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COMMENTARY

Context-specific dependence on FERONIA kinase activity

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FERONIA (FER) is the most extensively studied member of the malectin-like domain containing receptor kinases in plants distinguished by their extracellular domain homology with the animal diglucose-binding protein malectin [1,2]. Since its original identification as a key regulator of female fertility [3–6], FER has been found crucial for a plethora of growth, developmental, and stress-related processes. The FER cytoplasmic domain interacts with ROP-GEFs, guanine exchange factors that activate RAC/ROPs (plant RHO GTPases and major molecular switches known to regulate diverse signal-response pathways) [7]. Its malectin-like domains interact with pectin, a major carbohydrate polymer in the plant cell wall [8]. These interactions have been linked to several major FER functions, including auxin- and RAC/ROP-mediated root hair growth and epidermal pavement cell patterning [7,9], and salt stress-induced cell growth responses [8].

While the FER cytoplasmic domain underlies its interaction with key signal mediators, such as RAC/ROPs [7], ABI2 phosphatase [10], RPM1-induced protein kinase [11], and the β-subunit of heterotrimeric G protein [12], it remains unclear how its kinase activity integrates with its intracellular signaling networks. A mutation in the conserved catalytic site converting Lysine565 to arginine, FER K565R, rendered the kinase domain inactive in autophosphorylation *in vitro* [4]. In homozygous *fer* mutants, typically ~80% of ovules failed to be fertilized due to loss of the female gametophytic ability to induce pollen tube rupture and sperm release [7,13]. FER K565R along with two additional mutants with the less conservative substitution of glutamic acid or alanine at K565 were also comparably active as wild-type FER in reducing the percentage of unfertilized ovules in *fer-1* to ~40%, suggesting that the kinase activity might be dispensable [13]. In seedling root growth responses, requirement of a fully active kinase domain is apparently conditional [14]. In this study, FER K565R was partially active relative to FER in restoring in *fer* mutant seedlings the ability to mediate a bending (which imposed mechanical stimuli)-induced increase in root surface pH. This observation implies that the FER kinase activity, while not required, apparently contributes to events in mechanical signal transduction. On the other hand, FER K565R acted just like wild-type FER in complementing *fer* mutant seedlings in root growth responses to mechanical barriers implying that the kinase activity is dispensable.

In 2014, Haruta *et al.* [15] discovered that Rapid Alkalinization Factor 1 (RALF1) functions as a ligand for FER and that RALF1 induced several responses in seedlings, including a transient calcium spike and root growth inhibition. In the current study, Haruta *et al.* used the classical assay of *in-gel* phosphorylation of myelin basic protein to confirm that FER (K565R) had no detectable kinase activity *in vitro*. They then carried out a side-by-side study comparing the efficacy of this 'kinase-dead' FER relative to wild-type FER in reversing defects in the T-DNA knockout mutant *fer-4* [7] in RALF1-regulated calcium spike and root growth responses in seedlings and in ovule fertilization. They found, as in Kessler *et al.* [13], that FER (K565R) approximated wild-type FER in mitigating ovule fertilization deficiency in *fer-4*. On the other hand, in seedlings grown under normal light condition, while wild-type FER rendered *fer-4* almost fully responsive to RALF1-induced calcium spike and root growth inhibition, FER (K565R) approximated only 25% of the wild-type FER efficacy in rendering *fer-4* sensitive to RALF1 in these responses. Haruta *et al.* further tested the biological effect of FER (K565R) on FER-mediated root growth suppression under dim light conditions. They found that the root length of FER (K565R)-transformed *fer-4* was intermediate between

Abbreviations
FER, FERONIA; RALF1, rapid alkalinization factor 1.

that of *fer-4* and wild-type FER-transformed *fer-4*, which approximated wild-type. These results imply that while FER kinase activity is not solely responsible for FER functions in these seedling processes, the kinase domain, either enzymatically or structurally, is at the least partially required. It is also possible that a kinase deemed 'dead' under *in vitro* protein phosphorylation conditions might still retain adequate activity to differentially meet the *in vivo* needs of different cell and tissue types.

Implications from the differential ability exhibited by FER (K565R) in fully and partially restoring FER functions, respectively, in reproduction and root growth processes [13,14,16] share some similarity with those implicated by differential phenotypes observed in *fer-4* and a *fer-5*, a considerably weaker mutant. *fer-5* was induced by T-DNA insertion located close to the end of the FER cytoplasmic domain, producing truncated FER-related transcripts [7]. *fer-5* displays highly notable root hair defects that are weaker than those in *fer-4* but is negligibly affected in growth and reproduction, contrasting severe growth and female deficiency in *fer-4*. These observations imply that root hairs have strict dependence on a fully functional FER, including an intact C-terminal region the loss of which is somehow tolerated by other cell and tissue types. Perhaps the relatively short and defined pathway of FER-ROPGEP-RAC/ROP-ROS-dependent polarized root hair growth [7] is similar to the relatively rapid ionic changes induced by RALF1 and mechanical stimuli in that these processes have little compensatory opportunity from other cellular processes, rendering them highly dependent on uncompromised FER early signaling ability. In contrast, root growth and pollen tube–female gametophyte interaction are considerably more complex processes involving broader sets of cellular processes and regulators and early FER signaling deficiency might be compensated by other participating events. Differential threshold needs of FER signaling activity in different cell types might underlie whether and how much a kinase-compromised FER could adequately meet these needs and differences in the efficacy of FER (K565R) reported in studies based on this FER variant.

Haruta *et al*. suggested that cell type-specific regulatory mechanisms mediating downstream signaling events underlie differential FER functions in different cell types. They also speculated that FER could function with other protein kinases, including other members of the same receptor kinase family as implicated in a recent study involving several pollen-expressed homologs of FER [17]. FER functioning as a scaffold for the assembly of signaling complexes has also been proposed [18]. Identification of cell type-specific kinase substrates and other interacting proteins will be needed to fully understand the functional contributions made by the FER cytoplasmic domain in fulfilling its diverse biological roles.

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