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Growth Control: A Saga of Cell Walls, ROS, and Peptide Receptors

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Despite an increasingly detailed understanding of endogenous and environmental growth-controlling signals and their signaling networks, little is known on how these networks are integrated with the cell expansion machinery. Members of the CrRLK1L family control cell wall properties and cell expansion in a variety of developmental and environmental contexts. Two recent reports provide exciting new insights into the mode of action of these RLKs. One study shows that one family member, FERONIA (FER), is required for the production of hydroxyl radicals in the female gametophyte, which causes pollen tube rupture and sperm cell release during fertilization. Another study shows that FER is a receptor for a signaling peptide (Rapid Alkalinization Factor 1 [RALF1]) that triggers cell wall alkalization and growth arrest, possibly through the inhibition of plasma membrane H⁺-ATPase activity. RALF1 belongs to a large gene family, with a wide range of expression patterns. Other CrRLK1L family members therefore may also be receptors for RALF-like peptides. These findings have important implications for our understanding of the control of cell wall integrity during growth and raise new intriguing questions.

FERONIA CONTROLS CELL EXPANSION AND CELL-TO-CELL COMMUNICATION

The receptor-like kinase FERONIA (FER), named after an Etruscan goddess of fertility, was originally identified as a factor required for the communication between the male and female gametophytes during fertilization. Ovules lacking FER fail to induce pollen tube bursting and sperm cell release and attract supernumerary pollen tubes leading to a characteristic phenotype in which pollen tubes pile up inside the mutant female gametophyte (Huck et al., 2003; Rotman et al., 2003; Escobar-Restrepo et al., 2007). Later, it was shown that FER is required for correct polar targeting of NORTIA, a multi-spanning membrane protein of the mildew resistance locus O (MLO) family, which is also required for pollen tube bursting and fertilization (Kessler et al., 2010). Furthermore, FER has fertilization-independent functions, as *fer* mutants generally show reduced growth, defective root hairs, and increased resistance to powdery mildew (Guo et al., 2009; Duan et al., 2010; Kessler et al., 2010; Duan et al., 2014).

FER belongs to a 17-member family of proteins in *Arabidopsis thaliana* named CrRLK1L (named after the first member characterized in *Catharanthus roseus* cell cultures), several members of which have been implicated in the regulation of cell elongation (see Table 1 for a summary of phenotypes observed in CrRLK1L mutant alleles). CrRLK1L family members that have been studied are part of a signaling module that involves the recruitment of guanidine nucleotide exchange factors of the

plant-type Rho GTPase, the activation of NADPH oxidases and, thus, the generation of extracellular reactive oxygen species (ROS), and an increase in intracellular Ca²⁺ levels (Duan et al., 2010, 2014; Denness et al., 2011; Boisson-Dernier et al., 2013). Depending on the cellular context, this can have various consequences for cell elongation and cellular integrity (see below). The function of CrRLK1L family proteins has been discussed by excellent recent reviews (Boisson-Dernier et al., 2011; Cheung and Wu, 2011; Lindner et al., 2012a). Here, we concentrate on new perspectives and questions that have been raised by several exciting recent studies.

FER FUNCTIONS

FER Controls Hydroxyl Radical Production by the Female Gametophyte, Which Induces Rupture of the Pollen Tube

FER is expressed in most tissues except for the male gametophyte. In the female gametophyte, FER is highly expressed, in particular in the synergid cells that line the ovular aperture, consistent with its role in inducing pollen tube rupture. Recently, Duan et al. (2014) investigated the molecular mechanism of this process. They confirmed previous observations (Martin et al., 2013) that ROS transiently accumulate at the so-called filiform apparatus, a domain in synergid cells with abundant membrane protrusions at the entrance of the female gametophyte. ROS accumulation depends on plasma membrane-associated NADPH-oxidase activity, correlates in space and time with FER protein accumulation (as shown with a FER-GFP fusion), and is

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Table 1. Overview of CrRLK1L Family Member Mutant Phenotypes

Allele	Phenotype	Mutation	Ecotype	Reference
FER				
<i>fer</i>	Pollen tube overgrowth, multiple pollen tubes, reduced stature, increased cell death, increased ROS burst and MAPK activation upon <i>flg22</i> in leaves, reduced bacterial proliferation, resistant to powdery mildew, defective NTA localization	4-bp insertion, frame shift Ds transposon	<i>Ler</i>	Huck et al. (2003) ; Escobar-Restrepo et al. (2007); Keinath et al. (2010) Kessler et al. (2010)
<i>sm/sir</i>	Pollen tube overgrowth, multiple pollen tubes, defective root hairs, larger seeds, bigger cotyledons, reduced ROS in female gametophyte, hypersensitive to ABA, autonomous endosperm development	1-bp deletion, frame shift γ -Ray irradiation	C24	Rotman et al. (2003) Escobar-Restrepo et al. (2007); Rotman et al. (2008); Duan et al. (2010, 2014); Yu et al. (2014)
<i>fer-2</i>	Reduced hypocotyl length, brassinosteroid insensitivity, ethylene hypersensitivity	tDNA insertion Activation tagging	Col-0	Deslauriers and Larsen (2010)
<i>fer-3</i>	Reduced hypocotyl length	tDNA insertion in 5' part	Col-0	Deslauriers and Larsen (2010)
<i>fer-4</i>	Reduced stature, defective root hairs, defective trichomes, reduced fertility, reduced auxin and RAC/ROP signaling, reduced ROS in roots/female gametophyte, larger seeds, bigger cotyledons, RALF insensitive, longer root length under blue light, increased capacity to acidify medium, hypersensitive to LiCl, pollen tube overgrowth, reduced ROS in female gametophyte, hypersensitive to ABA	tDNA insertion in 5' part	Col-0	Duan et al. (2010) ; Yu et al. (2012, 2014); Huang et al. (2013); Duan et al. (2014); Haruta et al. (2014)
<i>fer-5</i>	Reduced stature, defective root hairs, reduced auxin and RAC/ROP signaling, reduced ROS in roots/female gametophyte, partially insensitive to RALF, hypersensitive to ABA	tDNA insertion in 3' part	Col-0	Duan et al. (2010) Haruta et al. (2014) Yu et al. (2014)
<i>fer amiRNA</i>	Reduced growth, rounded leaves, reduced cell elongation in hypocotyl and petioles	amiRNA under control of <i>BRI1</i> promoter	Col-0	Guo et al. (2009b)
THE1				
<i>the1-1</i>	Suppresses growth defect and ectopic lignification of <i>prc1-1</i> , reverts gene expression changes in <i>prc1-1</i>	G110A EMS allele	<i>prc1-1</i> (Col-0)	Hématy et al. (2007) Denness et al. (2011)
<i>the1-2</i>	Suppresses growth defect of <i>prc1-1</i>	G448A EMS allele	<i>prc1-1</i> (Col-0)	Hématy et al. (2007)
<i>the1-3</i>	Suppresses growth defect and ectopic lignification of several cell wall-damaging mutants, reverts gene expression changes in <i>prc1-8</i>	tDNA	Ws	Hématy et al. (2007)
<i>the1-4</i>	Reduced stature and cell elongation when combined with <i>herk</i> , enhances <i>bri1-5</i> when combined with <i>herk1</i> , suppresses <i>bes1-1D</i> when combined with <i>herk1</i> , altered gene expression when combined with <i>herk1</i>	tDNA	Col-0	Guo et al. (2009b)
ANX1/2				
<i>anx1,2</i>	Reduced fertility, pollen tube burst	tDNAs	Col-0	Miyazaki et al. (2009)
<i>anx1-2, 2-2</i>	Reduced fertility, pollen tube burst	tDNAs	Col-0	Boisson-Dernier et al. (2009) ; Boisson-Dernier et al. (2013)

(Continued)

not observed in *fer* mutants. Using elegant assay systems (a pistil feeding assay and a semi-in vivo pollen tube growth system), the authors show that promoting superoxide production, adding H₂O₂ or promoting \cdot OH formation triggers rapid pollen tube bursting.

Inversely, the pharmacological inhibition of NADPH oxidase activity (which produces superoxide) as well as the scavenging of H₂O₂ or, most significantly, \cdot OH in the female gametophyte prevents pollen tube bursting. These results indicate that superoxide

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Table 1. (continued).

Allele	Phenotype	Mutation	Ecotype	Reference
HERK1/2				
<i>herk1</i>	Reduced stature and cell elongation when combined with <i>the1-4</i> , enhances <i>bri1-5</i> when combined with <i>the1-4</i> , suppresses <i>bes1-1D</i> when combined with <i>the1-4</i> , altered gene expression when combined with <i>the1-4</i>	tDNA	Col-0	Guo et al. (2009b)
<i>herk2</i>	Reduced stature when combined with <i>herk1</i> and <i>the1-4</i>			Guo et al. (2009a)
ERULUS				
<i>eru1</i>	Defective root hairs	tDNA	Col-0	Haruta et al. (2014)
<i>eru2</i>	Defective root hairs	tDNA	Col-0	Haruta et al. (2014)

References in bold denote first description of the respective mutant alleles. Col-0, Columbia-0; *Ler*, Landsberg *erecta*; *Ws*, Wassilewskija; amiRNA, artificial microRNA.

or H₂O₂ primarily act via their conversion into hydroxyl radicals, which therefore are the most likely reactive oxygen species to induce pollen tube rupture in this context. Since ·OH radicals are very short-lived (the average diffusion distance is <10 nm; Roots and Okada, 1975), they would constitute an effective messenger to signal the close proximity of the pollen tube to its target. The identity of the female gametophyte-expressed NADPH-oxidase(s) responsible for FER-dependent ROS production remains to be determined. Finally, the authors show that Ca²⁺ influx into the pollen tube is induced by ROS application and is required for ROS-induced bursting. It is intriguing in this context that *rboh*-deficient mutant pollen tubes (see below; Boisson-Dernier et al., 2013; Lassig et al., 2014) and root hairs (Duan et al., 2010) with low internal levels of ROS also display loss of integrity. It is possible, however, that the tip-localized bursting of the pollen tube and the subsequent extrusion of the sperm cells involves a specialized process that is distinct from that underlying the premature loss of integrity of the pollen tube observed in *rboh*-deficient mutants.

The exact link between ·OH production and the observed Ca²⁺ influx and bursting during fertilization is not clear. Is the hydroxyl radical acting directly as a Ca²⁺ channel agonist, or is it acting indirectly through its loosening effect on cell walls? Previous work has shown that ·OH radicals are generated from H₂O₂ in the cell wall in the presence of trace elements of transition metals, such as copper or iron, through Fenton chemistry. These radicals can very locally cleave polysaccharides and promote wall relaxation (Fry, 1998; Fry et al., 2001; Schopfer, 2001). In this scenario, ·OH-mediated wall weakening might facilitate bursting once the pollen tube enters the presumably Ca²⁺-rich environment of the synergid cytoplasm, perhaps combined with the opening of stretch-activated Ca²⁺ channels in the pollen tube. It should be noted that ·OH-mediated cell wall loosening and peroxidase-mediated tightening (e.g., through tyrosine cross-linking of wall proteins or phenolic acids) would compete for available H₂O₂. ·OH radicals in principle should be produced in most cell

walls, given the fact that most walls contain sufficient copper or iron and that very high amounts of scavenging solutes (e.g., cysteine or salicylate) are needed to remove ·OH completely. This raises the possibility that the balance between the growth-inhibiting or -promoting effect of ROS production depends on the peroxidase activity in the wall, perhaps as part of a complex with the NADPH-oxidase as observed during the formation of the Casparian strip in the endodermis (Lee et al., 2013). In this context, it will be interesting to investigate peroxidase activity in synergid cells during fertilization.

FER Is a RALF Receptor, Causing Cell Wall Alkalinization

Another recent study sheds a new and unexpected light on the role of FER (Haruta et al., 2014). The authors show that FER is a receptor for Rapid Alkalinization Factor 1 (RALF1), a 49-amino acid peptide that had been identified in tobacco (*Nicotiana tabacum*) leaf extracts by its ability to induce the rapid alkalinization of the culture medium of tobacco suspension-cultured cells (Pearce et al., 2001). RALF-like peptides are conserved among land plants, including mosses and lycophytes. In *Arabidopsis*, RALF1 is released from a 120-amino acid pre-pro-peptide by proteolytic processing (Matos et al., 2008) and overexpression or external application of the peptide results in growth inhibition (Bergonci et al., 2014; Haruta et al., 2014). In their quest for the mode of action of RALF1, Haruta et al. used an elegant quantitative phosphoproteomics approach to monitor early changes in protein phosphorylation in *Arabidopsis* seedlings treated with epitope-tagged RALF1. Within 5 min of RALF1 addition, a 2.5-fold or greater change in abundance in phosphopeptides was observed for five proteins, including FER, which was phosphorylated at three positions in the C terminus of the kinase domain.

The hypothesis that FER plays a role in RALF1 signaling was corroborated by the absence of RALF1-induced root growth inhibition in the loss-of-function mutant *fer-4* and the reduced

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response in the hypomorphic mutant *fer-5*. Another RALF1-induced immediate response, the transient increase of cytosolic Ca^{2+} , was also impaired in *fer-4*. The H^+ -ATPase AHA2 was also among the RALF1-induced phosphorylated proteins, however, with a phosphorylation site that is suggested to promote activity (Rudashevskaya et al., 2012), indicating that more work is needed to elucidate the mechanism of the proposed FER-mediated inhibition of ATPase activity. Moreover, it remains to be seen whether the activated FER kinase directly phosphorylates the H^+ -ATPase or whether another protein kinase is involved, for instance, the Ca^{2+} -dependent protein kinase 9 (CPK9) that was also found to be phosphorylated upon RALF1 treatment. Finally, the authors show that FER is a bona fide RALF1 receptor by demonstrating the binding of RALF1 to FER produced in tobacco and to the FER ectodomain produced as a fusion protein in *Escherichia coli*.

Other CrRLK1L Family Members Also Play a Role in Growth Control

In addition to FER, several other CrRLK1L family members have been studied and shown to play a role in the control of cell expansion: ANXUR1 (ANX1), ANX2, THESEUS1 (THE1), HERKULES1 (HERK1), and HERK2.

ANX1 and 2 are pollen-specific RLKs that redundantly control polar growth of pollen tubes. Double mutant *anx1/2* pollen tubes burst prematurely, whereas *ANX1* overexpression causes pollen tube growth inhibition and the overaccumulation of secreted wall material (Boisson-Dernier et al., 2009, 2013; Miyazaki et al., 2009). Genetic analysis shows that ANX1-induced growth inhibition requires the activity of the partially redundant NADPH oxidases RbohH and RbohJ, which are responsible for ROS production close to the pollen tube tip (Boisson-Dernier et al., 2013). Both enzymes are located in the pollen tube tip and are activated by intracellular Ca^{2+} through direct binding (to a cytosolic EF hand motif) and Ca^{2+} -dependent phosphorylation (Kaya et al., 2014). A recent report used kinematic analysis to study the link between Ca^{2+} -induced NADPH oxidase activation, cell wall deposition, and growth control. Whereas wild-type pollen tubes in general elongate with a constant growth rate, *rbohH/j* mutants typically show oscillatory growth patterns, with rates exceeding that of the wild type, followed by growth arrest (Lassig et al., 2014). Interestingly, in *rbohH/j*, exocytosis of wall material, in particular pectate, appears to precede the growth burst, whereas intracellular Ca^{2+} levels peak together with, or slightly after, the growth peak (Lassig et al., 2014). These results are consistent with previous observations (brilliantly reviewed in Winship et al., 2011) showing that pectate deposition may have a cell wall-loosening and growth-promoting effect through the chelation of Ca^{2+} from load-bearing Ca^{2+} -pectate cross-links (Proseus and Boyer, 2006, 2008, 2012; Rojas et al., 2011). In addition, the results indicate that the growth-dependent Ca^{2+} increase may be part of a feedback mechanism that dampens growth oscillations in wild-type pollen tubes through the

activation of NADPH oxidases and ROS-dependent growth inhibition, thereby coordinating growth rate and exocytosis rate. ANX1/2 may impinge on this feedback loop through the promotion of NADPH oxidase activity either directly or indirectly for instance by promoting cytosolic Ca^{2+} increase.

THE1 was identified through a genetic screen for suppressors of reduced growth in the cellulose-synthase deficient mutant *cesa6^{prc1-1}* (Hématy et al., 2007). Strikingly, *the1* mutants do not restore cellulose content itself but diminish the secondary effects of reduced cellulose synthesis such as ROS production, growth inhibition, ectopic lignin accumulation, expression of stress-related genes, and the synthesis of defense compounds. In the absence of cell wall damage, *the1* mutants do not show an apparent phenotype; hence, THE1 is thought to sense the integrity of the cell wall and trigger ROS-mediated growth inhibition (Hématy et al., 2007; Denness et al., 2011). Another family member, HERK1, is thought to be partially redundant with THE1 in the absence of cell wall damage, as shown by the elongation defects in leaves and petioles of double mutants (Guo et al., 2009). The phenotype of these double mutants is very similar to that of *fer*, suggesting a role of the three RLKs in a common pathway. Finally, based on the gene expression patterns, other family members may have potential roles in responses to abiotic or biotic stresses (Lindner et al., 2012a).

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Open Questions on CrRLK1L Signaling

The identification of RALF1 as a FER ligand raises the question how the signal is transduced. Exposure to RALF1 leads to a rapid FER-dependent intracellular Ca^{2+} increase (Haruta et al., 2014). However, it is at present unclear whether this is mediated by RAC/ROP signaling or whether it depends on RALF1-induced, NADPH oxidase-dependent ROS production.

While extracellular signals are often linked to cell reprogramming involving transcription factor targets, it is at present unclear whether there is a direct, dedicated pathway from FER or other CrRLK1Ls to transcription factors. The transcriptome of plants treated with RALF1 has been analyzed (Haruta et al., 2014), but it would be interesting to know which proportion of the gene expression changes compared with the wild type are dependent on FER, AHA2, and NADPH oxidases, for example.

A number of additional proteins have been identified, which might be involved in CrRLK1L signaling pathways. For example, NORTIA (NTA), a member of the MLO family, was discovered in a screen for *fer*-like pollen tube overgrowth phenotypes (Kessler et al., 2010). Remarkably, NTA becomes polarly localized in synergid cells upon pollen tube arrival, and this relocalization strictly depends on the presence of FER, suggesting that the two proteins act together in pollen tube reception, possibly in the same complex. Other mutants also cause a pollen tube phenotype similar to that of *fer*, such as mutants for the

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glycosyl-phosphatidylinositol-anchored protein LORELEI (LRE) and the UDP-glycosyltransferase TURAN (Capron et al., 2008; Tsukamoto et al., 2010; Lindner et al., 2012b). Like FER, LRE was shown to bind the small GTPase ROP2 and to be required for the production of female gametophytic ROS, suggesting that FER and LRE act in the same pathway presumably through a transient ROP2 signaling complex (Duan et al., 2014). Furthermore, a similar phenotype was discovered in the mutant *abstinence by mutual consent* (*amc*), in which a protein with homology to yeast PEROXIN13 (PEX13) is affected (Boisson-Dernier et al., 2008). PEX13 is involved in peroxisomal protein import, which might suggest a link to the generation or scavenging of ROS. However, as the name suggest, *amc* differs from the above-mentioned mutants in that the mutant allele has to be present in both male and female gametophyte (Boisson-Dernier et al., 2008) to elicit an otherwise identical phenotype.

CrRLK1L Proteins with Multiple or Complex Ligands?

Based on the cellular phenotypes of *anx1,2* and *fer* mutants and the nature of the screen that led to the identification of *THE1*, it was originally suggested that CrRLK1L proteins might be sensing the cell wall state by interacting with extracellular carbohydrates. This is also suggested by the presence of GFP-tagged THE1 in so-called Hechtian strands that connect the protoplast to the cell wall of plasmolyzed cells. The discovery of malectin-like domains in CrRLK1Ls further supported this assumption (Boisson-Dernier et al., 2011). Indeed, malectin is a carbohydrate binding protein originally discovered in *Xenopus laevis*, whose homologs in mammals are involved in endoplasmic reticulum quality control through interaction with *N*-linked glycans (Schallus et al., 2008; Galli et al., 2011). The discovery that RALF1 binds to FER thus challenges the common view that a given receptor is matched with precisely one ligand. It is not known with which part of the 400-amino acid FER ecto-domain RALF1 interacts, and it is certainly possible that binding occurs outside of the two ~165-amino acid malectin-like domains. Furthermore, it is conceivable that the malectin domains function differently in plants and animals since the observed sequence conservation is only limited (Boisson-Dernier et al., 2011). What seems clear, however, is that glycosylation does not play a role in RALF1 function since (1) the mature peptide lacks predicted *N*-linked glycosylation sites, and (2) RALF1 peptide produced in *E. coli*, and thus in the absence of glycosylation, was biologically active and able to bind to FER (Haruta et al., 2014). Taken together, this opens the interesting possibility that CrRLK1Ls might have more than one ligand, as frequently observed with mammalian receptor proteins (Noy, 2007; Pi and Quarles, 2012). Alternatively, the malectin domain could function in tethering the proteins to the extracellular matrix, which could be tested for instance with fluorescence recovery after photobleaching experiments (Martinière et al., 2012).

Reconciliation of Cellular and Organismal Phenotypes

Possibly related to our incomplete understanding of CrRLK1L downstream signaling, it is challenging to reconcile the presumed role of these proteins at the cellular level with the phenotypes observed in mutant plants. For example, in pollen tubes and in roots, FER seems to have an inhibiting effect on cell elongation, whereas the phenotype of numerous *fer* mutants seems to suggest a positive effect on growth (Guo et al., 2009; Deslauriers and Larsen, 2010; Duan et al., 2010; Kessler et al., 2010) (see also Table 1). However, this discrepancy is also observed with well-characterized growth promoting agents, e.g., auxin or brassinosteroids, that have growth-inhibiting effects depending on their concentration and the tissue in question. Therefore, the capacity to promote growth is certainly context dependent. In addition, one of the immediate results of CrRLK1L activation is the generation of ROS, which can be rapidly inter-converted and can also have opposing effects depending on the precise species produced. As an alternative explanation for the apparent incongruity of the different *fer* phenotypes in light of the new evidence for FER being a negative regulator of growth (Haruta et al., 2014), reduced stature of *fer* mutants might be a secondary effect on development, perhaps through an imbalance in ROS production, increased cell death (Keinath et al., 2010), or hyperpolarization of the plasma membrane (Haruta et al., 2014). Thus, despite the recent advances, more insight into CrRLK1L signaling is needed to understand the biological functions of these receptors.

Regulation of RALFs

In light of the findings by Haruta et al. (2014), attempts to understand the physiological role of FER, and possibly other CrRLK1Ls, must take into account the observation that RALF family members are subject to complex regulation. Unfortunately, only 18 of at least 34 family members are represented on the commonly used ATH1 gene chip. But among those few, interesting patterns of expression can be found in public databases. Several *Arabidopsis* RALF peptide genes (*RALFL4*, *RALFL12*, *RALFL17*, *RALFL25*, and *RALFL26*) are strongly and specifically expressed in pollen where they potentially play a role in growth control (Covey et al., 2010). These peptides are candidate ligands for ANX1 and 2. Interestingly, expression of several members of the RALF family is also increased upon pollen–pistil interaction, which would be in line with a role in pollen tube integrity control or reception (Boavida et al., 2011). With respect to the latter process, it is interesting to note that according to a recent study, at least two RALFs are specifically expressed in synergid cells (*RALFL14* and *RALFL18*; Wuest et al., 2010) where FER exerts its pollen tube reception control.

Environmental, hormonal, and defense-related cues also affect RALF transcript abundance. Germination and abscisic acid (ABA) treatment induce a number of RALF members, whereas the expression of others is reduced by the same conditions.

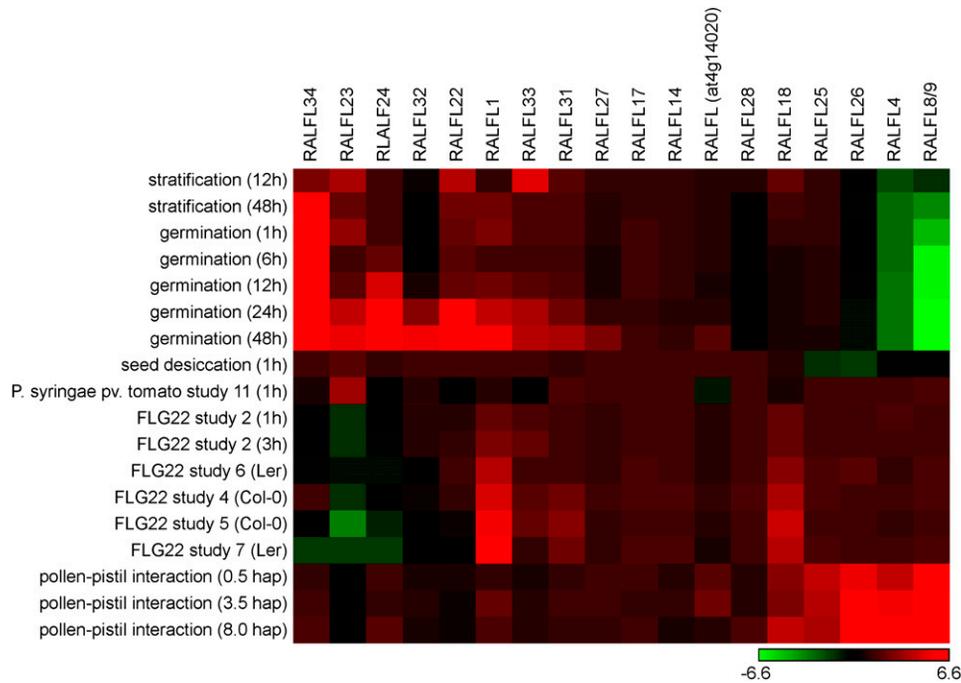


Figure 1. Cluster Analysis of RALF-Like Peptide Expression under Influence of Selected Stimuli.

A heat map was generated using matrix2png (<http://www.chibi.ubc.ca/matrix2png/bin/matrix2png.cgi>) and data extracted from public databases with the Genevestigator tool (Hruz et al., 2008). Color code indicates fold change in expression compared with control.

Some RALFs, including RALF1, are upregulated upon treatment with the bacterial elicitor flg22 (Figure 1). Interestingly, FER mutants appear to be indistinguishable from the wild type with respect to flg22-induced growth inhibition (Haruta et al., 2014), but have a strongly increased response to the elicitor in terms of ROS production and mitogen-activated protein kinase (MAPK)

activation (Keinath et al., 2010), also suggesting a link between RALF1 and elicitor responses.

Another interesting aspect of RALF regulation concerns the maturation of the pro-peptide into the active form. RALFs have conserved basic amino acid motifs, which suggest potential processing by members of the subtilase protease family. Indeed,

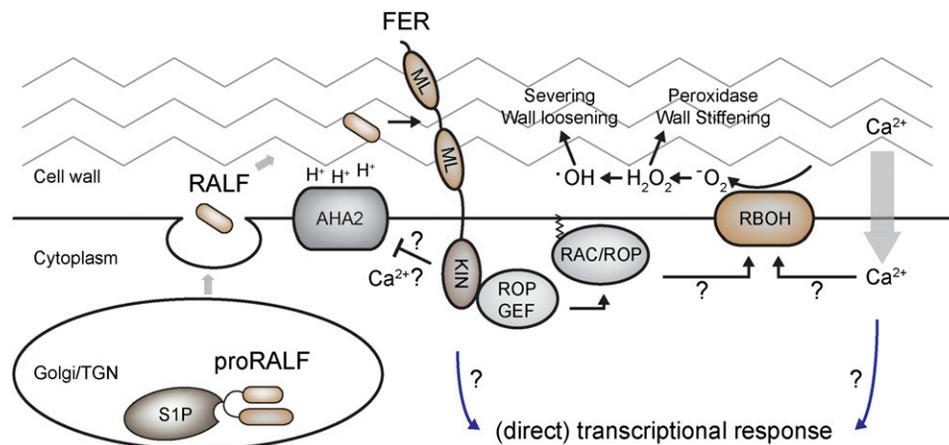


Figure 2. Schematic Representation of FER/RALF1 Signaling Components.

Speculative steps are indicated by question marks. See text for explanations.

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RALF23 is processed by Site-1 protease (S1P) at the motif RRIL (Srivastava et al., 2009). Intriguingly, the same motif is present in the FER ligand RALF1 and was shown to be required for RALF1 processing (Matos et al., 2008). S1P is localized in the Golgi apparatus and possibly also in other endomembrane compartments, such as the *trans*-Golgi network, but seems to be absent from the plasma membrane (Liu et al., 2007). This raises the question whether RALF processing occurs “per default” or whether the activity of S1P (or that of other subtilases putatively involved in RALF processing) is somehow controlled, adding another layer of regulation to this growth controlling module.

As pointed out above, CrRLK1L/RALF function seems to be associated with cell wall integrity maintenance. In this respect, it is interesting to note that at least RALF4 is highly coregulated with pectin modifying enzymes (our unpublished data). Around 20% of the primary cell wall is homogalacturonan, the most abundant pectin species. Homogalacturonan is synthesized and secreted in an almost entirely methyl-esterified state, but can be selectively demethylated with a high spatio-temporal resolution by enzymes called pectin methyl esterases (PMEs), a process that is associated with growth transitions (Peaucelle et al., 2008; Pelletier et al., 2010). As PMEs have an alkaline pH optimum, and the removal of methyl groups leads to cell wall acidification and thus PME inhibition, it is possible that RALF receptor activation participates in pH homeostasis of the cell wall during growth.

Intriguingly, S1P not only processes RALFs as noted above, but also mediates the proteolytic processing of one type of PMEs, which is a prerequisite for their secretion to the apoplast (Wolf et al., 2009), again suggesting complex networks involving CrRLK1L/RALF signaling for the regulation of cell wall maintenance.

CONCLUSION

The CrRLK1L receptor family is involved in the regulation of cell expansion and cell-to-cell communication. At least some of the family members promote ROS production, which may be translated into wall loosening or wall stiffening depending on the ROS produced and the composition of the wall. We now know that FER is a receptor for a signaling peptide, RALF1, that induces Ca²⁺ influx and alkalization of the apoplast through the inhibition of the H⁺-ATPase (summarized in Figure 2). This discovery opens up the possibility that other CrRLK1L family members may also be regulated by RALF-like peptides. The identification of the ligands for these receptors, for instance, using the approaches discussed in the accompanying Perspective (Butenko et al., 2014), should provide new insights into how various endogenous and environmental signals are translated into growth-controlling changes in cell wall properties.

AUTHOR CONTRIBUTIONS

S.W. and H.H. conceived and wrote the article.

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