breeding. The elucidation of biosynthetic pathways and identification of regulatory elements provide insights guiding more targeted breeding efforts towards the selection of desired traits. In turn, understanding the molecular mechanisms behind domestication could accelerate continued breeding efforts. Taking classical breeding for the sweet kernel taste in almonds as an example, it takes 2–3 years until the first fruit set occurs. Only at this stage are the breeders able to check whether an almond tree carries sweet or bitter almonds and then to proceed with crossings to incorporate other traits [4,6]. Now the process can be greatly accelerated because the selection for the sweet kernel trait can be performed at the seedling stage. The fascinating study by Sánchez-Pérez *et al.* not only documented the detailed molecular mechanisms by which bitter wild almond was domesticated into sweet kernel almond, but also advanced our understanding of the evolution of metabolism.

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https://doi.org/10.1016/j.tplants.2019.09.001

References

1. Olsen, K.M. and Wendel, J.F. (2013) A bountiful harvest: genomic insights into crop domestication phenotypes. *Annu. Rev. Plant Biol.* 64, 47–70
2. Sanchez-Perez, R. *et al.* (2008) Bitterness in almonds. *Plant Physiol.* 146, 1040–1052
3. Sánchez-Pérez, R. *et al.* (2007) Mapping major genes and quantitative trait loci controlling agronomic traits in almond. *Plant Breed.* 126, 310–318
4. Thodberg, S. *et al.* (2018) Elucidation of the amygdalin pathway reveals the metabolic basis of bitter and sweet almonds (*Prunus dulcis*). *Plant Physiol.* 178, 1096–1111
5. Wurtzel, E.T. and Kutchan, T.M. (2016) Plant metabolism, the diverse chemistry set of the future. *Science* 353, 1232–1236
6. Sanchez-Perez, R. *et al.* (2019) Mutation of a bHLH transcription factor allowed almond domestication. *Science* 364, 1095–1098
7. Gross, B.L. and Olsen, K.M. (2010) Genetic perspectives on crop domestication. *Trends Plant Sci.* 15, 529–537
8. Fang, C. *et al.* (2019) Exploring the diversity of plant metabolism. *Trends Plant Sci.* 24, 83–98
9. Wu, Y. *et al.* (2012) Presence of tannins in sorghum grains is conditioned by different natural alleles of *Tannin1*. *Proc. Natl. Acad. Sci. U. S. A.* 109, 10281–10286
10. Shang, Y. *et al.* (2014) Biosynthesis, regulation, and domestication of bitterness in cucumber. *Science* 346, 1084–1088
11. Huang, D. *et al.* (2018) Subfunctionalization of the Ruby2–Ruby1 gene cluster during the domestication of citrus. *Nat. Plants* 4, 930–941
12. Butelli, E. *et al.* (2019) Noemi controls production of flavonoid pigments and fruit acidity and illustrates the domestication routes of modern citrus varieties. *Curr. Biol.* 29, 158–164

Spotlight

How CrRLK1L Receptor Complexes Perceive RALF Signals

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RALFs are secreted peptides that are perceived by various CrRLK1L–LRE/LLG receptor complexes. The mechanistic basis of this perception has now been elucidated showing that the co-receptor LLG binds RALF23 to nucleate a FER receptor complex. This interaction likely occurs in other tissues where RALFs meet CrRLK1L receptors and LRE/LLG co-receptors.

CrRLK1Ls Act as Cell-Wall Sensors

Plant cells constantly sense their environment during growth and development, reproduction, abiotic stress, and biotic interactions. Their walls play a crucial role in environmental sensing by acting as a reservoir of signaling molecules including hormones, peptide ligands, and sugars, among others. Moreover, the structure and composition of the cell wall change during growth and various interactions. To precisely sense these signals, plants have evolved a battery of transmembrane receptors, notably various classes of receptor-like kinases (RLKs). The arabidopsis (*Arabidopsis thaliana*) genome, for example, encodes more than 600 RLKs, each containing an extracellular domain for signal perception, an intracellular kinase domain for downstream signaling activation, and a transmembrane domain to locate and anchor the receptor in the plasma membrane [1].

RLKs are stabilized and/or structurally modified at the cell surface by co-receptors or chaperones such as glycosylphosphatidylinositol-anchored proteins (GPI-APs) [2,3]. Various members of the CrRLK1L (*Catharanthus roseus* receptor-like kinase 1-like proteins) subclass of RLKs appear to be involved in cell-wall sensing [4]. Members of the RAPID ALKALINIZATION FACTOR (RALF) peptide family serve as extracellular ligands for CrRLK1Ls. RALF1 and RALF23 promote root and seedling growth after binding to the CrRLK1L receptor FERONIA (FER), whereas RALF17 and RALF23 regulate FER-dependent immune responses to pathogens [4,5]. RALF34 interacts with the THESEUS 1 (THE1) receptor that controls lateral root initiation and defense responses [6], whereas RALF4 and RALF19 are necessary for the control of pollen tube growth and cell-wall integrity. The latter act as ligands for a pollen tube-expressed CrRLK1L receptor complex that consists of ANX1/2 (ANXUR 1/2) and BUPS1/2 (Buddha's Paper Seal 1/2). RALF34, which is capable of interacting with the same receptor complex, shows

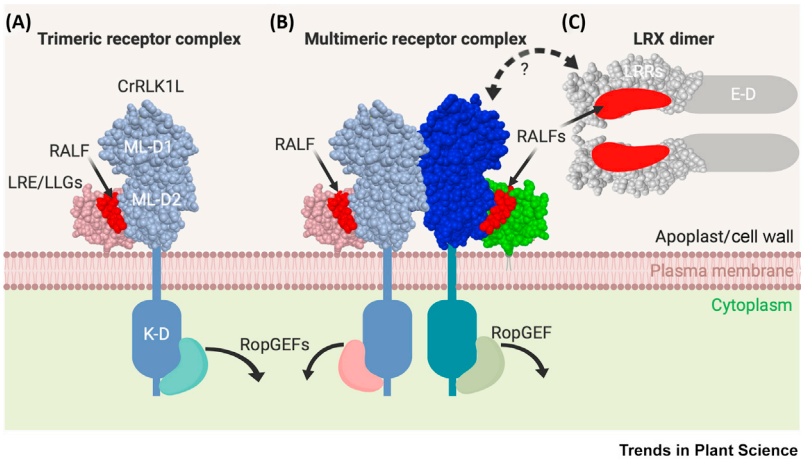

Figure 1. Insights into RALF Perception in *Arabidopsis* by Using Structural Biology.
(A) During RALF perception, receptor-like kinases of the CrRLK1L family interact with LRE/LLG GPI-APs in a ligand-induced manner. RALF peptides serve as a molecular glue that strengthens the interaction between receptor and co-receptor, thereby forming a trimeric signaling complex which activates a RopGEF, leading to intracellular signaling events. Note that the structure of the complex is restricted to the extracellular domain of the CrRLK1L kinase FER as well as to part of the co-receptor LLG2 and the N-terminal domain of RALF23 [8]. The structure was drawn based on PDB 6A5E that lacks parts of LLG2 and RALF23. Missing components of the large FER protein were drawn based on secondary structure predictions. (B) Trimeric CrRLK1L receptor complexes may form multimeric higher-order complexes in this hypothetical model because the extracellular domains of the CrRLK1Ls ANX and BUPS, as well as of FER, ANJEA, and HERK1, are able to interact with each other. The color code indicates that different RALFs, LLGs, and CrRLK1Ls may exist in a multimeric receptor complex. (C) Leucine-rich repeat extension proteins (LRX) are also involved in RALF binding in the cell wall where they form a tetramer. Whether LRX proteins are linked to the membrane by replacing, for example, LRE/LLGs in the CrRLK1L receptor complexes is unclear. The hypothetical model is based on a crystal structure of RALF4 in complex with the N-terminal domain of LRR domain of LRX2 [12]. Because the reported structure (PDB 6QXP) has not yet been released, the complex was drawn using a similar LRR domain (PDB 1D08). The figure was created using BioRender (https://biorender.com). Abbreviations: CrRLK1L, *Catharanthus roseus* RLK1-like (CrRLK1L) receptor; E-D, extensin domain; GPI-AP, glycosylphosphatidylinositol-anchored protein; K-D, kinase domain; LLG, LRE-like GPI-AP; LRE, LORELEI; LRX, leucine-rich repeat extension; LRRs, leucine-rich repeats; ML-D1/2, malectin-like domains 1 and 2; RALF, RAPID ALKALINIZATION FACTOR; RopGEF, Rho of plant guanine nucleotide-exchange factors.

an opposite effect and is required for pollen tube rupture [7]. Although CrRLK1L receptors are able to directly bind RALF peptides [5–7], the perception of RALF strongly relies on GPI-APs, namely LRE (LORELEI) and LLGs (LRE-like GPI-APs), which serve as chaperones/co-receptors [2–4]. There are four LRE-related GPI-APs in arabidopsis, named LRE and LLG1–3. LLG1 is ubiquitously expressed in many tissues, LRE predominately in ovules, and LLG2/LLG3 specifically in pollen grains and tubes, respectively [2,3]. It was shown that the synergid cell-expressed LRE genetically interacts with FER [2]. Biochemical studies revealed that LRE and LLG1 are able to directly interact with FER to control root development and pollen tube reception, whereas the interaction of LLG2/LLG3 with ANX/BUPS is required for maintaining pollen tube integrity [2–4].

The FER–LLG2–RALF23 Complex

However, the detailed mechanism of how RALF peptides are perceived by CrRLK1L receptors and the role of LRE-related GPI-APs in RALF–CrRLK1L signaling complexes has remained unclear. Xiao *et al.* now report the structure of the extracellular FER–LLG2–RALF23 complex [8], which sheds further light on RALF signaling in plants (Figure 1A). It was previously reported that FER recognizes RALF23 in arabidopsis immune responses [5]. To investigate whether LLG1 is also involved in FER/RALF23-mediated immunity, Xiao *et al.* first demonstrated that the *llg1* mutant phenocopies the *fer* mutant both in plant growth and in the immune response following RALF23 treatment [8]. They further showed that RALF23 directly interacts not only with the ectodomain of FER but also with LLG1. Notably, LLG1 did not show affinity for the FER ectodomain, but their association could be induced by the addition of RALF23.

Xiao *et al.* found that LLG1–3, but not LRE, exhibit similar binding affinity for RALF23 and for the RALF23–FER complex in analytical ultracentrifugation (AUC) and isothermal titration calorimetry (ITC) assays. A doctoral student in our laboratory then raised the question – what do we learn from the structure of the FER–LLG2–RALF23 complex given that FER and LLG2 are not coexpressed *in vivo*? One answer to this key question is that the structure casts light on the molecular specificity of the interaction and identifies the amino acids required for the interaction, whether these are conserved, and which domains of the binding partners are still available for interactions with other proteins. The N-terminal region of RALF23, for example, which includes the conserved 'YISY' motif that is essential for RALF activity and FER binding, is also directly involved in binding to the C terminus of LLG2 [8]. Moreover, the N-terminal 17 residues of mature RALF23 are

sufficient to recapitulate the activity of RALF23 binding to LLG1–3 and the induction of FER–LLG1–3 complex assembly [8]. Because the 'YISY' motif is conserved among multiple RALF peptides, this finding further supports the assumption that LLGs are general co-receptors of RALF peptides in various CrRLK1L signaling pathways [8], and justifies the generation of a structure that may not exist in this composition *in vivo*. Moreover, it was shown that the highly conserved 'KEGKEGLD' domain in LLG1–3 and LRE flips through 90° following RALF23 binding. However, when the first glycine of the conserved domain in LRE is replaced by an arginine, this leads to loss of LRE binding affinity for RALF23 and FER–RALF23 [8]. This finding implies that LRE probably adopts a distinct mechanism in FER-mediated pollen tube reception.

Concluding Remarks

In conclusion, the report by Xiao *et al.* [8] represents a major breakthrough in showing that the N terminus of RALF23 possesses high affinities for LLG1–3 as well as for FER, serving as a molecular glue to promote the association between LLGs and FER. The authors further propose that different RALF peptides containing the conserved N-terminal motifs might be recognized in a similar manner, facilitating our understanding of how different RALF peptides mediate numerous signaling pathways involving similar or distinct CrRLK1L receptors and LRE-related GPI-APs. The structure will also help to elucidate the opposing effects of RALF4/19 and RALF34 activity on pollen tube growth and rupture [8], indicate how RALF17 and RALF23 are recognized by FER but generate opposing plant immune responses [5], and contribute to understanding how fungal F-RALF homologs suppress immunity by inhibiting the formation of active receptor complexes [4].

Despite this advance, several open questions remain. It is unclear whether the described complex represents an active or inactive complex, and whether its assembly is only transient and induces, for example, receptor internalization. Treatment of roots with RALF22/23 was recently shown to induce FER internalization [9], indicating that receptor complex formation is highly dynamic. Moreover, LLG2 and LLG3 are predominately intracellular and are not found at the plasma membrane, indicating that formation of the RALF4/19–LLG2/3–ANX/BUPS receptor complex likely occurs only transiently [3]. Whether LLGs are biologically mutual equivalents also remains unclear. In addition, the role of the C-terminal region of RALF peptides is not known. This region, which is also important for FER binding, is not present in the structure of the RALF23–LLG2–FER complex [8]. Moreover, two additional CrRLK1L receptors, HERK1 and ANJEA, were recently reported to potentially form a heteromeric complex (Figure 1B) with FER to control pollen tube reception [10]. Whether the C terminus of RALF peptides and LLGs/LRE are involved in the heterodimerization/hetero-oligomerization of multiple CrRLK1L receptors will need to be determined in future experiments.

Finally, leucine-rich repeat extensin (LRX) proteins, which are required for pollen tube growth and cell-wall integrity, also interact with RALF peptides [11]. A recently released crystal structure of the LRX8–RALF4 complex showed that the N-terminal LRR domain of LRX8 interacts with RALF4 to generate a 2 + 2 heteromeric complex [12] (Figure 1C). Whether LRX proteins and CrRLK1L receptors compete for RALFs, or whether a supramolecular receptor complex for RALF sensing and signaling physically links LRX cell-wall proteins to CrRLK1L membrane receptor complexes, represents an exciting question for future studies.

Acknowledgments

This work is supported by the National Natural Science Foundation of China (31830004, 31620103903, and 31621001) and the China Postdoctoral Science Foundation (2018M640020, and 2019T120012). Financial support by the Peking–Tsinghua Joint Center for Life Sciences and the Research Council of Germany via SFB924 is gratefully acknowledged.

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https://doi.org/10.1016/j.tplants.2019.09.002

References

1. Liang, X.X. and Zhou, J.M. (2018) Receptor-like cytoplasmic kinases: central players in plant receptor kinase-mediated signaling. *Annu. Rev. Plant Biol.* 69, 267–299
2. Li, C. *et al.* (2016) FERONIA and her pals: functions and mechanisms. *Plant Physiol.* 171, 2379–2392
3. Ge, Z.X. *et al.* (2019) LLG2/3 are co-receptors in BUPS/ANX–RALF signaling to regulate *Arabidopsis* pollen tube integrity. *Curr. Biol.* https://doi.org/10.1016/j.cub.2019.08.032.
4. Franck, C.M. *et al.* (2018) Plant malectin-like receptor kinases: from cell wall integrity to immunity and beyond. *Annu. Rev. Plant Biol.* 69, 301–328
5. Stegmann, M. *et al.* (2017) The receptor kinase FER is a RALF-regulated scaffold controlling plant immune signaling. *Science* 355, 287–289
6. Gonneau, M. *et al.* (2018) Receptor kinase THESEUS1 is a rapid alkalinization factor 34 receptor in *Arabidopsis*. *Curr. Biol.* 28, 2452–2458
7. Ge, Z.X. *et al.* (2017) *Arabidopsis* pollen tube integrity and sperm release are regulated by RALF-mediated signaling. *Science* 358, 1596–1599

8. Xiao, Y. *et al.* (2019) Mechanisms of RALF peptide perception by a heterotypic receptor complex. *Nature* 572, 270–274
9. Zhao, C. *et al.* (2018) Leucine-rich repeat extensin proteins regulate plant salt tolerance in *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* 115, 13123–13128
10. Galindo-Trigo, S. *et al.* (2018) CrRLK1L receptor-like kinases HERK1 and ANJEA are female determinants of pollen tube reception. *bioRxiv*. Published online September 27, 2018. https://doi.org/10.1101/428854.
11. Mecchia, M.A. *et al.* (2017) RALF4/19 peptides interact with LRX proteins to control pollen tube growth in *Arabidopsis*. *Science* 358, 1600–1603
12. Moussu, S. *et al.* (2019) Structural basis for recognition of RALF peptides by LRX proteins during pollen tube growth. *bioRxiv*. Published online July 8, 2019. https://doi.org/10.1101/695874.

Spotlight

The PHYTOGLOBIN-NO Cycle Regulates Plant Mycorrhizal Symbiosis

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The production of the redox-active signaling molecule, NO, has long been associated with interactions between microbes and their host plants. Emerging evidence now suggests that specific NO signatures and cognate patterns of *PHYTOGLOBIN1* (*PHYTOGB1*) expression, a key regulator of cellular NO homeostasis, may help determine either symbiosis or pathogenicity.

Mycorrhizal fungi spend a minor portion of their life cycle as free living organisms and a majority of their life cycle associated with their respective host plant. It has been estimated that over 90% of land plants are associated with mycorrhizal fungi [1]. Among various mycorrhizal associations, the arbuscular mycorrhizal (AM) association with plants is one of the most important, as they play a major role in shaping both agricultural and natural ecosystems and their associated productivity. AM fungi establish themselves in root cortical cells, facilitating the uptake of key molecules, especially phosphorous, to their host plant, thus providing a unique source of essential micronutrients under limiting conditions, promoting plant growth. In turn, AM fungi receive photosynthate from their host plants [1]. AM fungi also convey additional advantages to their host plants, such as increasing disease resistance due to the presence of 'elicitor' molecules on their surface, which trigger microbial-associated molecular pattern (MAMP) immunity [2] and, further, activation of the symbiotic regulatory (SYM) pathway, which partially suppresses the host immune response, facilitating colonization [3].

The accumulating evidence suggests that the free radical signaling molecule, nitric oxide (NO), plays a key role in plant symbiotic interactions [3]. AM fungi have also been reported to induce disease resistance in soybean against *Phytophthora sojae*, an economically significant pathogen of this plant. Further, NO is thought to be a key component in the signaling network establishing this resistance [4]. In the association between leguminous plants and rhizobium bacteria, both partners contribute to NO production [5]. Significantly, NO plays a key role from the initial stages of the interaction through to the development of mature root nodules and their subsequent senescence [5]. In this context, nitrate reductase (NR), mitochondrial electron transport chain-mediated nitrite NO reduction and nitric oxide synthase-like (NOS-like) activity have all been proposed to generate the observed NO production. Further, an important function for NO turnover has also been studied [5]. Thus, a delicate balance between NO production and removal is thought to determine key signaling outputs associated with plant–microbe symbiosis [5].

The major NO scavenging pathways are thought to be mediated by phytoglobin (Pgb) and S-nitrosoglutathione reductase (GSNOR). Pgbs are a group of nonsymbiotic hemoglobins. These hexacoordinate hemoglobins are functionally and genetically distinct from symbiotic hemoglobins and possess high affinity for both oxygen and NO under certain conditions such as hypoxia, thereby functioning as effective molecular scavengers for these molecules [5]. The generated nitrate via oxygenation of NO via Pgb can subsequently become a substrate for NR to produce nitrite and concomitantly, NO. This cycling of NO mediated by Pgb is termed the 'Pgb-NO cycle' [5]. Although NO is known to play a key signal in the establishment of AM fungal–plant interactions, the underpinning molecular details have remained enigmatic.

Excitingly, Martinez-Medina *et al.* [6] now demonstrate that NO-dependent regulation of *PHYTOGB1* (class 1 hemoglobin) transcription plays a key role in these mycorrhizal–plant interactions. Significantly, they also identify specific NO-based signatures that precede colonization by *Rhizophagus irregularis*, employed as a soil inoculant in agriculture and horticulture, that regulate *PHYTOGB1* expression. Using transgenic tomato hairy roots, these authors demonstrated that PHYTOGB1 controls the levels of NO in tomato roots during colonization of the AM fungus, *R. irregularis*. Further, PHYTOGB1