FERONIA as an upstream receptor kinase for polar cell growth in plants

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Polarized cell growth is important for morphogenesis and function of specialized cell types in eukaryotes. Typical tip-growing cells in plants are root hairs and pollen tubes (Fig. 1). A root hair is a highly polarized tubular structure emerging from a special type of cell on the root surface, from which water and nutrients are absorbed and supplied to the plant body (Fig. 1A). A root hair also functions in nodule formation during *Rhizobium* legumes symbiosis. A pollen tube is a male reproductive organ that delivers sperm cells to a female gametophyte (Fig. 1C). Rapid elongation of these cells occurs in response to external cues. It is well established that RAC/ROP (Rho-related small GTPase from plants) signaling, reactive oxygen species (ROS), and calcium homeostasis regulate polarized cell growth in plants. However, the nature of the cell surface receptor transducing external signals for tip growth has remained poorly characterized. In PNAS, Duan et al. (1) report the identification of a receptor-like kinase responsible for ROP signaling and ROS-mediated root hair elongation. Surprisingly, the receptor is FERONIA (FER), a well-known regulator of female fertility (2–4).

ROP guanidine exchange factor (ROPGEF) converts ROP from the inactive GDP-bound form to the active GTP-bound form. A yeast two-hybrid screen in search of ROPGEF1 interactors in *Arabidopsis* identified a CrRLK family of receptor-like kinases, including FER. The interaction of FER and ROPGEF1 occurs in plant cells. An active form of ROP is reduced in the loss-of-function *fer* mutant plants, suggesting that FER is required for ROP activation. Duan et al. (1) further demonstrate that FER, ROPGEF, and ROP form a protein complex in vitro and that association of FER and ROP is enhanced by the presence of GDP. This is consistent with the known activation mechanism of ROPs: ROPGEFs preferentially interact with the GDP-bound inactive form of ROPs, and on nucleotide exchange, the GTP-bound active form of ROPs is released from the ROPGEF complex to transduce downstream signaling (5) (Fig. 1B).

fer and its allele *sirene* (*sm*) were originally discovered as female gametophytic mutants defective in proper communication between male (pollen tube) and female

Fig. 1. Signaling pathway models control polar cell growth in root hairs and pollen tubes. (A) Illustration of growing root. A root hair emerges from an epidermal cell. (B) Higher-magnification image of the dotted square region in A. FER interacts with ROPGEF via its cytosolic kinase domain. ROPGEF interacts with and activates RAC/ROP via exchanging GTP from GDP. Downstream signaling activates NADPH oxidase to produce ROS, leading to influx of Ca^{2+} from extracellular space. ROS and Ca^{2+} enhance tip growth. Auxin likely activates ROP signaling to promote tip growth. (C) Illustration of a pollen tube reaching an ovule. An ovule contains a female gametophyte consisting of an egg cell (EC), a central cell (CC), three antipodal cells (AC), and two synergid cells (SC). (D) Higher-magnification image of dotted square region in C. Based on the molecular similarity, ANX1/2 may function as a membrane-localized receptor that activates downstream ROP signaling for pollen tube elongation. FER expressed in synergid cells may release a signal or may sequester putative ligands for ANX1/2, leading to the arrest of pollen tube growth and the rupture of the pollen tube.

(synergid cell) gametophytes (2–4). However, FER is expressed widely throughout vegetative tissues, suggesting that FER function is not limited to gametophyte interactions. Indeed, Duan et al. (1) find that the loss-of-function *fer* alleles conferred short root hairs, which often bulged or ruptured.

How does FER promote root hair growth? Root hair growth is stimulated by plant hormone auxin and secondary messenger ROS, both acting via ROP signaling (6). Production of ROS by the NADPH oxidase RHD2 leads to stimulation of Ca^{2+} channels, elevated cytoplasmic Ca^{2+} concentrations, and subsequent tip growth (Fig. 1B). The root hair defects in *fer* closely resemble those of *rhd2* (7). Given that FER physically interacts with and acts upstream of ROP, it is predicted to mediate auxin- and ROS-mediated root hair growth. Sure enough, Duan et al. (1)

demonstrate that accumulation of ROS is negligible in *fer* root hairs and that *fer* mutants are not responsive to auxin-stimulated ROS accumulation. The root hair and ROS defects were countered by overexpression of *ROP2-GFP*. Moreover, overexpression of FER, ROPs, or ROPGEF1 resulted in the increased accumulation of ROS in the root. Taken together, Duan et al. (1) establish FER as a cell surface receptor acting upstream of ROP, which targets NADPH oxidase to accumulate ROS for root hair elongation (Fig. 1B).

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The finding of FER as an upstream receptor for ROP signaling in root hair morphogenesis raises important questions. During double fertilization, the pollen tube enters an embryo sac along the guidance cue emitted from a synergid cell, in which *FER* is expressed, and subsequently ruptures to release sperm nuclei (Fig. 1C). In the absence of *FER*, the pollen tube fails to rupture; hence, fertilization does not occur (2–4). Does the pollen tube grow through a conserved molecular mechanism as in root hair growth? If so, what is the role of FER in pollen tube tip growth and rupture?

Lines of evidence support the notion that Ca^{2+} -mediated ROS production by NADPH oxidase is necessary for pollen tube elongation. Down-regulation of pollen tube-specific NADPH oxidase caused lower oxidase activity and reduced pollen tube elongation (8). This growth inhibition was rescued by adding exogenous hydrogen peroxide. A mutation in the *ACA9* gene, which is primarily expressed in the pollen tube and encodes the plasma membrane-localized calcium pump, caused less pollen tube elongation in *Arabidopsis* (9). Finally, ROP signaling acts upstream of Ca^{2+} signals to control pollen tube tip growth (10) (Fig. 1D).

The ROP–ROS– Ca^{2+} signaling module is likely conserved in stimulated tip growth in root hairs and pollen tubes, suggesting the presence of an equivalent upstream receptor kinase in pollen tubes. In gametophytes, however, *FER* is expressed only on the female side (synergid cell) and not on the male side (pollen tube); thus, it cannot be the upstream receptor kinase for pollen tube tip growth. Are there any other receptors in growing pollen tubes? Recently, two sister proteins of FER, ANXUR1 (ANX1) and ANX2, have been

reported as CrRLKs specifically expressed in pollen tubes and preventing pollen tube rupture (11, 12). Although single knockout of *ANX1* or *ANX2* showed no detectable phenotype, *anx1;anx2* double knockout led to precocious pollen tube bursting, with a phenotype resembling that of root hairs in *fer*.

Polar cell growth of plant cells thus occurs through variations on a theme: equivalent pathways constituted by slightly different molecular components that may reflect unique specialized features of each cell type (Fig. 1). In the pollen tube, the plasma membrane-localized receptor-like kinases ANX1 and ANX2 may receive localized external cues. The signal could be transferred to their direct binding partner, ROPGEFs, which activate ROP GTPases (5). The active form, ROP, may stimulate respiratory burst oxidase homolog, a putative catalytic component of NADPH oxidase to produce ROS for pollen tube elongation (Fig. 1D). Pollen tube elongation initiates on the stigma and persists through transmitting tissues in the style until finally reaching the ovules. In tomatoes, LePRK2, a receptor-like kinase unrelated to FER, associates with ROP-GEF, known as KPP, to promote pollen-tube polar growth within styles (13, 14). This class of receptors, together with ANX1/2, may promote the proper tube growth in the pistil.

It is intriguing that FER acts in an opposite way from ANX1/2 in pollen tubes (and from itself in root hairs). *FER* is expressed in the synergid cell, and *fer* mutation impairs pollen tube rupture in the female gametophyte (2–4). One possibility is that the FER signaling pathway produces secreted signals to facilitate growth arrest and rupture of pollen tubes (Fig. 1D). An alternative more exciting scenario

would be that FER and ANX1/2 compete for the same ligands. In this case, FER on the surface of the synergid cells may sequester the ligands for ANX1/2 on the pollen tubes. This, in turn, quenches the ANX1/2 signaling, resulting in pollen tube rupture (Fig. 1D). In support of this idea, FER and ANX1/2 share an extracellular domain with some sequence similarity.

The biggest unanswered question is the identity of ligands for FER. FER belongs to the CrRLK family, and its extracellular domain possesses some sequence similarities to lectin proteins, which bind maltose and related oligosaccharides (15). One can speculate that FER interacts with and/or is affected by cell wall components or apoplastic materials. In this regard, it is striking that a CrRLK related to FER, THESEUS1 (THE1), senses the status of the cell wall environment and coordinates growth: a loss-of-function *the1* mutation suppresses the growth defects in a cellulose synthase mutant (16). Recent reports further link FER, THE1, and yet another CrRLK, HERCULES1, in hormone-mediated seedling growth (17, 18). Some receptors may interact with a number of ligands to modulate signaling outputs (19). Does FER sense different signals in each developmental context? Future identification of ligands for FER and its related CrRLKs will shed light on the mechanism of coordinating external signals for ROP and ROS-mediated polarized cell growth.

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