Viewpoint

Keeping up with the RALFs: how these small peptides control pollen–pistil interactions in Arabidopsis

Summary

The pollen and pistil RALF peptides, along with multiple receptor-like kinases and leucine-rich repeat extensins, regulate pollen tube growth and the final burst within the ovule, where sperm cells are released for fertilisation to occur. This review introduces some new questions that arose about the regulation of this complex process.

Introduction

In flowering plants, pollen grains develop in the stamen and, once they mature, they are released from the anthers and interact with the stigma where they hydrate and eventually germinate, generating the pollen tube. The pollen tube is responsible for delivering the two sperm cells to the ovule, where double fertilisation takes place. This process requires a very complex and coordinated communication between pollen tubes and the different tissues of the pistil, so that the pollen tube grows rapidly and efficiently until reaching the ovule where it discharges its cytoplasmic content (for more details see Johnson *et al.*, 2019).

In recent years, significant progress has been made in the study of how RALF peptides affect the growth and development of different types of plant cells. This review focuses on the latest findings on how pollen and pistil RALFs influence the fertilisation process in Arabidopsis. This starts with pollen germination and pollen tube growth, and then continues through the discharge of its content within the synergid cell, analysing in all cases the downstream effects triggered by the action of RALF peptides. As these are very complex processes and there are many proteins involved in their regulation, we also incorporate new questions that arose from the details that are already known.

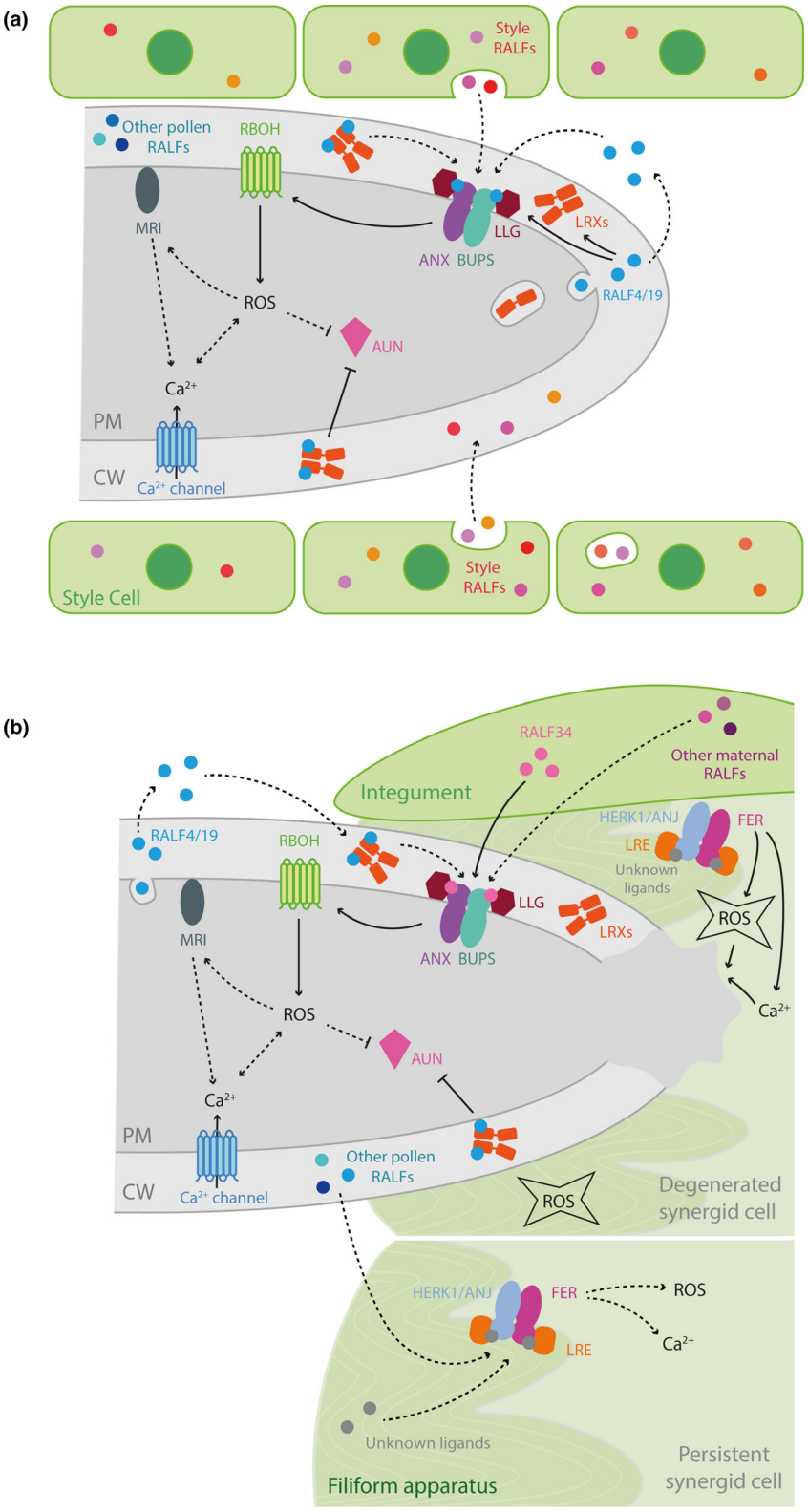
Pollen RALFs: RALF4 and RALF19 control proper pollen tube growth within the style

Pollen tubes exhibit rapid polarised growth throughout the style that ends with bursting of the pollen tube tip within one of the synergid cells. These processes must be strictly controlled, as premature bursting of the pollen tube during growth or the lack of bursting in the synergid cell would impair fertilisation.

It has been reported that some of the mechanisms of plant pollination and fertilisation are controlled by cysteine-rich peptides of the Rapid Alkalinization Factor (RALF) family together with their respective receptors of the *Catharanthus roseus* Receptor-Like Kinase 1-like (CrRLK1L) family. This interaction maintains efficient pollen tube growth along the pistil and coordinated pollen tube tip bursting within one of the synergid cells (Ge *et al.*, 2017). The only pollen RALFs that have been characterised to date are RALF4 and RALF19 (RALF4/19), which have 85% amino acid identity (Mecchia *et al.*, 2017).

What is the role of these RALF4 and RALF19 pollen peptides during pollen tube growth? Homozygous plants that lack *RALF4* are completely fertile due to the presence of RALF19. However, in *ralf4* mutant plants, almost half of the pollen tubes grown *in vitro* burst after an initial period of fast growth (Mecchia *et al.*, 2017). This phenomenon is most likely to be due to the increased growth rate that prevents an adequate assembly of the cell wall components, causing pollen tube bursting. By contrast, RALF4 overexpression leads to a drastic increase in pollen grain explosion, resulting in a reduced seed set (Mecchia *et al.*, 2017) while the addition of synthetic RALF4 peptide inhibits *in vitro* pollen germination (Morato do Canto *et al.*, 2014; Mecchia *et al.*, 2017; Ge *et al.*, 2019) and pollen tube growth (Mecchia *et al.*, 2017). All these findings suggest that the function of RALF4 is to activate a suitable downward speed change to maintain an appropriate growth rate.

The integrity of the pollen tube during polarised growth is maintained by the concerted action of RALF4/19 peptides, their CrRLK1L family receptor-like kinases ANXUR1 and ANXUR2 (ANX1/2) and Buddha's Paper Seal 1 and 2 (BUPS1/2), and two pollen LORELEI-Like-Glycosylphosphatidylinositol (GPI)-anchored proteins (LLG2/3) (Ge *et al.*, 2017, 2019; Zhu *et al.*, 2018; Feng *et al.*, 2019). RALF4 and RALF19 are synthesised in pollen and delivered to the apoplast in order to bind directly to the extracellular domains of ANX1, ANX2, BUPS1 and BUPS2. LLG2/3 also interact with RALF4/19 and with the extracellular domains of ANX–BUPS, suggesting that they are all involved in the formation of a ligand–receptor complex in the plasma membrane of growing pollen tubes (Fig. 1a). In addition, RALF4/19 interact with LRX8, LRX9, LRX10 and LRX11, four pollen-specific proteins from the Leucine-Rich repeat Extensin (LRX) family that participate in cell wall assembly during pollen tube growth (Mecchia *et al.*, 2017; Fabrice *et al.*, 2018; Sede *et al.*, 2018; Wang *et al.*, 2018; Moussu *et al.*, 2020) (Fig. 1a). The N-terminal domain of several vegetative LRXs (LRX1, LRX2, LRX4, and LRX5) has been reported to interact with FERONIA (FER) (Dünser *et al.*, 2019; Herger *et al.*, 2020), another receptor of the CrRLK1L family, ubiquitously expressed in all tissues of Arabidopsis except pollen. Additionally, high affinity binding sites for RALF1 have



been found in LRX1, LRX4, and LRX5 (Herger *et al.*, 2020). Accordingly, LRXs could function as a physical link between the intracellular and extracellular components, but in pollen it remains to be tested if LLG2/3, ANX1/2 and BUPS1/2 physically interact with LRX8–11.

When any of the aforementioned pollen proteins are missing, as occurs in double mutants *anx1 anx2*, *bups1 bups2*, or in RNAi knock-down or knock-out mutants of *RALF4/19* and *LLG2/3*, or in multiple pollen-expressed *lrx* mutants, pollen tubes burst after germination, which severely compromises male fertility. When the

Fig. 1 Role of RALF peptides throughout the different stages of plant fertilisation. (a) Maintenance of proper polarised growth of the pollen tube throughout the style. The RALF4 and RALF19 pollen peptides are secreted into the apoplast of the pollen tubes, where they interact with the ANX1/2–BUPS1/2–LLG2/3 complex and, through MRI and RBOH, activate a signalling pathway that regulates cytosolic levels of ROS and calcium, supporting polarised growth. Simultaneously, the interaction between RALF4/19 and LRX8/9/10/11 activates a parallel pathway necessary to maintain cell wall integrity, which is negatively regulated by AUN1/2. The role of the style and the 'other pollen' RALFs in maintaining proper pollen tube growth is still unknown. (b) Induction of pollen tube bursting within one of the synergid cells. The ovule peptide RALF34, and probably 'other maternal' RALFs, compete with RALF4/19 by binding to the ANX1/2–BUPS1/2–LLG2/3 complex. This interaction leads to changes in cytoplasmic ROS and calcium levels of pollen tubes triggering tip bursting inside the synergid cell. The synergid complex formed by HERK1, ANJ, FER and LRE, when activated by a still unknown ligand, possibly a pollen RALF/s, generates an increase in the levels of calcium and ROS necessary for pollen tube bursting. Abbreviations: ANJ, CrRLK1L receptor-like kinase ANJEA; ANX, refers to ANX1/2, CrRLK1L receptor-like kinase ANXUR1/2; AUN, refers to AUN1/2, Type One Protein Phosphatases ATUNIS1/2; BUPS, refers to BUPS1/2, CrRLK1L receptor-like kinase Buddha's Paper Seal 1/2; CW, cell wall; FER, CrRLK1L receptor-like kinase FERONIA; HERK1, CrRLK1L Hercules Receptor-like Kinase 1; LLG, refers to LLG2/3, LORELEI-Like-Glycosylphosphatidylinositol (GPI)-anchored proteins 2/3; LRE, Glycosyl-phosphatidylinositol (GPI)-anchored protein LORELEI; LRXs, refers to LRX8/9/10/11, Leucine-Rich repeat Extensin 8/9/10/11; MRI, receptor-like cytoplasmic kinase MARIS; PM, plasma membrane; RALF4, Rapid Alkalinization Factor 4; RALF 19, Rapid Alkalinization Factor 19; RBOH, refers to RBOH H/J, Respiratory Burst Oxidase Homologue H/J; ROS, reactive oxygen species. Solid arrows denote direct positive actions, while dotted arrows denote still unknown actions.

cell wall composition of these lines was analysed, it was found that downregulation of *RALF4/19* or *LLG2/3* and the multiple knock-out mutants *lrx8–11* led to an abnormal deposit of pectin and deficient deposit of callose, suggesting a role of all of them in maintaining the integrity of the pollen tube cell wall (Boisson-Dernier *et al.*, 2013; Ge *et al.*, 2017; Mecchia *et al.*, 2017; Fabrice *et al.*, 2018; Sede *et al.*, 2018; Feng *et al.*, 2019; Ge *et al.*, 2019). The functional relationship between LRX and RALF is also supported by the fact that pollen tube growth of the triple mutant *lrx8 lrx9 lrx10* is no longer inhibited by the addition of exogenous synthetic RALF4 peptide (Mecchia *et al.*, 2017).

The ANX1/2 pathway activates a Receptor-Like Cytoplasmic Kinase (RLCK) called MARIS (MRI), which is anchored to the plasma membrane of pollen tubes (Fig. 1a). A constitutively active form of MRI (MRIR240C, *mri-3D*) suppresses the infertile phenotype of the double mutant *anx1 anx2* (Boisson-Dernier *et al.*, 2015) and the downregulated pollen *RALF4/19* (Mecchia *et al.*, 2017), which indicates that RALF4/19 act upstream of the ANX1/2–MRI pathway. However, *mri-3D* cannot suppress the reduced male fertility of the multiple pollen-expressed *lrx* mutants, suggesting that the intracellular pollen tube response mediated by RALF4/19–ANX1/2–MRI occurs through a parallel pathway of RALF4/19–LRX8–11. Conversely, the Type One Protein Phosphatases ATUNIS1/2 (AUN1/2) are negative regulators of the ANX1/2 pathway and act downstream of RALF4/19 and LRX8–11 but not of MRI (Franck *et al.*, 2018) (Fig. 1a). All these results suggest that regulation of the mechanisms that control the integrity of the pollen tube occurs through complex and divided pathways.

More recently, studies have revealed the structure of the complex formed by pollen LRX8–RALF4 and the one containing RALF23, LLG1 and FER (Xiao *et al.*, 2019; Moussu *et al.*, 2020). The N-terminal region of RALF23 interacts with different LLGs (LLG1–3) and promotes the interaction between the LLGs and FER in a tertiary complex (Xiao *et al.*, 2019). Moreover, LRXs appear as dimers linked by a disulfide bond and their N-terminal region interact with RALF4 (Moussu *et al.*, 2020). It is possible that in the apoplast of the pollen tubes, LRX8–11 titrates the amount of free RALF4/19 in such a way that, during pollen tube growth, a defined percentage of RALF4/19 would disassociate from LRX8–11 through an unknown mechanism and bind to ANX–BUPS receptors activating a pathway that would maintain pollen tube integrity. A similar scenario would occur in roots of Arabidopsis plants that overexpress the N-terminal domain of LRX4, which are hypersensitive to the addition of exogenous RALF1 and exhibit a growth rate reduction presumably due to inhibition of formation of the LRX–RALF–FER complex (Dünser *et al.*, 2019).

All pollen LRXs, LLGs and RALFs are synthesised in the pollen tube and secreted to the apoplast of the pollen tube, this suggests that active autocrine signalling during growth would inform pollen tubes where they are in their journey to the ovules. In addition to RALF4 and RALF19, there are six other pollen-expressed RALFs: RALF8, RALF9, RALF15, RALF25, RALF26 and RALF30 (Loraine *et al.*, 2013). None of these six RALFs could replace the absence of both RALF4 and RALF19 in RNAi *RALF4/19* knock-down and *RALF4/19* knock-out mutant plants, this suggests that they act either during pollen development or later as potential ligands of FER, HERCULES RECEPTOR KINASE 1 (HERK1) and/or ANJEA (ANJ), which are three synergid receptor kinases of the CrRLK1L family that participate in pollen tube reception, along with the glycosyl-phosphatidylinositol (GPI)-anchored protein LORELEI (LRE) (Galindo-Trigo *et al.*, 2020). When the pollen tubes reach the *fer* single mutant or *herk1 anj* double mutant ovules, most of them overgrow inside the synergid cells and the rest of the embryo sac, without bursting (Escobar-Restrepo *et al.*, 2007; Galindo-Trigo *et al.*, 2020). HERK1 and ANJ were proposed to act redundantly in association with FER by promoting pollen tube bursting within the synergid cells (Galindo-Trigo *et al.*, 2020). Analysis of higher order mutants for these pollen RALFs would shed some light on their function in pollination and fertilisation (see Box 1).

Pistil RALFs: RALF34 triggers bursting of the pollen tube tip inside the synergid to release the two sperm cells and produce fertilisation

Once a pollen tube reaches an ovule, the male and female tissues must communicate with each other to ensure proper progression of fertilisation events, so at this stage maternal RALFs may play a role in the release of sperm cells within the synergid cells. According to Ge *et al.* (2017), there are nine *RALFs* expressed in the Arabidopsis pistil: RALF5, RALF14, RALF18, RALF24, RALF28, RALF29, RALF31, RALF33 and RALF34. RALF34 binds *in vitro* to the

Box 1 Despite all the findings mentioned above, there are still many questions to answer:

- What is the function of the other pollen and pistil RALFs? Why so many?
- Are some pollen RALFs potential ligands for synergid FER, HERK1 and/or ANJEA receptor-like kinases?
- Are some pistil RALFs potential ligands for pollen ANX/BUPS receptor-like kinases?
- What other 'maternal' RALFs work together with RALF34?
- RALF4/19 and RALF34 bind to the same pollen ANX/BUPS receptors but produce contrasting final responses. What are the pollen signal transduction pathways triggered by the two different RALFs that generate these opposite responses?
- If pollen tube tip bursting requires a high external concentration of ROS and calcium, why does addition of the synthetic RALF34 peptide in the germination medium also cause pollen tube bursting?

BUPS1/2 and ANX1/2 ectodomains by displacement of RALF4/19 from these receptors, and addition of synthetic RALF34 peptide induces bursting of the pollen tubes grown *in vitro* (Ge *et al.*, 2017). Based on this, it is proposed that once the pollen tubes reach the ovule, free RALF34 secreted by the inner integument, binds and activates the pollen tube ANX/BUPS pathway. Finally, RALF34 trigger pollen tube tip bursting in one of the synergid cells, discharging the two sperm cells (Fig. 1b). As loss of function *ralf34* mutants did not show any fertilisation defect (Ge *et al.*, 2017), it seems reasonable to consider that other maternal RALFs are involved in this paracrine process (see Box 1).

Conversely, despite the fact that pollen from plants *ralf4* is functional *in vivo*, it bursts when germinated *in vitro* (Mecchia *et al.*, 2017). Some stigma and/or style RALFs could replace the absence of RALF4 *in vivo*. Knowing the exact source and localisation of all pistil RALFs mentioned above will help to understand the precise interactions that occur between pollen and pistil tissues.

Another striking feature of this complex regulation is that RALF4/19 and RALF34 bind to the same pollen ANX/BUPS receptors, but their final responses are completely opposite. Coincidently, a similar type of action has been previously described for FER, in which two of its ligands, RALF17 and RALF23, trigger contrasting immune responses (Stegmann *et al.*, 2017). While pollen RALF4/19 binding maintains proper pollen tube growth, maternal RALF34 binding induces bursting of the pollen tube tip. It is even more intriguing that very similar and evolutionarily very close peptides such as RALF4/19 and RALF34 trigger these two opposite final responses by activating the same set of pollen receptors (ANX/BUPS). It is feasible that these distinct responses could be caused by different affinities of these RALFs to their respective receptors and/or by different concentrations of these RALFs in the apoplast of the pollen tubes. In summary, it remains to be understood whether these two contrasting pollen responses are triggered by the same set of cytoplasmic pollen proteins or by two fully diverging pollen pathways that involve different downstream proteins (see Box 1).

Downstream effectors triggered by pollen and pistil RALFs-activation pathway

Other crucial players involved in plant pollination and fertilisation are second messengers, such as Reactive Oxygen Species (ROS) and calcium. Any disturbance in the dynamics of either of them can inhibit pollen tubes growth or induce their bursting. Pollen NADPH oxidases RESPIRATORY BURST OXIDASE HOMOLOGUE H and J (RBOH H/J) are part of the ANX1/2 pathway and produce cytoplasmic ROS, essential for preserving pollen tube integrity (Boisson-Dernier *et al.*, 2013). It has been shown that upon activation of the FER-LLG1/LRE complex in the synergid cells, activated small GTP-binding proteins RAC/ROPs induce ROS production via NADPH oxidases, necessary to induce pollen tube bursting in a calcium-dependent manner (Duan *et al.*, 2014; Li *et al.*, 2015).

More recently, it was shown that pollen tube cytoplasmic ROS was increased by addition of RALF4 but was reduced in *LLG2/3* RNAi lines, presumably because there was no activation of RBOHH/J (Feng *et al.*, 2019). In seedlings and roots, calcium increases after addition of exogenous RALF1 (Haruta *et al.*, 2014), however there are still no reports on the influence of RALF4/19 or other pollen RALFs on calcium dynamics of growing pollen tubes. Regarding RALF34 and the other potential ovule secreted RALFs, it remains to be determined what biochemical response starts in the pollen tube cytoplasm after interaction with the ANX/BUPS receptor complex.

It is expected that pollen ROS and calcium levels will be modified in such a way that, in concerted action with high calcium and ROS content in the synergid cells, it generates the appropriate environment for pollen tube bursting. Measurement of calcium and ROS levels at the tip of pollen tubes growing *in vivo* and at the receiving synergid cell will provide the necessary information to understand the mechanisms that control pollen tube bursting and release of sperm cells.

Concluding remarks

RALF peptides are involved in autocrine and paracrine regulation during pollen–pistil interactions. As the pollen tubes travel through the pistil, a pollen RALF-governed autocrine regulation activates the ANX/BUPS pathway to maintain proper pollen tube growth. Once the pollen tubes reach the ovules, the maternal paracrine RALF peptides displace the pollen RALFs from binding to the ANX/BUPS receptors and trigger pollen tube bursting within the synergid cell, allowing fertilisation to occur. Here, we bring together the latest advances in RALF regulation in this field and include some still unanswered questions that will help to shed some light on our understanding of this complex signalling pathway.

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Author contributions

JPM conceived the project; SCS, ARS and NAB reviewed the text, references, and figure; SCS, ARS, NAB and JPM made the figure and wrote the article. SCS, ARS and NAB contributed equally to this work.

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References

Boisson-Dernier A, Franck CM, Lituiev DS, Grossniklaus U. 2015. Receptor-like cytoplasmic kinase MARIS functions downstream of CrRLK1L-dependent signaling during tip growth. *Proceedings of the National Academy of Sciences, USA* **112**: 12211–12216.

Boisson-Dernier A, Lituiev DS, Nestorova A, Franck CM, Thirugnanarajah S, Grossniklaus U. 2013. ANXUR receptor-like kinases coordinate cell wall integrity with growth at the pollen tube tip via NADPH oxidases. *PLoS Biology* **11**: e1001719.

Duan Q, Kita D, Johnson EA, Aggarwal M, Gates L, Wu HM, Cheung AY. 2014. Reactive oxygen species mediate pollen tube rupture to release sperm for fertilization in Arabidopsis. *Nature Communications* **5**: 3129.

Dünser K, Gupta S, Herger A, Feraru MI, Ringli C, Kleine-Vehn J. 2019. Extracellular matrix sensing by FERONIA and Leucine-Rich Repeat Extensins controls vacuolar expansion during cellular elongation in *Arabidopsis thaliana*. *EMBO Journal* **38**: 1–12.

Escobar-Restrepo JM, Huck N, Kessler S, Gagliardini V, Gheyselinck J, Yang WC, Grossniklaus U. 2007. The Feronia receptor-like kinase mediates male-female interactions during pollen tube reception. *Science* **317**: 656–660.

Fabrice TN, Vogler H, Draeger C, Munglani G, Gupta S, Herger AG, Knox P, Grossniklaus U, Ringli C. 2018. LRX proteins play a crucial role in pollen grain and pollen tube cell wall development. *Plant Physiology* **176**: 1981–1992.

Feng H, Liu C, Fu R, Zhang M, Li H, Shen L, Wei Q, Sun X, Xu L, Ni B *et al.* 2019. LORELEI-LIKE GPI-ANCHORED PROTEINS 2/3 Regulate pollen tube growth as chaperones and coreceptors for ANXUR/BUPS receptor kinases in Arabidopsis. *Molecular Plant* **12**: 1612–1623.

Franck CM, Westermann J, Bürssner S, Lentz R, Lituiev DS, Boisson-Dernier A. 2018. The protein phosphatases ATUNIS1 and ATUNIS2 regulate cell wall integrity in tip-growing cells. *The Plant Cell* **30**: 1906–1923.

Galindo-Trigo S, Blanco-Touriñán N, DeFalco TA, Wells ES, Gray JE, Zipfel C, Smith LM. 2020. Cr RLK 1L receptor-like kinases HERK 1 and ANJEA are female determinants of pollen tube reception. *EMBO Reports* **21**: e48466.

Ge Z, Bergonci T, Zhao Y, Zou Y, Du S, Liu M, Luo X, Ruan H, García-Valencia L, Zhong S *et al.* 2017. Arabidopsis pollen tube integrity and sperm release are regulated by RALF-mediated signaling. *Science* **358**: 1596–1600.

Ge Z, Zhao Y, Liu MC, Zhou LZ, Wang L, Zhong S, Hou S, Jiang J, Liu T, Huang Q *et al.* 2019. LLG2/3 Are co-receptors in BUPS/ANX-RALF signaling to regulate Arabidopsis pollen tube integrity. *Current Biology* **29**: 3256–3265.e5.

Haruta M, Sabat G, Stecker K, Minkoff BB, Sussman MR. 2014. A peptide hormone and its receptor protein kinase regulate plant cell expansion. *Science* **343**: 408–411.

Herger A, Gupta S, Kadler G, Franck CM, Boisson-Dernier A, Ringli C. 2020. Overlapping functions and protein-protein interactions of LRR extensins in Arabidopsis. *PLoS Genetics* **16**: e1008847.

Johnson MA, Harper JF, Palanivelu R. 2019. A fruitful journey: pollen tube navigation from germination to fertilization. *Annual Review of Plant Biology* **70**: 809–837.

Li C, Yeh FL, Cheung AY, Duan Q, Kita D, Liu MC, Maman J, Luu EJ, Wu BW, Gates L *et al.* 2015. Glycosylphosphatidylinositol-anchored proteins as chaperones and co-receptors for FERONIA receptor kinase signaling in Arabidopsis. *eLife* **4**: 1–21.

Loraine AE, McCormick S, Estrada A, Patel K, Qin P. 2013. RNA-seq of Arabidopsis pollen uncovers novel transcription and alternative splicing. *Plant Physiology* **162**: 1092–1109.

Mecchia MA, Santos-Fernandez G, Duss NN, Somoza SC, Boisson-Dernier A, Gagliardini V, Martínez-Bernardini A, Fabrice TN, Ringli C, Muschietti JP *et al.* 2017. RALF4/19 peptides interact with LRX proteins to control pollen tube growth in Arabidopsis. *Science* **358**: 1600–1603.

Morato do Canto A, Ceciliato PHO, Ribeiro B, Ortiz Morea FA, Franco Garcia AA, Silva-Filho MC, Moura DS. 2014. Biological activity of nine recombinant AtRALF peptides: Implications for their perception and function in Arabidopsis. *Plant Physiology and Biochemistry* **75**: 45–54.

Moussu S, Broyart C, Santos-Fernandez G, Augustin S, Wehrle S, Grossniklaus U, Santiago J. 2020. Structural basis for recognition of RALF peptides by LRX proteins during pollen tube growth. *Proceedings of the National Academy of Sciences, USA* **117**: 7494–7503.

Sede AR, Borassi C, Wengier DL, Mecchia MA, Estevez JM, Muschietti JP. 2018. Arabidopsis pollen extensins LRX are required for cell wall integrity during pollen tube growth. *FEBS Letters* **592**: 233–243.

Stegmann M, Monaghan J, Smakowska-Luzan E, Rovenich H, Lehner A, Holton N, Belkhadir Y, Zipfel C. 2017. The receptor kinase FER is a RALF-regulated scaffold controlling plant immune signaling. *Science* **355**: 287–289.

Wang X, Wang K, Yin G, Liu X, Liu M, Cao N, Duan Y, Gao H, Wang W, Ge W *et al.* 2018. Pollen-expressed leucine-rich repeat extensins are essential for pollen germination and growth. *Plant Physiology* **176**: 1993–2006.

Xiao Y, Stegmann M, Han Z, DeFalco TA, Parys K, Xu L, Belkhadir Y, Zipfel C, Chai J. 2019. Mechanisms of RALF peptide perception by a heterotypic receptor complex. *Nature* **572**: 270–274.

Zhu L, Chu LC, Liang Y, Zhang XQ, Chen LQ, Ye D. 2018. The arabidopsis CrRLK1L protein kinases BUPS1 and BUPS2 are required for normal growth of pollen tubes in the pistil. *The Plant Journal* **95**: 474–486.

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