REVIEW PAPER

The walls have ears: the role of plant CrRLK1Ls in sensing and transducing extracellular signals

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Abstract

In plants, organ formation and cell elongation require the constant adjustment of the dynamic and adaptable cell wall in response to environmental cues as well as internal regulators, such as light, mechanical stresses, pathogen attacks, phytohormones, and other signaling molecules. The molecular mechanisms that perceive these cues and translate them into cellular responses to maintain integrity and remodelling of the carbohydrate-rich cell wall for the coordination of cell growth are still poorly understood. In the last 3 years, the function of six membrane-localized receptor-like kinases (RLKs) belonging to the CrRLK1L family has been linked to the control of cell elongation in vegetative and reproductive development. Moreover, the presence of putative carbohydrate-binding domains in the extracellular domains of these CrRLK1Ls makes this receptor family an excellent candidate for coordinating cell growth, cell–cell communication, and constant cell wall remodelling during the plant life cycle.

Key words: Arabidopsis, cell wall, CrRLK1L, malectin-like domain, plant reproductive development, pollen tube reception, receptor-like kinase, signaling.

Introduction

Plant cells are surrounded by an extracellular matrix known as the cell wall. Cell walls are composed of a variety of carbohydrate polymers (including cellulose, hemicellulose, and pectins), and associated structural and regulatory proteins (Cosgrove, 2005). The rigid cell wall is responsible for resisting the high turgor pressure in plant cells and also provides protection against influences from the environment. Cell wall modifications are required for plant growth and development. The loosening of cell wall components determines the direction of growth and, as cells expand, new wall components must be synthesized in a coordinated manner. For example, during pollination, the pollen tube (PT) must sense signals from the surrounding transmitting tract of the pistil and coordinate cell wall modifications so that the sperm cells can be carried towards the ovules by polarized tip growth. When the PT reaches the ovary, it must change its direction of growth in order to enter the ovule and, finally, the PT must perceive a signal to alter its cell wall properties in order to cease its growth and rupture to release the sperm cells. Signaling between the cell wall and the inside of the cell is critical for proper development and the sensing of environmental cues, but little is known about the molecular mechanisms involved (Ringli, 2010; Seifert and Blaukopf, 2010). The plant-specific Catharanthus roseus RLK1-like (CrRLK1L) family, named after the first member which was identified from C. roseus cell cultures (Schulze-Muth et al., 1996), has recently emerged as a candidate group for sensing changes at the cell wall and translating this information to cellular responses during both the reproductive and vegetative phases of the plant life cycle. The model plant Arabidopsis thaliana has 17 CrRLK1L family members (Hématy and Höfte, 2008). In this review, the functions of a subset of the CrRLK1L-encoding genes (Table 1) are explored and the significance of putative carbohydrate-binding domains in their extracellular domains is discussed.
During plant reproduction, the tip-growing PT grows through the female reproductive tissue to reach the female gametophyte (FG). Communication between the male and female occurs at several steps during reproduction (Dresselhaus, 2006). First, a pollen grain lands on a receptive stigma and hydrates, extending a PT that carries the two sperm cells towards the ovary. Next, the PT grows through the transmitting tract, absorbing nutrients from female tissues, from which the PT finally exits and is attracted to the FG.

### Table 1. The six characterized Arabidopsis CrRLK1Ls have been proposed to function in cell growth regulation

<table>
<thead>
<tr>
<th>Name</th>
<th>AGI</th>
<th>Expression pattern</th>
<th>Subcellular localization</th>
<th>Kinase activity</th>
<th>KO or KD mutant phenotypes</th>
<th>OX phenotypes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>THE1</td>
<td>At5g54380</td>
<td>• All vegetative tissues and more particularly in petioles, hypocotyls and root elongation zone</td>
<td>THE1–GFP, functional, uniform PM signal in hypocotyl epidermal cells</td>
<td>Yes</td>
<td>• the1 no obvious phenotypes</td>
<td>• p35S::THE1–GFP</td>
<td>a, b, c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Up-regulated by BL, down-regulated in brn1-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FER</td>
<td>At3g51550</td>
<td>• All vegetative tissues, more particularly in petioles and hypocotyls</td>
<td>FER–GFP, functional, uniform PM signal in leaf epidermis, but polarly localized towards the FA of synergids</td>
<td>Yes</td>
<td>• fer or amiRNA-FER lines very strong stunted phenotypes</td>
<td>• ND</td>
<td>b, c, d, e, f</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Strong in the synergids of the embryo sac</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Up-regulated by BL or in bes1-D, down-regulated in brn1-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HERK1</td>
<td>At3g46290</td>
<td>• All vegetative tissues and more particularly in petioles and hypocotyls</td>
<td>HERK1–GFP, functionality not demonstrated, uniform PM signal in hypocotyl epidermal cells</td>
<td>Yes</td>
<td>• herk1 the1 strong stunted phenotypes</td>
<td>• p35Se-HERK1::HERK1</td>
<td>b, c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Up-regulated by BL or in bes1-D, down-regulated in brn1-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HERK2</td>
<td>At1g30570</td>
<td>• Slightly up-regulated by BL</td>
<td>ND</td>
<td>ND</td>
<td>• herk1 thes1 strong stunted phenotypes</td>
<td>• ND</td>
<td>c</td>
</tr>
<tr>
<td>ANX1</td>
<td>At3g04690</td>
<td>• Preferential expression in pollen</td>
<td>ANX1/2–YFP, functional, polarly enriched in PM of the PT tip</td>
<td>ND</td>
<td>• single anx1 or anx2 no obvious phenotypes</td>
<td>• pACA9::ANX1/2–YFP</td>
<td>g, h, i</td>
</tr>
<tr>
<td>ANX2</td>
<td>At5g28680</td>
<td></td>
<td></td>
<td></td>
<td>• anx1 anx2 PTs burst prematurely before reaching the embryo sacs</td>
<td>• inhibits PT growth in WT</td>
<td></td>
</tr>
</tbody>
</table>

BL, brassinolide; FA, filiform apparatus; KD, knock-down; KO, knock-out; ND, not determined; PM, plasma membrane; PT, pollen tube; OX, overexpression; 35S, CaMV 35S promoter; 35Se, CaMV 35S enhancers; WT, wild type.

CrRLK1Ls in reproductive development

FERONIA in the female gametophyte

During plant reproduction, the tip-growing PT grows through the female reproductive tissue to reach the female gametophyte (FG). Communication between the male and female occurs at several steps during reproduction (Dresselhaus, 2006). First, a pollen grain lands on a receptive stigma and hydrates, extending a PT that carries the two sperm cells towards the ovary. Next, the PT grows through the transmitting tract, absorbing nutrients from female tissues, from which the PT finally exits and is attracted to the FG.
Attracted by a chemotactic signal (Okuda et al., 2009), the PT enters one of the synergids, stops growth, and bursts to release the two sperm cells to effect double fertilization. The CrRLK1L family member FERONIA (FER) plays a key role in PT reception, the final step of communication between the male and female gametophytes. In fer mutant FGs, the PT is attracted and penetrates the receptive synergid, but continues to grow and fails to rupture and release the sperm cells (Huck et al., 2003), a phenotype also observed in sircéne mutants, which are alleleic to fer (Rotman et al., 2003; Escobar-Restrepo et al., 2007). A FER–green fluorescent protein (GFP) fusion protein that complements the fer mutation is localized to the plasma membrane in leaf epidermal cells, but, more importantly for its role in fertilization, FER–GFP is localized at the micropylar pole of synergid cells, a region known as the filiform apparatus (FA) (Escobar-Restrepo et al., 2007). The FA is made up of a highly invaginated plasma membrane and a thickened cell wall, and constitutes the entry point of PTs into the FG (Higashiyama, 2002). FER could either act as receptor for a specific ligand from the PT, or could sense and respond to release the sperm cells (Huck et al., 2003). Alternatively, it has been proposed that the final step of communication between the male and female gametophytes is the role in PT reception, the proper maturation of the synergids, allowing them to interact (Rotman et al., 2008). However, this hypothesis does not explain why fer-like phenotypes are observed in some interspecific crosses (see below), where the synergids are wild type and fully mature.

Interspecific crosses between A. thaliana and A. lyrata or Cardamine flexuosa exhibit defects in PT reception similar to those observed in fer mutants (Escobar-Restrepo et al., 2007). The extracellular domains of FER in these species exhibit high sequence variation that could indicate that FER may recognize species-specific ligands. Recently, three other mutants with fer-like phenotypes have been reported. scylla (unidentified; Rotman et al., 2008), lorelei [lre; a putative glycosylphosphatidylinositol (GPI)-anchored protein; Capron et al., 2008; Tsukamoto et al., 2010], and nortia [ntr; a mildew resistance locus o (MLO) family member; Kessler et al., 2010] are all female gametophytic mutants that affect PT reception, exhibiting fer-like PT overgrowth inside the FG. An NTA–GFP fusion protein becomes polarly localized to the filiform apparatus at PT arrival, but this polar localization does not occur in fer mutant FGs, indicating that an active FER pathway required for sensing PT arrival is necessary for the redistribution of NTA–GFP (Kessler et al., 2010). The mechanism through which FER, NTA, LRE, and other components cooperate in the synergid to control growth of the PT as it arrives at the FG remains elusive (Fig. 1). However, recent studies by two independent research groups have shed some new light on this intriguing male–female gametophytic communication by characterizing the functionally redundant CrRLK1Ls ANXUR1 (ANX1) and ANX2, which are required for normal PT growth (Boisson-Dernier et al., 2009; Miyazaki et al., 2009). ANXI and ANX2 were analysed based on their preferential expression in pollen and/or their high sequence similarity to FER. Indeed, they are most closely related to FER in the Arabidopsis genome, and while FER is expressed everywhere in the plant except for pollen, ANXI and ANX2 display the exact opposite expression pattern (Boisson-Dernier et al., 2009; Miyazaki et al., 2009). PTs carrying T-DNA insertions in both ANXI and ANX2 are able to germinate but burst prematurely in vitro and in vivo, preventing them from reaching and fertilizing the FG (Boisson-Dernier et al., 2009; Miyazaki et al., 2009). Consequently, double homozygous anx1 anx2 mutant plants were only rarely found in the progeny of selfed anx1/anx1 anx2/ANX2, or anx1/ANXI anx2/lanx2 plants and were almost completely male sterile. Interestingly, ANXI–yellow fluorescent protein (YFP) and ANX2–YFP fusions are polarly localized in the plasma membrane of the PT tip (Boisson-Dernier et al., 2009). Transforming anx1/lanx1 anx2/ANX2 plants with either an ANXI–YFP or an ANX2–YFP fusion under the control of a strong pollen promoter rescues the mutant phenotypes, indicating that these protein fusions are fully functional, while overexpressing them in wild-type PTs considerably slows PT growth (A. Boisson-Dernier and U. Grossniklaus, unpublished). Therefore, an appropriate threshold of functional ANX–RLK protein levels at the PT tip is required for PT growth, with an excess leading to growth inhibition while depletion impairs PT tip stability causing PT rupture.

The bursting of PTs in vitro is a common phenomenon as 2–10% of wild-type PTs rupture depending on the growth conditions. However, mutations that consistently lead to PT rupture are rare. Interestingly, anx1 anx2 mutant PTs display the same phenotype as PTs lacking the cell wall-localized pectin methylesterase (PME; EC 3.1.1.11) encoded by VANGUARD1 (VGD1; Jiang et al., 2005). PMEs catalyse the specific demethylesterification of the linear homopolymer (1,4)-linked-α-D-galacturonic acid homogalacturonan (HGA), a major pectic constituent of the cell wall (Pelloux et al., 2007). HGA is deposited in the apoplast through the secretory pathway in a predominantly methyl-esterified state (Mohnen, 2008). HGA is subsequently demethylesterified within the cell wall by PMEs, the activities of which are regulated by the cell wall’s pH, PME inhibitor (PMEI) proteins, or even intramolecularly by PME-like domains (Bosch et al., 2005; Juge, 2006; Rockel et al., 2008). Demethylesterified pectins are able to form Ca2+ bonds inducing gel formation that rigidifies the cell wall. Moreover, they are more accessible to degradation by pectinolytic enzymes, which affect cell wall composition and lead to the production of signaling molecules called oligogalacturonides. Therefore, the balance between methyl-esterified ‘loose’ and demethylesterified ‘rigid’ forms of HGA is essential for wall mechanics and must be tightly regulated during cell growth and development (Ridley et al., 2001; Willats et al., 2001; Pelloux et al., 2007; Hématy et al., 2009; Wolf et al., 2009). Thus, it is not surprising that many studies indicate a central role for asymmetrically distributed...
pectins, as well as PMEs and PMEIs during PT growth (Chebli and Geitmann, 2007; Cheung and Wu, 2008; Zonia and Munnik, 2009; Wolf et al., 2009).

Although the similar phenotypes of anx1 anx2 and vgd1 PTs could be merely circumstantial, the facts that (i) these mutant PTs consistently burst prematurely; (ii) ANX-RLKs and pollen-expressed PMEs/PMEIs are in close proximity to each other at the PT tip; and (iii) the synthesis, distribution, and degree of esterification of pectins play a major role in PT growth, indicate that ANX-RLKs and PMEs/PMEIs may cooperate during PT growth (Fig. 1).

For example, the ANX-RLKs could regulate the activity or the distribution of PMEs/PMEIs in PTs in order to readjust the balance of methylesterified and demethylesterified HGAs. Alternatively, HGAs might bind the extracellular domains of ANX-RLKs to regulate their activity and,}

Fig. 1. Molecular components that play a role in the male–female gametophytic dialogue during pollen tube reception. When the PT reaches the vicinity or comes into contact with the filiform apparatus of the receptive synergid, unknown pollen molecules and/or physical interactions between the two gametophytes are thought to signal the female gamete to prepare itself for penetration and fertilization through the activation of a female signaling module composed of the FA-localized FER-RLK, the putative GPI-anchored protein LRE, and the seven-transmembrane domain MLO protein, NTA. How these female components cooperate remains unclear, but FER activity is required to relocalize NTA to the membrane-enriched FA, the entry point for the PT. Subsequently, the FG signals back to its male partner so that it stops growth and ruptures to deliver the two sperm. This is achieved via synergid signals, such as the cysteine-rich small protein ZmES4, which triggers PT rupture possibly through an osmotic shock due to the opening of the PT-localized K+ channel KZM1 and subsequent K+ influx. Other male gametophytic components, such as the tip-polarized plasma membrane-localized ANX-RLKs or the cell wall-localized pectin methylesterase VGD1, promote PT growth within the female sporophytic tissues in order to prevent PT rupture before reaching the FG. However, unlike KZM1, it is still unclear whether the FG, via unknown synergid signals, is able to deactivate these male components to trigger PT growth arrest and sperm cell discharge. Finally, the PT-localized Ca2+ channel ACA9 appears to have a complex role by promoting both PT growth and sperm cell discharge. EC, egg cell; SY, synergids.
therefore, modulate the corresponding signaling cascade. In this respect, it is noteworthy that the extracellular regions of the RLKs WALL-ASSOCIATED KINASE1 (WAK1) and WAK2 have been shown to bind demethylsterified HGAs and that HGAs can regulate WAK2 activity (Kohorn et al., 2009). However, the extracellular regions of these RLKs share little similarity to those of the CrRLK1L family members.

For example, it is possible that cell wall-modifying enzymes are involved in triggering PT growth arrest and sperm discharge efficiently. The tip of the PT that grows into the FA would enable the FG to trigger PT growth arrest and sperm discharge efficiently. Therefore, disrupting the ANX-dependent pathway at the tip of the PT that grows into the FA would enable the FG to trigger PT growth arrest and sperm discharge efficiently. For example, it is possible that cell wall-modifying enzymes, such as pectin methylesterases, have a male-specific role in controlling PT growth through the female sporophytic tissue.

However, whether ANX-RLKs are also involved in the male–female gametophytic dialogue that controls PT reception, respectively, is still a matter of debate. The CrRLK1Ls and vegetative development

Recently, several members of the CrRLK1L family of RLKs have also been shown to be involved in controlling cell growth during different stages of vegetative development. Plant steroid hormones known as brassinosteroids (BRs) regulate cell elongation as well as many other developmental processes (Belkhadir and Chory, 2006). BR treatment induces cell elongation, while mutations in BR signaling components such as BRRI1 (BRASSINOSTEROID INSENSITIVE 1), the BR receptor, cause dwarf phenotypes. The CrRLK1L receptor kinases HERCULES RECEPTOR KINASE1 (HERK1), HERK2, THESEUS1 (THE1), and FER were all identified by microarray analysis as genes whose expression is induced by the BR pathway. Homozygous feronia mutant plants (Kessler et al., 2010), transgenic plants with induced down-regulation of FER by an artificial microRNA (amiRNA) (Guo et al., 2009a), double mutants between herk1 and the1, and herk1 herk2 the1 triple mutants (Guo et al., 2009b) all have stunted growth phenotypes with shorter petioles and hypocotyl cells than the wild type.

The interaction between CrRLK1L genes and the BR pathway was investigated to determine if the genes are acting in the same or in parallel pathways (Guo et al., 2009a). Triple mutants between herk1 the1 and the weak bril-5 loss-of-function allele have an enhanced bril dwarf phenotype, while the combination of herk1 the1 with bes1-D, a gain-of-function mutant with constitutive BR responses including excessive cell elongation, showed partial suppression of the cell elongation phenotype. This genetic analysis indicated that HERK1 and THE1 cooperate with the BR pathway to regulate cell elongation. Likewise, homozygous fer-2 loss-of-function mutants have recently been shown to modulate the BR response in a light-dependent manner (Deslauriers and Larsen, 2010). Hypocotyls of light-grown fer-2 seedlings showed an enhanced BR response, while dark-grown hypocotyls were partially BR insensitive and displayed an enhanced ethylene response.

Cell elongation requires modifications of the plant cell wall (Darley et al., 2001). BR-induced cell elongation is thought to occur through the up-regulation of cell wall-loosening enzymes (Becnel et al., 2006; Palusa et al., 2007; Darley et al., 2001). Consistent with a role for HERK1 and THE1 in cell elongation, the transcription of cell wall-loosening enzymes, including expansin, pectin lyase-like, and xyloglucan endotransglycosylase/hydrolase genes, is down-regulated in a herk1 the1 double mutant (Guo et al., 2009a). However, the function of CrRLK1Ls is not simply to modify cell walls and induce cell elongation. The the1 mutant was originally identified as a suppressor of procuste1 (prcl), a mutation in the cellulose synthase gene CESAs6 (Hématy et al., 2007). The prcl mutants are cellulose deficient and have short hypocotyls when grown in the dark, while prcl the1 double mutants have hypocotyl lengths intermediate between the wild type and prcl, but the same cellulose deficiency as prcl mutants. The short hypocotyls and dwarf phenotypes of these other cellulose-deficient mutants,
cesA<sup>eli1-1</sup> and <i>pompon1</i>, are also partially suppressed by <i>the1</i>. Moreover, overexpression of <i>THE1</i> in these mutant backgrounds enhanced their dwarf phenotype, indicating that, in cellulose-deficient backgrounds, <i>THE1</i> actually suppresses growth. However, in a wild-type background, overexpression of <i>THE1</i> has no apparent effect on vegetative growth.

The seemingly opposite phenotypic effects of <i>the1</i> in a cellulose-deficient background and in a double mutant background with <i>herk1</i> indicate that the function of these RLKs is probably dependent on the cell wall context. When cellulose levels are lower than normal in the ces<i>A</i> mutant background, <i>THE1</i> senses the abnormal or weak cell wall and inhibits growth. However, if the cell wall is intact, <i>THE1</i> (along with <i>HERK1</i> and/or <i>FER</i>) could participate in cell wall loosening to allow BR (and probably other pathways) to direct cell elongation.

A cell wall-sensing role for the CrRLK1L family members is further supported by the recent discovery of a function for <i>FER</i> in powdery mildew susceptibility (Kessler et al., 2010). The link between <i>FER</i> and fungal invasion arose from the identification of NTA as an MLO protein family member. MLO genes were first identified as powdery mildew susceptibility genes in barley (Buschges et al., 1997), with mutations in some MLO genes leading to powdery mildew resistance in barley, <i>Arabidopsis</i>, and tomato (Piffanelli et al., 2004; Consonni et al., 2006; Bai et al., 2008). Since <i>fer</i> and <i>nta</i> mutants displayed a similar PT reception phenotype (Kessler et al., 2010) and, unlike <i>NTA</i>, <i>FER</i> is also expressed in leaf epidermal cells, <i>fer</i> mutants were examined for powdery mildew resistance. Interestingly, homozygous <i>fer</i> mutants showed a similar powdery mildew resistance phenotype to that seen in <i>mlo</i> mutants. These results indicate that <i>FER</i> may act as a sensor of cell wall alterations due to contact with tip-growing cells and may coordinate the function of distinct MLO proteins in a tissue-dependent manner to allow entry of fungal hyphae and PTs, respectively.

### Putative ligands of the CrRLK1L family

The huge family of RLKs in plants is classified into 45 subfamilies that can be differentiated according to sequence similarity of their extracellular domains and their domain organization (Shiu and Bleecker, 2003). Although the presence of known domains in the extracellular half of RLKs can be used in some cases to predict the nature of the corresponding ligands (peptides, proteins, hormones, carbohydrates, etc.), so far only a few ligand–RLK interactions have been biochemically characterized (Butenko et al., 2009). The extracellular domain sequence for all CrRLK1Ls was recently submitted to a Pfam 24.0 analysis (http://pfam.sanger.ac.uk; Finn et al., 2010) to search for similarities to domains of known function. Surprisingly, this analysis showed that most of the CrRLK1Ls contain two regions with similarity to the carbohydrate-binding domain of a newly characterized <i>Xenopus laevis</i> protein named malectin (Schallus et al., 2008; Fig. 2).

The <i>Malectin</i> gene is widely expressed throughout <i>Xenopus</i> development and encodes an endoplasmic reticulum (ER)-localized protein containing an N-terminal signal peptide (SP), followed by a globular domain and a C-terminal transmembrane helix (TM). The function of malectin has not been elucidated, but its globular domain shares a close structural similarity to carbohydrate-binding modules from prokaryotes. In addition, nuclear magnetic resonance-based screening demonstrated that malectin binds maltose and related oligosaccharides, while analyses with carbohydrate microarrays containing various mammalian glycans identified di-glucose-N-glycan (Glc<sub>2</sub>-N-glycan) as the preferential binding partner. Together, these data led the authors to suggest a role for malectin during the control of N-glycosylation in the ER (Schallus et al., 2008). The maltose-binding domain, hereafter referred to as the malectin domain (Pfam PF11721), is well conserved in animals (Schallus et al., 2008). In plants, although more divergent, malectin-like domains are found frequently in the extracellular halves of RLKs and in many other proteins with various domain combinations. Interestingly, the association of an extracellular malectin-like domain with an intracellular kinase seems to be unique to the plant kingdom. The plant malectin-like domain with highest similarity to malectin is found in the extracellular part of the leucine-rich repeat LRR-RLK At1g53430, where identity and similarity reach 21% and 35% over 160 amino acids, respectively (Fig. 2B). According to Pfam analysis, 10 CrRLK1L members have two significant malectin-like domains, three have one significant and one non-significant malectin-like domain, while only two members contain two non-significant malectin-like domains (Fig. 2A). The domain with highest similarity to malectin in the CrRLK1L family is the malectin-like domain adjacent to the TM of ANX2 with identity and similarity scores of 19% and 36%, respectively (Fig. 2). It is noteworthy that the tandem domain organization does not correspond to a duplication of the same malectin-like domain, i.e. the conservation between two domains at the same position (adjacent to the SP or the TM) for different CrRLK1L proteins can reach as high as ~90% (e.g. ANX1 and ANX2), while, within the same protein, identity between the two malectin-like domains is very low (e.g. ~10% for the two domains of ANX1).

Considering (i) the low homology between <i>X. laevis</i> malectin and the CrRLK1L malectin-like domains; (ii) the weak conservation in CrRLK1L malectin-like domains of the residues that mediate the malectin–Glc<sub>2</sub> interaction; and (iii) the unique tandem-domain organization in the CrRLK1L members and their plasma membrane localization, it is unlikely that the CrRLK1L family members play a similar role to the one proposed for <i>X. laevis</i> malectin in the quality control of N-glycosylated proteins in the ER. However, it is likely that the extracellular part of the CrRLK1L members mediates binding to carbohydrates. It is too early to predict whether carbohydrates that may be
Fig. 2. Phylogenetic tree of the 17 members of the Arabidopsis thaliana CrRLK1L family with their domain organization and alignment of the CrRLK1L malectin-like domains. (A) Multiple alignments were performed with ClustalW 1.83 and the phylogenetic tree was
bound by CrRLK1L extracellular domains are oligo- or polysaccharides from cell wall constituents, by-products of cell wall degradation, or membrane-associated or secreted glycosylated proteins. However, development of a carbohydrate microarray binding assay that would include plant oligosaccharides, glycosylated proteins, and cell wall degradation products could allow the identification of the preferential binding partners for the CrRLK1L members similarly to what was reported for the X. laevis malectin (Schallus et al., 2008).

**Partial functional redundancy among CrRLK1L family members**

Expression analyses of CrRLK1L family members indicate that some display overlapping expression patterns in vegetative tissues, some in pollen, while others are similarly induced by stresses and hormones, suggesting the existence of functional redundancy between some members (Guo et al., 2009a; Hématy and Höfte, 2008). Indeed, functional redundancy has been reported in pollen for ANX1 and ANX2, which is not surprising as their extracellular domains share 83.2% identity at the amino acid level, suggesting that ANX1 and ANX2 could bind the same ligand (Boisson-Dernier et al., 2009; Miyazaki et al., 2009). However, the partial redundancy observed between THE1, HERK1, and HERK2 during vegetative growth (Guo et al., 2009a, b) is more intriguing as they belong to different subclasses and their extracellular domains share more limited identity (38.7% for HERK1–THE1, 30.3% for THE1–HERK2, and 28.2% for HERK1–HERK2; Fig. 2A). Moreover, because FER-RNAi (RNA interference) lines display the same severe stunted growth phenotype as the triple herkl herk2 the1 mutant (Guo et al., 2009a, b), Guo and colleagues hypothesized that in vegetative tissues CrRLK1Ls could act cooperatively through heterodimerization, with FER acting as a co-receptor for HERK1, HERK2, and THE1. Alternatively, considering the divergence in the extracellular domains of these CrRLK1Ls and the compensatory mechanisms that occur in cell wall remodelling, it is proposed that each CrRLK1L may bind a specific cell wall carbohydrate or glycoprotein to monitor cell wall integrity and that loss of this monitoring for some constituents can be partially compensated by other CrRLK1L-dependent pathways, while loss of the FER-dependent monitoring cannot. Cell wall components such as the rhamnogalacturonan II (RG-II) pectin or glycosylated proteins can carry very complex and diverse carbohydrate decorations. Thus, in another model, CrRLK1Ls could bind different sugar decorations of the same complex cell wall constituent and the lack of recognition could have more drastic consequences for some decorations than that for others.

**Conclusion**

Since the characterization of THE1 and FER, the first Arabidopsis CrRLK1L members, 3 years ago, the hypothesis that the CrRLK1L family could function in the control of cell wall integrity becomes more plausible with each new CrRLK1L-related study. Simultaneously, the complexity of such a cell wall integrity sensing system is becoming more and more apparent. In Fig. 3, a model for the function(s) of the CrRLK1Ls is presented. During normal developmental processes, these RLKs could be responsible for monitoring cell wall integrity, probably in terms of molecular composition, in order to fine-tune the responses from other signaling pathways such as those of phytohormones. Alternatively, CrRLK1Ls could specifically recognize carbohydrate ligands produced in response to environmental...
cues or developmental processes that have rapid effects on cell wall composition and integrity, such as mechanical stress, fungal invasion, or male–female gametophyte interactions. The next challenge in the elucidation of CrRLK1L function is to identify not only some specific ligands but also other signaling components involved in CrRLK1L-mediated control of cell wall regulation and integrity. Regulators of secretion, distribution, or regulation of cell wall biosynthetic or modifying enzymes are good candidates for downstream effectors of CrRLK1L signaling pathways. Moreover, the potential binding of the tandem malectin-like domains of CrRLK1Ls to carbohydrates opens up the possibility that the variation in the CrRLK1L extracellular domains (both within the family and between species) could have evolved to reflect both tissue-dependent and species-dependent variations in the composition of cell wall components or glycosylated proteins (Knox, 2008; Sarkar et al., 2009).

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