two proteins in rice that are localized specifically to the aperture domains in the membrane of developing pollen and are involved in aperture formation.

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In flowering plants, pollen grains deliver sperm cells to ovules by forming pollen tubes[1]. Specific regions on the pollen surface, known as pollen apertures, are important for this process. At aperture sites, pollen wall exine is deposited in much smaller amounts, if at all, compared to the rest of the pollen surface, allowing these sites to serve as portals for pollen hydration and pollen tube germination[2]. Apertures are also a research model for the establishment of cellular polarity and distinct plasma membrane domains[3]. Apertures of different plant species vary widely in their positions, numbers and morphology, thus creating species-specific patterns. For example, *Arabidopsis* pollen develops three equidistant, longitudinal, furrow-shaped apertures with centres at the pollen equator[3] (Fig. 1a). In contrast, rice pollen develops a single circular aperture located at the distal pole, surrounded by a bulging ring-like area of exine (annulus) and covered by a lid-like operculum[4] (Fig. 1b). The mechanisms through which the sites for apertures are selected and exine formation at such sites is prevented are poorly understood. Previously, two proteins with important roles in aperture formation, D6 PROTEIN KINASE-LIKE3 (D6PKL3) and INAPERTURATE POLLEN1 (INP1), have been identified in *Arabidopsis*. The membrane-associated protein kinase D6PKL3 interacts with phospholipids and likely helps create three distinct aperture domains within the plasma membrane[5]. The protein of unknown function INP1 aggregates at these domains (Fig. 1c) and prevents deposition of exine by ensuring that the plasma membrane there stays in close contact with the callose wall that covers developing pollen grains[6]. However, the mechanisms guiding aperture formation in species with other aperture patterns are mostly unknown. In this issue of *Nature Plants*, Zhang et al.[7] report the identification of the first aperture factors from rice.

Through a forward genetic screen in rice, the authors identified two male-sterile mutants with identical defects in pollen aperture formation. Their apertures lacked the annulus but retained the operculum, suggesting that the affected gene is specifically involved in annulus formation. Without the annulus, pollen tubes fail to germinate. Both mutations were mapped to a lectin receptor-like kinase, dubbed DEFECTIVE IN APERTURE FORMATION1 (OsDAF1). Strikingly, at the tetrad stage of pollen development, the OsDAF1 protein was found assembling into a tiny ring at the distal pole of each

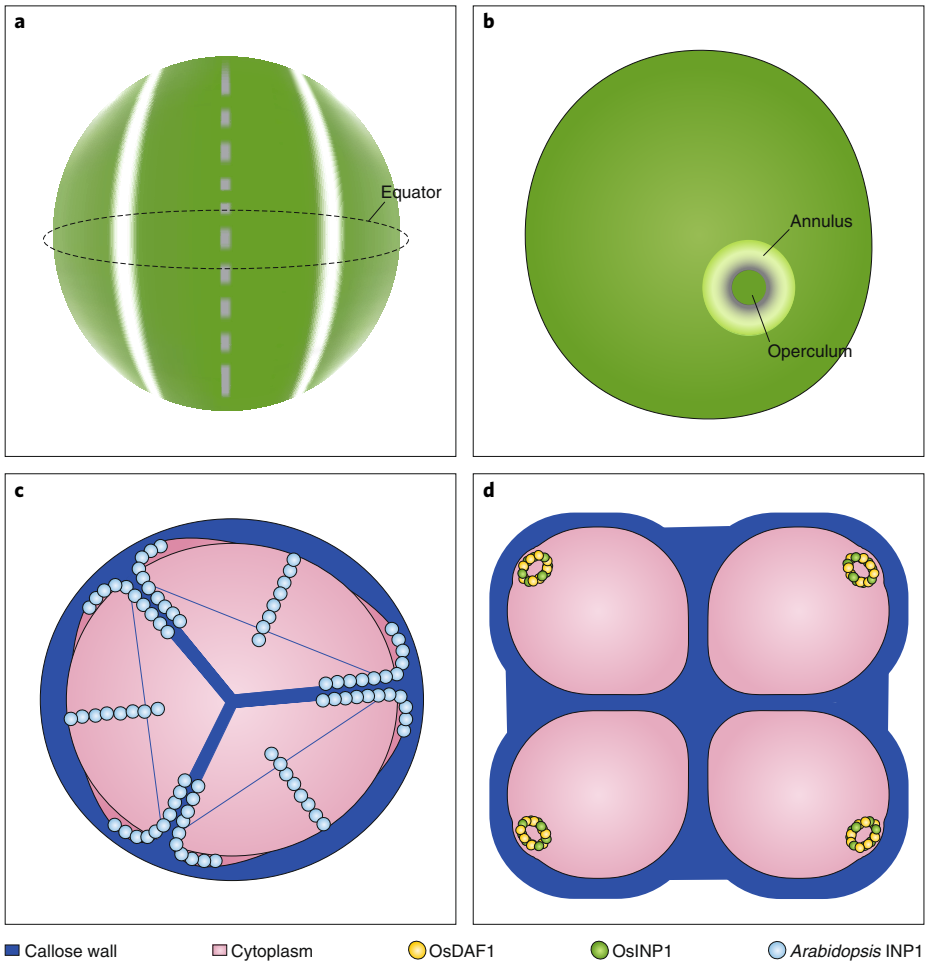

Fig. 1 | Aperture factors mark future aperture sites in *Arabidopsis* and rice. a, Pollen grains of *Arabidopsis* have three apertures shaped like furrows that are equidistantly placed at the pollen equator. **b**, Rice pollen grains have a single round aperture that is surrounded by a bulging ring-like annulus and covered with a lid-like operculum. **c**, In each developing pollen grain at the tetrad stage, *Arabidopsis* INP1 assembles into three equidistant lines that mark the aperture domains of the plasma membrane. **d**, Both OsDAF1 and OsINP1 assemble into tiny rings at the distal poles of tetrad-stage pollen, which coincide with the future aperture sites.

grain, corresponding to the positions and morphology of apertures in rice pollen (Fig. 1d).

OsDAF1 localizes to aperture membrane domains and is involved in aperture formation; this is similar to *Arabidopsis* INP1, which also marks sites of future apertures[6,8]. The authors then explored the function of the rice homologue of INP1, OsINP1, by generating its CRISPR knockouts. Although INP1 homologues can be recognized in many angiosperms, these proteins are not well conserved: proteins from *Arabidopsis* and grasses share less than 40% sequence identity. Despite significant sequence divergence and dramatic differences in aperture patterns, the involvement of INP1 proteins in aperture formation appears to be conserved. Just like the previously described *inp1* mutants from *Arabidopsis* and maize[8,9], the *osinp1* mutants lack any signs of apertures. The temporal patterns of OsINP1 expression and localization also strongly resemble the patterns of *Arabidopsis* INP1 (ref. [6]), indicating that specification of aperture domains occurs at the same time in rice as in *Arabidopsis*. Although OsINP1 is present in the cytoplasm as early as the pollen mother cells, it is not until the late tetrad stage that the protein becomes enriched at the plasma membrane. Notably, just like OsDAF1, OsINP1 specifically aggregates into a tiny ring located at the distal pole in each developing pollen (Fig. 1d). Similar to *Arabidopsis*, in which the INP1-decorated plasma membrane domains form protrusions to the callose wall[6], OsINP1–YFP localization coincides with the plasma membrane protrusions in rice, suggesting that in both species the membrane at aperture domains might be kept in tight contact with the overlying callose wall to prevent exine deposition.

The similar localizations of OsINP1 and OsDAF1 suggest that these proteins might work together to regulate formation of apertures in rice. Indeed, OsINP1 was found to interact with the intracellular kinase domain of OsDAF1. The authors further demonstrated that the correct localization of OsDAF1 depends on the presence of OsINP1, but not vice versa. In the absence of OsINP1, OsDAF1 reached the plasma membrane but failed to become enriched at the ring-shaped polar aperture domain. A similar loss of distinct localization was observed when OsDAF1 lacked its kinase region, but not when it lacked the extracellular lectin domain. This suggests that OsINP1 acts upstream of OsDAF1 and likely recruits it to the future aperture sites by interacting with its kinase region.

The study leads to many avenues for future inquiry. One area of interest is the apparent difference in localization of INP1 proteins relative to the plasma membrane surfaces. In *Arabidopsis*, INP1 appears to assemble on the outer membrane surface, potentially facilitating contact between the membrane and the callose wall[6]. Yet in rice, OsINP1 interacts with the cytoplasmic domain of OsDAF1, suggesting localization to the inner surface of the membrane. Another is the search for factors and mechanisms in rice that select sites for ring-shaped aperture domains and attract OsINP1 there. *Arabidopsis* mutants affecting INP1 localization have been recently discovered[5,10] and, once the affected genes are identified, might offer some clues to the rice mechanism. Also, as OsINP1 regulates multiple aspects of aperture formation, including formation of the operculum, it might require other partners besides OsDAF1 to control these processes. Finally, the identification of a lectin receptor-like kinase as an aperture factor brings the questions of which ligands and downstream signalling pathways help build the annulus, and whether any lectin receptor kinases participate in aperture formation in other plant species. Future studies should allow more insights into the fascinating mechanisms that guide the formation of these amazingly diverse portals in the pollen of different species. ❐

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Published online: 13 April 2020
https://doi.org/10.1038/s41477-020-0641-3

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Competing interests

The authors declare no competing interests.