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# How many receptor-like kinases are required to operate a pollen tube

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Successful fertilization depends on active molecular dialogues that the male gametophyte can establish with the pistil and the female gametophyte. Pollen grains and stigmas must recognize each other; pollen tubes need to identify the pistil tissues they will penetrate, follow positional cues to exit the transmitting tract and finally, locate the ovules. These molecular dialogues directly affect pollen tube growth rate and orientation. Receptor-like kinases (RLKs) are natural candidates for the perception and decoding of extracellular signals and their transduction to downstream cytoplasmic interactors. Here, we update knowledge regarding how RLKs are involved in pollen tube growth, cell wall integrity and guidance. In addition, we use public data to build a pollen tube RLK interactome that might help direct experiments to elucidate the function of pollen RLKs and their associated proteins.

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## Introduction

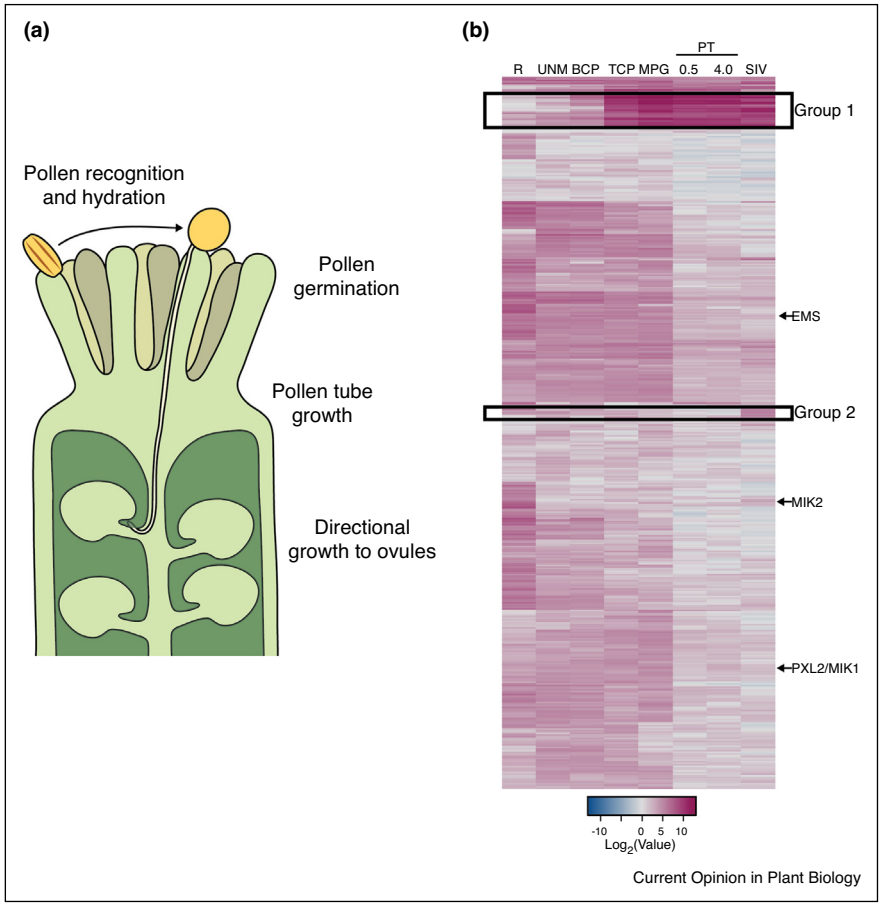
In vascular plants, most cells are bound to their neighbors from birth till death. This restriction demands a strict and complex coordination of developmental programs to create proper tissues and organs. In contrast, the male gametophyte, the pollen grain, is a simple structure that contains three cells when terminally differentiated: a vegetative cell that develops a tip-growing pollen tube to deliver the two sperm cells to the ovule. However, the apparent structural simplicity of the male gametophyte underlies a highly complex life history. Pollen tubes change neighbors throughout their lifetime and the tissues they encounter vary depending on the type of pistil on which they are competent to germinate. These can comprise dry or wet stigmas involved in pollen recognition, hydration and germination; solid or hollow styles that let pollen tubes to grow; and placental or ovule types that produce chemical signals that attract pollen tubes (Figure 1a). Each provides specific challenges that pollen tubes need to overcome in order to achieve fertilization. One of the main goals in pollen developmental biology is to uncover the mechanisms that enable pollen tube navigation in all these changing landscapes. While somatic cells use cell-to-cell communication to decide cell fate, pollen uses self-generated (autocrine) and foreign (paracrine) signals to modulate $Ca^{2+}$ dynamics, actin polymerization, $H^+$ pump activity, ROS (reactive oxygen species) production, cell wall assembly and other processes that control pollen tube growth, guidance and sperm cell discharge [1]. In this context, signals and receptors have central roles in the life of pollen tubes.

In this review, we will focus on the putative roles of receptor-like kinases (RLKs) in mediating cell-to-cell communication during the life of pollen tubes. In our last section, we use public protein–protein interaction data to predict possible RLK functions based on their interactors. We hope the pollen development community will find our hypotheses useful as starting points for future research.

## RLKs in Arabidopsis pollen

The Arabidopsis genome encodes 610 RLKs [2]. Of them, 492 are transmembrane receptor-like proteins and 118 are receptor-like cytoplasmic kinases (RLCKs). Which of these transmembrane RLKs participate in pollen signaling? Analysis of RLK gene expression throughout pollen development can provide some clues (Figure 1b). Two expression profiles stand out from the diverse patterns shown by *RLK*-encoding genes. Group 1 consists of 23 pollen-enriched RLKs whose expression increases throughout pollen development, reaching their expression maxima in mature pollen grains and/or pollen tubes. Group 2 is a small group of RLKs specifically induced after pollen tubes traverse stigmas, suggesting that these RLKs could be involved in pollen–pistil interactions. Group 1 includes some receptors with proven roles in pollen tube development, such as ANXUR1/2 (ANX1/2) [3,4], POLLEN RECEPTOR-LIKE KINASE2/3/6 (PRK2/3/6) [5••] and MALE DISCOVERER1/2 (MDIS1/2) [6••] (see below). Other RLKs in this group are RECEPTOR-LIKE

**Figure 1**




RLKs in pollen development. **(a)** Pollen–pistil interaction stages where pollen RLKs might be involved. **(b)** Expression profiles for 492 RLK-encoding genes show a cluster of pollen-enriched RLK-encoding genes (Group 1) and a cluster of stigma-induced pollen RLK-encoding genes (Group 2). Expression levels in unicellular microspore (UNM), bicellular pollen (BCP), tricellular pollen (TCP), mature pollen grain (MPG) [74], two stages of *in vitro*-germinated pollen tubes (PT 0.5 and 4 hours) and semi *in vivo*-germinated pollen tubes (SIV) [42••] were obtained from the Bio-Analytic Resource (BAR, University of Toronto, Canada) and hierarchically clustered using hclust in R. Expression levels for rosette leaves (R) were included in the analysis as an indicator for baseline expression in the sporophyte. Arrows indicate other RLK-encoding genes with roles in pollen development that do not belong to Group 1 or 2.

KINASE IN FLOWERS (RKF1/2), members of the PROLINE-RICH EXTENSIN-LIKE RECEPTOR KINASES (PERKs) family [7] and some other uncharacterized RLKs. Interestingly, several RLKs that function in microgametogenesis and pollen development are not in Group 1 or 2 (indicated with arrows in Figure 1b). For example, the expression of *EXCESS MICROSPOROCYTES 1* (*EMS1*) [8•] and *MDIS1-INTERACTING RECEPTOR LIKE KINASE1/2* (*PXL2/MIK1* and *MIK2*) (see below) is not particularly enriched in the male lineage, yet mutants for these genes show abnormal male segregations. This behavior could imply that certain RLKs act as signaling modules that are recurrently recruited in various tissues (such as MIK2 [9•,10]).

Two fundamental questions in plant signaling hold true for pollen development: what is the meaning of having so many RLKs? What are the ligands for all these RLKs? In the next sections, we discuss the few identified ligands involved in pollen tube development and growth, and their corresponding RLKs, case by case.

## RLKs controlling pollen tube growth through the pistil

Pollen-enriched RLK-encoding genes were first found in petunia (*PRK1*, *POLLEN RECEPTOR-LIKE Kinase 1*) [11] and tomato *LePRK1/2* (*Solanum lycopersicum*, formerly *Lycopersicon esculentum*, *POLLEN RECEPTOR-LIKE KINASE 1* and *2*) [12] when searching for plasma membrane-localized kinases potentially involved in pollen development. Although similar in structure, PRK1 and LePRK1/2 have different functions. PRK1 has a role in microspore development [11] while LePRK1/2 participate in pollen tube growth and/or pollen–pistil

interactions [13–15]. Studies of LePRK1/2 have laid the foundations to understanding certain pollen RLK signaling mechanisms. First, LePRK1/2 can form hetero-oligomeric complexes with dynamic responses to pistil components [16]. Second, several autocrine (LAT52, LATE ANTHER TOMATO 52) and paracrine (LeSTIG1, STIGMA-SPECIFIC PROTEIN 1 and STIL, STYLE INTERACTOR FOR LePRKs) ligands compete for the same RLKs [17–19]. LAT52 is a pollen cysteine-rich protein that interacts with LePRK2 only before pollen germinates [17–19]; LeSTIG1, a small style cysteine-rich protein, is a second potential ligand that can bind the extracellular domains (ECD) of LePRK1 and LePRK2, displacing LAT52 [17–19]. STIL is a compound that promotes pollen tube growth presumably through LePRK2 dephosphorylation and LePRK complex disassembly [17–19]. Third, RLKs can regulate pollen tube growth rate by modulating the actin cytoskeleton through ROP-GEFs (GUANINE NUCLEOTIDE-EXCHANGE FACTORS for RHO-LIKE SMALL GTPASES OF PLANTS, ROPs) that activate ROPs [20,21]. As in tomato, Arabidopsis PRK2 interacts with and activates ROP-GEF12 [22]. In another report, PRK2 was shown to phosphorylate ROP-GEF1 *in vitro* as part of a PRK2/ROP-GEF1/ROP1 protein complex [23]. In contrast to the situation in tomato, however, the Arabidopsis PRK and ROP-GEF protein families seem to show higher levels of redundancy. Only *prk1 prk2 prk3* triple and *rop-gef1 rop-gef9 rop-gef12 rop-gef14* quadruple mutants produce abnormal pollen tubes compared to wildtype [23]. The current model suggests that RLKs bind to extracellular ligands and control pollen tube growth through phosphorylation-dependent activation of ROP-GEFs and ROPs, ultimately affecting actin cytoskeleton dynamics.

## RLKs controlling cell wall integrity during pollen tube growth

During polarized growth, pollen tubes secrete new cell wall material to the surface of the pollen tube tip. This material is enriched in esterified pectin, resulting in a flexible cell wall that sustains tip expansion, while pectin de-esterification and callose deposition in the subapical wall provides rigidity preventing lateral expansion. In this context, a sensing mechanism for cell wall physicochemical properties is predicted to exist.

In plants, receptor-like kinases of the *Catharanthus roseus* RLKs (*Cr*RLK1L) subclass have been implicated in diverse processes [24]. The most studied member of the CrRLK1L family is FERONIA (FER), an RLK expressed in all plant tissues but pollen [24,25]. ANX1 and ANX2 are two other CrRLK1Ls proposed to regulate cell wall integrity in pollen tubes [3,4]. *ANX1* and *ANX2* are the closest homologues of *FER* and, together with three other yet uncharacterized CrRLK1L-encoding genes (*At4g39110*, *At2g21480* and *At5g61350*), are preferentially expressed in pollen tubes [24]. Single *anx1* and *anx2* loss-of-function mutants do not show any visible pollen phenotype. However, *anx1 anx2* double mutant is essentially sterile because pollen bursts immediately after germination [3,4]. In contrast, *ANX1/2* overexpression inhibits pollen tube growth by causing overaccumulation of cell wall material and invagination of the plasma membrane [26••]. Furthermore, ANX1-GFP and ANX2-GFP localize to the pollen tube tip membrane, a logical localization for a protein with a putative role in maintaining cell wall integrity. As for FER [27], genetic experiments place ROS production downstream of ANX1/2 signaling. In double mutants for *RESPIRATORY BURST OXIDASE HOMOLOGUES H* and *J* (*RBOHH* and *RBOHJ*), encoding two NADPH oxidases that regulate ROS production, pollen bursts when germinated both *in vitro* and *in vivo*, like pollen of the *anx1 anx2* double mutant [26••]. Moreover, *ANX1/2* overexpression-induced pollen tube growth inhibition depends on RBOHH/J, suggesting a functional link between ANX1/2 and RBOHH/J [26••]. The current model is that ANX1/2 regulates ROS production in a RBOHH/J-dependent manner, affecting calcium dynamics and subsequent deposition of new cell wall material by exocytosis [26••]. This model predicts that *anx1 anx2* pollen tubes should have thinner cell walls at the tips, and for this reason, burst with increasing turgor pressure. All these findings suggest that ANX1/2 act as sensors of cell wall integrity at the tip of growing pollen tubes.

A central and still unanswered question is the identity of ANX1/ANX2 ligands. The extracellular domain of the CrRLK1L family contains two malectin-like domains [28]. In animals, malectins bind di-glucose-high mannose N-glycan (Glc2-N-glycan) [28]. If this binding capacity is functionally conserved, ANX1/2 might bind polysaccharides from the pollen tube cell wall. Contrary to this hypothesis, FER was found to bind secreted peptides of the RAPID ALKALINIZATION FACTORS family (RALF), such as RALF1 [29] and RALF-LIKE23 (RALFL23) [30••]. There is a plethora of RALFLs peptides that could bind ANX1/2 and thereby play some role during pollination: RNAs encoding *RALFL4/8/9/15/19/25* and *26* are preferentially expressed in pollen and RNAs encoding *RALFL14/18/28* and *34* are expressed in the pistil. The presence of candidate ligands for ANX1/2 both in pollen and pistil is reminiscent of the LePRK1/2 autocrine and paracrine ligands [17,18]. Is there additional pollen autocrine regulation, as reported for LAT52-LePRK2 [17]? Is it possible that pistil RALFLs compete with pollen RALFLs for RLK binding, as described for tomato STIG1/LAT52-LePRK2 [18], or are all these RALFLs acting simultaneously? To answer such questions, it will be imperative to determine whether pollen and/or pistil RALFLs bind ANX1/2.

Other RLKs that might be involved in sensing at the pollen cell wall are the PERKs (PROLINE-RICH

EXTENSIN-LIKE RECEPTOR KINASES). The PERK family in Arabidopsis has 15 members and all have a proline-rich extracellular domain. *PERK4/5/6/7/11* and *12* RNAs are highly expressed in pollen and, most of them are hardly detected in sporophytic tissues [7]. The link between PERKs and cell wall homeostasis was first established for PERK4, a receptor that negatively regulates root growth, sensitivity to abscisic acid (ABA) and apical dominance [31]. In membrane protein preparations from root extracts, PERK4 was released from the cell wall only after pectinase treatments. This result suggests that PERK4, and maybe all PERKs, interact with cell wall components as demonstrated for other RLKs such as WAKs (WALL-ASSOCIATED KINASE family) [32]. Future work addressing PERK functions and the nature of their ligands will reveal the potential roles of these receptors in perceiving changes in the cell wall of pollen tubes.

## Short-range guidance

Plants must carry out fertilization rapidly and efficiently, minimizing the time between pollen hydration and sperm cells discharge. In their voyage to the ovary, pollen tubes need to be guided. LURE ('bait' in Japanese), cysteine-rich peptides produced by synergid cells and released at the ovule micropyle, are the most extensively characterized pollen tube chemo-attractants to date [33]. In 2016, two independent research groups identified LURE1 receptors, but surprisingly, they identified different RLKs [5••,6••].

The Higashiyama group postulated that PRK6 (POLLEN RECEPTOR-LIKE KINASE6) is the *bona fide* LURE1 receptor because *prk6* mutant pollen tubes failed to be attracted to LURE1 *in vitro* and because *prk3 prk6 prk8* triple mutants showed abnormal fertilization *in vivo* [5••]. They also showed that PRK6 accumulates on the pollen tube side closer to the LURE1 source before the tube changes growth direction. They demonstrated that PRK6 interacts with PRK3 and ROP-GEF12 and showed that PRK6 kinase activity is important for pollen tube growth. Moreover, PRK6 interacts with LOST IN POLLEN TUBE1 and 2 (LIP1 and LIP2, respectively), two RLCKs implicated in pollen tube growth and LURE1 signaling [34]. On the basis of the biochemical similarities to LePRK1/2, we speculate that LURE1 activates PRK6-PRK3 signaling, thereby modulating ROP-GEF12 and LIP1/2 activity and affecting the actin cytoskeleton. However, it remains to be elucidated whether LURE binding to PRK6 recruits PRK3, ROP-GEF12 and LIP1/2 into a complex, or induces complex dissociation.

In a companion article, Wang and co-workers described two pairs of pollen RLKs, MDIS1 and MDIS2 (MALE DISCOVERER1/2), and MIK1 and MIK2 (MDIS1-INTERACTING RECEPTOR LIKE KINASE1/2) as LURE1 receptors [6••]. They showed that *mdis1* and *mik1* *mik2* mutant pollen were less sensitive to LURE1 guidance, although mutant pollen tube growth was otherwise normal (remarkably similar to the *prk6* mutant). Interaction of LURE1 with the extracellular domains of MDIS1, MIK1 and MIK2 was demonstrated biochemically by co-immunoprecipitation and microscale thermophoresis. Moreover, LURE1 induced MDIS1–MIK1 oligomerization, activation of MIK1 kinase activity and MIK1 autophosphorylation. They also reported that LURE1 triggers down-regulation of MDIS1, by removing it from the cell membrane. In the absence of other interactors, it is difficult to predict the mechanism downstream of MDIS-MIK signaling.

It is puzzling that two overlapping complexes appear to perceive the LURE1 signal. Since *prk6* and *mdis mik* mutants show similar abnormal guidance phenotypes, it is possible that both complexes belong to a higher order signaling unit or that they act sequentially in a complex signaling cascade. Alternatively, both complexes could regulate different aspects of pollen tube guidance. Future work elucidating downstream effectors of PRK6/PRK3 and MDIS-MIK complexes will contribute to revealing the intricacies of tip growth orientation in plant cells.

## Pollen phosphoproteome

Signaling pathways frequently rely on protein phosphorylation to regulate protein-protein interactions, function, degradation and subcellular localization. Typically, ligand binding induces RLK auto-phosphorylation or trans-phosphorylation and signaling pathway activation. A phosphoproteomic analysis of Arabidopsis mature pollen identified 962 phosphorylated peptides that correspond to 598 phosphoproteins [35•]. This list included only three pollen RLKs: two CrRLK1L pollen-specific RLKs, At2g21480 and At4g39110, and PERK6. Considering that 45 RLKs are specifically enriched in mature pollen, in addition to the presence of many other non-enriched RLKs, it is curious that only three phosphorylated receptors were identified. RLK expression levels, phospho-isoform abundance or ligand-dependent phosphorylation might explain differences between expectation and observation. In any case, future analysis of RLK phosphorylation dynamics will certainly have the potential to contribute to a better understanding of signal transduction in pollen. As shown for LePRK2 [15] and the RLKs FLAGELLIN-SENSING2/BRASSINOSTEROID INSENSITIVE1 (FLS2/BRI1) [15,36], a phosphosite mutant analysis can be used to demonstrate whether phosphorylatable residues control pollen RLK functions.

## Finding downstream connections for pollen RLKs

Liu and collaborators reported that the expression of 76 RLK-encoding genes is significantly enriched in pollen tubes [34] but that only 19 of these genes affected

male transmissions when knocked out or overexpressed. Even for these, however, the signaling mechanisms that lie downstream are mostly unknown. A common strategy for implicating proteins of unknown function in any process, is to identify their interactors, some of which may have a known activity. We took advantage of available high-throughput protein-protein interaction data [37–39] to make such predictions. Most interactions were identified from yeast two-hybrid experiments and showed different degrees of reproducibility [40,41]. For this reason, only those interactions obtained in at least three out of four replicates were included. To restrict the analysis to putative biologically relevant interactions, only interactors with relative gene expression intensities >50 in semi *in vivo*-germinated pollen tubes were included [42••]. Interactors for which no microarray probes are available, were also included. We also restricted the search to RLKs with a canonical structure composed of extracellular, transmembrane and kinase domains (45 out of the 76 RLKs). Only 25 of these RLKs had previously reported interactions. Finally, proteins with promiscuous interactors were eliminated. For example, At5g59650 and WALL ASSOCIATED KINASE3 (WAK3) were eliminated because they promiscuously interact with 183 and 223 proteins, respectively. A total of 125 interactions between 23 RLKs and 77 interactors remained (Figure 2). Below we highlight selected hubs and interactors whose mutants show abnormal fertility, and interactions that suggest roles for RLK activities in pollen. Information about other interactors is provided as Supplemental Information.

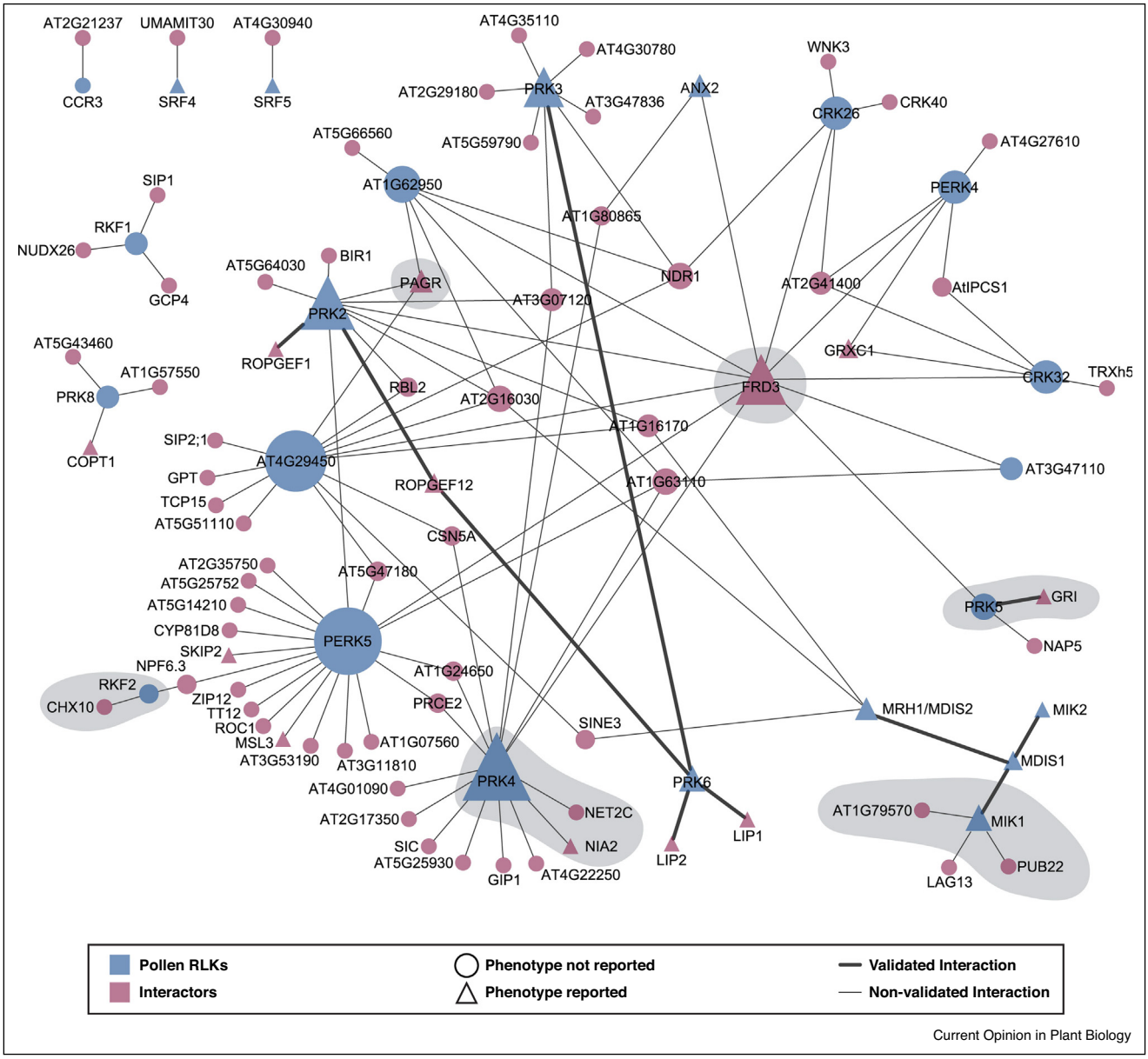
## Hubs

- FERRIC REDUCTASE DEFECTIVE3 (FRD3) appears as a hyper-connected node in the network, interacting with ANX2, CRK26, CRK32, PRK2, PRK4, PRK5, PERK4, PERK5, At1g62950, At3g47110 and At4g29450. FRD3 is a citrate efflux transporter involved in iron nutrition. Interestingly, microspore development aborts in *frd3* mutants, but can be recovered by iron supplementation [43]. Pollen tube growth, however, was not evaluated at different iron concentrations. When not bound to citrate, free Fe participates in ROS regulation (reviewed in [44,45]). Thus, RLKs could act at three levels: by affecting iron metabolism, by regulating extracellular ROS production through regulation of citrate efflux or by controlling extracellular pH, since *frd3* mutant shows an increase in apoplastic $H^+$ concentration [46].
- *PECTIC ARABINOGALACTAN SYNTHESIS-RELATED* (*PAGR*) encodes an O-fucosyltransferase involved in pectin synthesis. *PAGR*/*pagr* heterozygotes do not segregate homozygotes due to a defect in pollen germination [47•]. PAGR localizes to the Golgi apparatus [47•] and to vesicles like EXPOs (Exocyst-Positive Organelles) [48]. These vesicles localize in distinct puncta in the plasma membrane that are thought to release their contents to the apoplast and they contain arabinogalactan glycosyltransferases [49]. In pollen tubes, the EXPO vesicle components, SEC3 (SUBUNIT OF EXOCYST COMPLEX3), SEC8 (SUBUNIT OF EXOCYST COMPLEX8) and EXO70A1 (EXOCYST SUBUNIT EXO70 FAMILY PROTEIN A1) localize to the tip and their mutants have defective pollen germination and tube growth [47•,50,51].

## RLK interactors

- PRK5 interacts with GRIM REAPER (GRI) [52]. GRI is highly expressed in flowers and is processed to generate a secreted 11 amino acid peptide [52], which triggers cell death in a superoxide-dependent manner upon binding PRK5 [53]. Knockout *gri* plants showed reduced seed set [52], consistent with GRI-PRK5 having a role in pollen tube development. Interestingly, PRK5 also interacts with FRD3, establishing a possible downstream link to ROS production.
- CYSTEINE-RICH RLK32 (CRK32) interacts with THIOREDOXIN h5 (TRX h5) and GLUTAREDOXIN CYTOPLASMIC1 (GRXC1), two proteins from related families and with overlapping functions. TRX h5 is a cytoplasmic thioredoxin that provides resistance to $H_2O_2$ and regulates protein activity by reducing disulfide bridges in target proteins (reviewed in [54]). In the sporophyte *TRX h5* expression is increased in response to oxidative stress conditions [55] and *TRX h5* is highly expressed in pollen tubes. GRXC1 is a glutathione-dependent, cytoplasmic glutaredoxin that also catalyzes disulfide oxido-reduction. The double mutant *grxc1 grcx2* has partially empty siliques, indicating that fertilization might be affected [56]. TRXs and GRXs are connected by the systems that control their redox states. Glutathione was shown to indirectly maintain the pool of reduced TRXs in plants [57]. Interestingly, when *NADPH-DEPENDENT TRX REDUCTASES A* and *B* (*NTRA* and *NTRB*) are knocked out, wildtype pollen outcompetes *ntra ntrb* mutant pollen tubes, even though double mutants are still fertile [57]. *GLUTATHIONE REDUCTASE1* (*GR1*) encodes one of the two GRs in Arabidopsis. It is notable that a triple mutant *ntra ntrb gr1* fails to transmit through pollen [58]. The roles of TRXs and GRXs in pollen tubes might be controlling redox potential, protecting from ROS-induced damage or regulating the activity of interacting proteins. Alternatively, TRXs and GRXs could be acting as scaffolds for signaling, regardless of their enzymatic activity, as shown in the resistance mechanism to *Cladosporium fulvum* in tomato [59,60].
- Two interactors of the MDIS1/2-MIK1/2 signaling complex might connect this module to the MITOGEN-ACTIVATED PROTEIN KINASE (MAPK or MPK) cascade. MIK1 interacts with At1g79570, one of seven MKKKs (MAP KINASE KINASE KINASE) in group B4 [61], although no pollen phenotype has been

**Figure 2**




Predicted RLK pollen tube interactome. After filtering, 677 pairwise interactions remained, including those of the promiscuous RLKs, WAK3 and At5g59650, which accounted for 183 and 223 of these interactions respectively. Twenty-seven interactors were reported to interact with a large number of proteins. For example, UBQ3, At3g12180 and IQD6 interacted with 1347, 534 and 478 proteins, respectively. We eliminated proteins that had more than 100 interactors; At5g59650 and WAK3 were also eliminated. The network was generated using Cytoscape. Nodes and interactions of special interest are highlighted in grey and described in the text. Nodes sizes are proportional to their connectivity. *Validated interactions* are reported in the literature as pairwise interactions and often supported by genetic experiments. *Non-validated interactions* were mostly obtained from yeast two-hybrid experiments and in some cases, are supported by transient interaction assays *in planta*.

reported for mutants of this group. However, there is some support for a function of downstream MPKs in pollen tube guidance. Guan *et al.*, showed that funicular, but not micropylar, guidance in *mpk3 mpk6* mutants is defective [62••]. These results argue against a role of MPK3 and MPK6 in LURE1 signaling, but do not rule out that *MPK8/9/17* and *19*, which show higher expression levels than *MPK3/6* in pollen tubes, could be involved. Furthermore, MKK2/3/4/5 might connect MKKKs and MPKs. MIK1 also interacts with PLANT U-BOX 22 (PUB22), establishing a second connection to MAPK signaling. PUB22, a cytoplasmic U-box E3 ubiquitin ligase, is a regulator of the immune response. PUB22 oligomerizes and auto-ubiquitinates in the

absence of pathogens [63], but MPK3 phosphorylates and stabilizes PUB22 upon pathogen attack. Cytoplasmic accumulation of PUB22 leads to ubiquitin-mediated target degradation, with targets including the EXPO component EXO70B2 [64].

- PRK4 interacts with NETWORKED2C (NET2C) and NITRATE REDUCTASE2 (NIA2). *NET2C* is highly expressed in pollen tubes and shares homology with the actin-binding protein NET2A [65]. NET2A is involved in actin-plasma membrane interactions and is distributed as foci at the plasma membrane of the pollen tube shank. The N-terminus of NET2 family proteins is similar to petunia KINASE-INTERACTING PROTEIN 1 (KIP1) [66], a PRK1 interactor. NIA2, together with NIA1 and NOA1 (NITRIC OXIDE ASSOCIATED1), participate in nitric oxide (NO) synthesis; fertility is affected in the *nia1 nia2 noa1* triple mutant [67].
- RKF2 interacts with CATION/$H^+$ EXCHANGER10 (CHX10), a cation/$H^+$ exchanger. CHX10 is highly expressed in pollen tubes that is predicted to be localized in the plasma membrane/vacuoles. CHX10 is related to CHX21 and CHX23, two $K^+/H^+$ transporters also expressed in pollen tubes and with roles in guidance to ovules [68,69].

## Conclusions and perspectives

RLKs are important for regulating proper pollen tube growth and responding to guidance cues. The great diversity of putative RLK ligands involved in pollen tube biology, both autocrine and paracrine, suggest that competitive binding might provide positional cues that inform pollen tubes that their growth rate or path needs to be adjusted. These signals could affect $Ca^{2+}$ flux, actin polymerization, $H^+$ pump activity, ROS production, cell wall integrity and other processes, all of which need to be synchronized and balanced to sustain tip growth and guidance. For example, exocytosis secretes cell wall precursors and other material to the plasma membrane tip, but at the same time, endocytosis maintains the vesicle stock.

Most pollen-expressed RLKs, however, have no associated function, mainly because they belong to families with high redundancy. New technologies for knocking out multiple genes at once [70], together with the use of pollen-specific promoters might provide the genetic tools for analyzing the function of complex RLK families. Moreover, to date, most RLKs are orphan receptors. What are the *bona fide* ligands for these RLKs? The Arabidopsis genome encodes for more than 1000 putative secreted peptides [71]. The challenge to find ligand-receptor pairs is immense. It will be necessary to design alternative, scalable techniques for high-throughput screens (e.g. [72]).

Guidance can depend on mechanisms of localized stimulation (such as PRK6-mediated growth) or inhibition (such as NO effects on growth [73]). But with >45 RLKs expressed in pollen tubes and a myriad of candidate ligands, how is all the information integrated in the cytoplasm? Is there a hierarchy of RLKs, with some receptors transducing more important information than others? Combinatorial mutants for several types of RLKs seem to support the idea that not all receptors are equal. For example, among PRK genes, mutations of PRK6 alone were sufficient to disrupt LURE1-mediated *in vitro* pollen tube guidance, while *prk3 prk6 prk8* pollen tubes were less efficient than wildtype in leaving the transmitting tract (12 hours postpollination) [5••]. But how can such hierarchies be established when different RLKs converge on the same downstream processes? Besides *prk6* mutants, *mdis1 mdis2* pollen tubes are also insensitive to LURE1. Considering these observations, it is evident that we are just beginning to understand the mechanisms of how RLKs regulate pollen tube development.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.pbi.2017.09.008.

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- • of special interest
- •• of outstanding interest

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