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Current Opinion in
Plant Biology

Structural insights of cell wall integrity signaling during development and immunity

Hyun Kyung Lee and Julia Santiago

Abstract

A communication system between plant cells and their surrounding cell wall is required to coordinate development, immunity, and the integration of environmental cues. This communication network is facilitated by a large pool of membrane- and cell-wall-anchored proteins that can potentially interact with the matrix or its fragments, promoting cell wall patterning or eliciting cellular responses that may lead to changes in the architecture and chemistry of the wall. A mechanistic understanding of how these receptors and cell wall proteins recognize and interact with cell wall epitopes would be key to a better understanding of all plant processes that require cell wall remodeling such as expansion, morphogenesis, and defense responses. This review focuses on the latest developments in structurally and biochemically characterized receptors and protein complexes implicated in reading and regulating cell wall integrity and immunity.

Addresses

The Plant Signaling Mechanisms Laboratory, Department of Plant Molecular Biology, University of Lausanne, 1015 Lausanne, Switzerland

Corresponding author: Santiago, Julia (julia.santiago@unil.ch)

Current Opinion in Plant Biology 2023, **76:**102455

This review comes from a themed issue on **Cell Biology and Cell signaling 2023**

Edited by **Bert De Rybel** and **Juan Dong**

For complete overview of the section, please refer the article collection - Cell Biology and Cell signaling 2023

Available online 20 September 2023

https://doi.org/10.1016/j.pbi.2023.102455

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Introduction

Over the past decades, significant progress has been made in unveiling the molecular players involved in plant 'cell wall signaling'. The term encompasses a wide range of processes implicating the direct sensing of the cell wall or its products through the outer-inner crosstalk via receptors or mechano-sensing channels at the cell surface. As such, the complexities of cell-to-cell wall crosstalk are increasing rapidly and present a very dynamic yet tightly controlled network [1–4]. Mechanical and chemical properties of the cell wall fluctuate during cell expansion, morphogenesis, and when exposed to biotic/abiotic stressors [3,5–7]. These changes in the cell wall trigger a feedback mechanism that elicits a response from the cell surface. A tight surveillance system at the cell surface and the cell wall is required for adjusting to the constantly changing surroundings. Failure to adapt is often detrimental and ultimately perturbs developmental, defense, and reproductive processes.

In this review, we describe the recent advancements in the field of 'cell wall signaling and assembly' via the lens of structural biology. Part of this review offers potential functional or signaling scenarios based on the structural analysis of proteins that are proposed to be involved in cell wall sensing and architecture.

Signaling and structural proteins in maintaining cell wall integrity

The term 'cell wall integrity protein' is mainly coined for the two different protein families. The membrane-spanning *Catharanthus roseus* receptor-like kinase 1 (RLK1)-like (CrRLK1L) receptor kinases and the cell-wall-anchored leucine-rich repeat extensins (LRXs) [8,9]. Genetic analysis of these two protein families shows that they are not only required to maintain cell wall integrity during cell expansion, but they are also predicted to bind to cell wall components, namely pectin, while serving as bona fide receptors, or binding proteins, for the same group of signaling peptides: rapid alkalinization factors (RALFs) [10••–24]. Structures of the RALF4-LRX8 and LORELEI-LIKE GLYCOSYLPHOSPHATIDYLINOSITOL-ANCHORED PROTEIN (LLG2)-RALF23-FER complexes revealed two distinct peptide binding modes that facilitate complex formations in a mutually exclusive manner [10••,11••] (Figure 1a–c). The processed RALFs consist of a short N-terminal alpha-helix followed by a long loop shaped by disulfide bonds [10]. The LLG-RALF-FER ternary complex only requires the conserved N-terminal alpha-helix (YISY motif) of the peptide to nucleate the complex assembly [10••,11••] (Figure 1a, 1c). Contrastingly, the LRX proteins recognize the fully mature peptide, where two conserved tyrosine residues in the long loop anchor the peptide to the binding pocket, while the YISY motif is shown to be

Figure 1

Mechanistic insights into RALF binding to cell-wall receptor families. (a) LLG-RALF-FER complex formation is mediated by the RALF N-terminal alpha helix. The conserved YISY motif in RALF (cyan cartoon) nucleates the LLG protein to recruit the FER ectodomain. (b) LRXs display a dimeric conformation, with each monomer binding a RALF peptide to their LRR core, thus creating a hetero-tetradimer. (c) Alignment of clade I RALFs. LLG and LRX conserved binding motifs are depicted in blue and red, respectively. (d) Amino acid conservation heatmap of 17 members of the *Cr*RLK1L receptor kinases. The “cleft” region is highlighted as a box. (e) LRX-extensin domain alignment from root hair and pollen tubes. LRX1 and LRX2 possess the YxY motif, whereas the pollen tube LRXs only possess a single Tyr, which may indicate a divergent mode of network assembly at the cell wall. (f) Cartoon

unnecessary in LRX binding [10••]. This mutually exclusive mode of peptide binding confers RALFs dual functionality in regulating cell wall integrity via two different protein families.

CrRLK1L membrane receptors and cell wall monitoring

The member of the family THESEUS1 (THE1) was first identified as a cell-wall sensor for its ability to detect cell wall changes during cellulose synthesis inhibition [21]. THE1, like other *Cr*RLK1Ls, has two tandem malectin-like ectodomains (MAL) that sandwich together, creating a "cleft" between the domains [11••,25•,26] (Figure 1d). As the *Cr*RLK1L ectodomain was named after a *Xenopus* malectin that binds to N-glycans, it was hypothesized that it would interact with an unknown cell wall component and facilitate signaling inside the cell[27]. Recent evidence has pointed to pectin as the potential ligand of FERONIA, ANXUR1, and Buddha's Paper Seal 1 (FER, ANX1, BUPS1) [12,16,28,29••]. Genetic evidence suggests that these receptors perceive the de-esterified form of pectin and activate the intracellular secretion machinery to secrete modified cell-wall polymers [30]. In supporting this, direct and indirect associations have been shown for several *Cr*RLK1L members with experiments like protein localization post-plasmolysis, dot-blot assays, pectin shift-assays, and pull-downs [12,28]. Furthermore, the N-terminal half of the malectin-like domain (MalA) has been proposed by the authors to be the main contributor to pectin binding [28]. However, further evidence is required to prove that the two single malectin-like domains fold properly when produced independently, since analysis of the determined structures on ANX1/2 and FER would indicate otherwise [11••,25•,26]. Interestingly, structural and biochemical analysis on ANX1 revealed no conserved carbohydrate binding site or direct high affinity interaction with the number of sugars that had been previously described as potential ligands, including pectin, suggesting a different carbohydrate recognition mechanism. Their potential weaker interaction with the wall matches their recent proposed role as mechanosensors upon cell wall stress [12,29••].

The "cleft" region of the *Cr*RLK1Ls is speculated to be the platform for protein or cell wall polymer binding [25•,26]. The structure of ANX1 displayed the "cleft" region to be 30 Å in length, offering ∼1200 Å of accessible surface area [25•]. The family presents a nonconserved loop composition forming the cleft, containing numerous polar and aromatic amino acids that could serve to recognize a cell wall component or protein (Figure 1d). This variability may reflect the diversification of the *Cr*RLK1Ls "cleft" region, providing a specific interaction platform for tissue-specific cell-wall epitopes or proteins.

Structural leucine-rich repeat extensin (LRX) proteins and the cell wall architecture

Structures of reproductive LRX8 and vegetative LRX2 in complex with RALF4 have been elucidated [10••]. RALF4 tightly binds to LRX8 with a low ∼2.5-nM affinity. When bound to LRX8, RALF4 adopts a newly defined conformation with the long loop packed against the leucine-rich repeat (LRR) core [10••]. Interestingly, the heterologous expression of the LRX8-RALF4 complex forms a stable hetero-tetradimer with a central disulfide bridge reinforcing the dimer interface (Figure 1b, f). This creates a protein module where the LRR backbones of the two LRX monomers are facing back-to-back, exposing both peptide ligands to the solvent (Figure 1d). *In vivo* bioactivity validation of the hetero-tetradimer revealed that the protein complex is only active as a dimer and in the presence of the peptide ligand [10••]. The LRXs have a C-terminal extensin domain that is proposed to self-assemble via cross-linking with Tyr residues, as seen with extensin proteins *in vitro* [31,32] (Figure 1e,f). The dimeric nature of the protein complex lays the foundation of an interesting concept: a potential interconnected web-like LRX network that could be modulated to bind specific cell wall epitopes or proteins upon peptide activation, potentially changing cell wall properties. The proposed dendritic-like assembly of extensins via alternating hydrophobic and hydrophilic domains can likely mediate interactions with cell wall polymers such as pectin, which may indicate the mode of cell wall anchorage of LRXs to the cell wall [23,24]. Furthermore, the cysteine-rich tail located between the LRR and the extensin domain folds back onto the LRR domain via five cysteine-mediated disulfide bridges [10••]. This conformation stabilizes the LRR-extensin interface and reduces flexibility, which may add another modulatory layer to the cell-wall structural network formation of LRXs. Interestingly, the YxY motif required for forming a prerequisite isodityrosine for self-assembly is missing in pollen tube expressed LRX extensin domains that only contain one Y but present in root hair expressed LRXs [33] (Figure 1e). Single tyrosine residues can accept cross-linkers from other extensin proteins, suggesting that pollen LRXs may use other extensin scaffolds to form networks. This reflects divergent modes of LRX assembly required for two plant cell types, correlating with cell-specific pools of cell wall polymers.

representation of potential RALF-LRX hetero-tetradimer network assemblies via their extensin domain. (g) Electrostatic surface representation of RALF4-bound to LRX. Solvent-accessible surface electrostatic potential has been calculated using the adaptive Poisson-Boltzmann solver (APBS) plugin (PyMOL) at pH 5. The potential is given with the negative (red) and positive (blue) contour levels in the range from −5.0 to +5.0 kBT. Modified from [10••]. (h) Surface representation of RALF Clade I colored according to the members sequence conservation. The plot shows that RALFs have a highly variable exposed surface when bound to LRX proteins.

RALF4 binding to LRX8 is required to maintain cell wall integrity. Disruption of peptide binding to the LRR domain of LRX proteins, either by mutating the peptide or the LRX binding pocket, results in impaired fertility and disrupted pollen tube growth via bursting [10••]. Interestingly, RALF4 when bound to LRX8 adopts a defined conformation stabilized by the two cysteine disulfide bonds as opposed to the flexible free peptide, exposing a polycationic interaction surface [10••] (Figure 1g). This surface is speculated to potentially mediate a targeted interaction with other cell wall proteins or charged cell-wall polymers [10••]. Interestingly, this surface is highly variable within the RALF clade I members [10••], likely representing specific binding partners/cell wall components in different plant tissue types (Figure 1h).

Outstanding questions for CrRLK1Ls and LRXs in cell wall assembly and signaling

A tremendous amount of progress has been made in understanding the nature of how cell wall integrity is maintained. Yet there are some key questions that need to be addressed to understand how these two protein families read and shape the cell wall matrix to orchestrate core developmental processes in plants.

Are LRXs cell-wall structural proteins part of the cell-wall integrity system, or are they an integral part of the membrane signaling complex? The direct association of these two protein families and the formation of a big signaling complex are sources of debate [14,34,35]. On the one hand, LRX-FER has been shown to form a complex in various pull-down assays, while the pollen tubes LRX-ANX1 or BUPS1 have failed to display any direct physical interaction [10••,14,34]. Furthermore, a constitutively active MARIS kinase, a downstream component of the *Cr*RLK1L pathway, has either failed to complement the pollen tube *lrx* knock-outs [10••,36] or only partially in root hair [14]. In addition, the role of LLGs in this potential LRX-FER complex has not been defined. These results indicate cell type variability in diverging protein functions and the potential existence of an intermediate protein partner or cell wall component that could facilitate the LRX-FER interaction.

How do *Cr*RLK1Ls or LRXs interact with the matrix? Can they read and integrate mechanical cell wall signals? How do they integrate with canonical mechanosensors such as membrane channels? *Cr*RLK1Ls have been described as mechanosensors and shown to associate with pectin [12,29••], yet the exact molecular mechanism and precise polysaccharide species are currently unknown. Identification of specific pectin epitopes followed by structural determination of the different *Cr*RLK1L-RALF-LLG-pectin complexes will help to understand receptor activation, cell wall perception, and signal transduction. Similarly, the mechanism of LRX-RALFs binding to the cell wall needs to be elucidated. Interestingly, LRX proteins present a modulatory architecture, with a RALF-binding LRR domain and a cell-wall-connecting extensin domain [10••,23]. The binding of different RALFs would provide LRXs with distinct chemical surfaces capable of mediating specific interactions with different cell wall proteins or cell wall components.

Another central question would be to determine the molecular mechanisms by which cell wall polymers assemble to maintain cell wall integrity to sustain cell expansion, providing the right physico-chemical properties, and whether any of these two protein families may have a role in patterning the cell wall [6,7,37].

Immunity activation mechanisms by cell wall enzymes and cell-wall-derived DAMPs

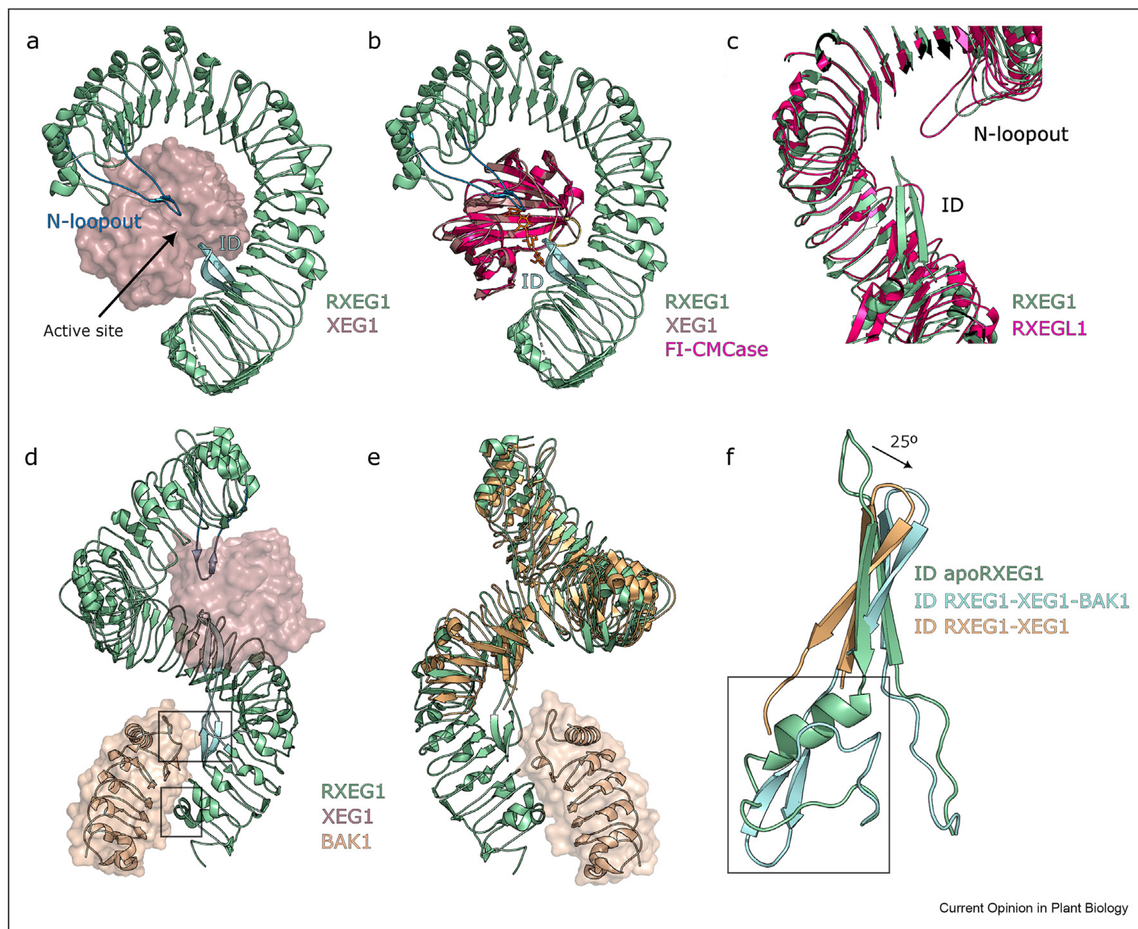
The plant cell wall acts as the first physical barrier to defend against pathogens, and it is also involved in sensing external stresses and transferring that information inside the cell to activate defense responses [3,38]. To penetrate the cell wall, plant pathogens secrete cell-wall degrading enzymes (CWDEs) to disrupt the main cell wall components (cellulose, hemicelluloses, and pectins) [39,40]. Plant cells have evolved a complex cell-surface surveillance system to detect pathogen- or host-derived danger signals to trigger an immunity response [41–44].

Binding of the xyloglucanase XEG1 and activation of the RLP-LRR receptor RXEG1

Leucine-rich repeat receptor like proteins (LRR-RLPs) constitute a subgroup of plasma-membrane-localized pattern recognition receptors (PRRs) and have been reported to play a key role in plant immunity [42,43,45]. This group of receptors lacks an intracellular kinase domain, and in the majority of cases, their extracellular LRR domain contains a loop-out region named the island domain (ID), located between the fourth and fifth LRR from the C-terminus [46]. To transduce the signal, LRR-RLPs constitutively interact with the LRR-RK suppressor of BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 (BAK1)-interacting receptor-like kinase (SOBIR) to form bi-partite heterodimers and recruit the LRR-RK somatic embryogenesis (SERKs) co-receptors upon ligand recognition [47•–52]. Cell-wall degrading enzymes have been described as microbe-associated molecular patterns recognized by LRR-PRRs [47•,51,53–57] to activate the immune signaling pathway. Recently, the crystal structure of the LRR-RLP receptor of xyloglucan-specific endo-beta-1,4-glucanase 1 (RXEG1) in complex with its ligand the xyloglucanase XEG1 (a glycoside hydrolase 12 protein) and the co-receptor SERK3/BAK1 has been determined, decoding a novel dual receptor activation and defense mechanism

[47•,58••]. Binding of the XEG1 to the RXEG1 triggers cell death as a plant defense response [47•]. The crystal structure of the RXEG1-XEG1-BAK1 complex uncovers a novel ligand recognition and receptor activation mechanism. The RXEG1 receptor uses two loop-out regions, an N-terminal loop-out and the C-terminal island domain, to recognize and anchor the enzyme-ligand to the inner surface of the super-helical ectodomain of the receptor (Figure 2a). Elegantly, these two out-loops happen to serve a dual function: on the one hand, they dock the enzyme to the receptor-binding site, and on the other hand, they inhibit the xyloglucanase catalytic activity by inserting into the enzyme active-site groove (Figure 2a), suppressing *Phytophthora* infection [58••]. Similar to LRR-RKs [59], RLP-ligand specificity relies on both the receptor binding site (mainly comprising the two out loops) and the structure of the enzyme. A structurally similar endo-1,4-β-d-glucanase (FI-CMCase) [60] with a longer loop at the entrance of its active site collides with the receptor island domain (Figure 2b), presumably preventing the receptor activation [58••]. Similarly, RXEG1-interacting loops are highly variable in the RXEG1 homolog, RXEGL1 (Figure 2c), explaining why RXEGL1 fails to recognize

Figure 2



LRR-RLPs ligand recognition and activation mechanisms. (a) The receptor RXEG1 docks the enzyme in the biding pocket and inhibits its xyloglucanase activity by inserting both out-loops (the N-loop and the ID) into the enzyme's active site. Structure of the receptor RXEG1 (in cartoon representation) in complex with XEG1 (surface representation) (PDB: 7DRB). (b−c) RXEG1 receptor homologs discriminate between different enzyme ligands through their out-loop conformation. (b) Superposition of the FI-CMCase in complex with cellotetraose (PDB: 5GM4) (dark pink) and XEG1 (light pink) in complex with RXEG1. The FI-CMCase enzyme gold loop closing the active site is longer than the XEG1-loop, clashing with the receptor's ID and preventing docking into the binding site. (c) Structural superimposition of RXEG1 (green) (PDB: 7W3X) and RXEGL1 (pink) (AlphaFold model). R.m.s.d of ~0.95 Å comparing 891 pairs of corresponding Cα atoms. Both receptors present different loop configuration, suggesting a distinct ligand recognition mode. (d−f) Activation mechanism of the RLP RXEG1 upon XGE1 binding. Interaction of the xyloglucanase XEG1 with RXEG1 promotes a series of conformational changes in the receptor to recruit the co-receptor BAK1. (d) Overall structure of RXEG1 (in cartoon representation) in complex with XEG1 (light pink surface) and the co-receptor BAK1 (light brown) (PDB: 7DR). The boxes indicate the BAK1 anchoring points to the receptor. (e) XEG1 binding to RXEG1 induces flexibility of the last four receptor LRRs, which become well defined upon BAK1 binding. Superposition of the receptors from the RXEG1-XEG1 (yellow) and RXEG1-XEG1-BAK1 (green) complexes. (f) Structural comparison of the RXEG1 island domains (ID) in the apo (green), XEG1-bound (orange), and XEG1-BAK1 complex (blue). The box highlights the conformational change in the C-terminal of the ID upon XEG1 binding to recruit the co-receptor BAK1.

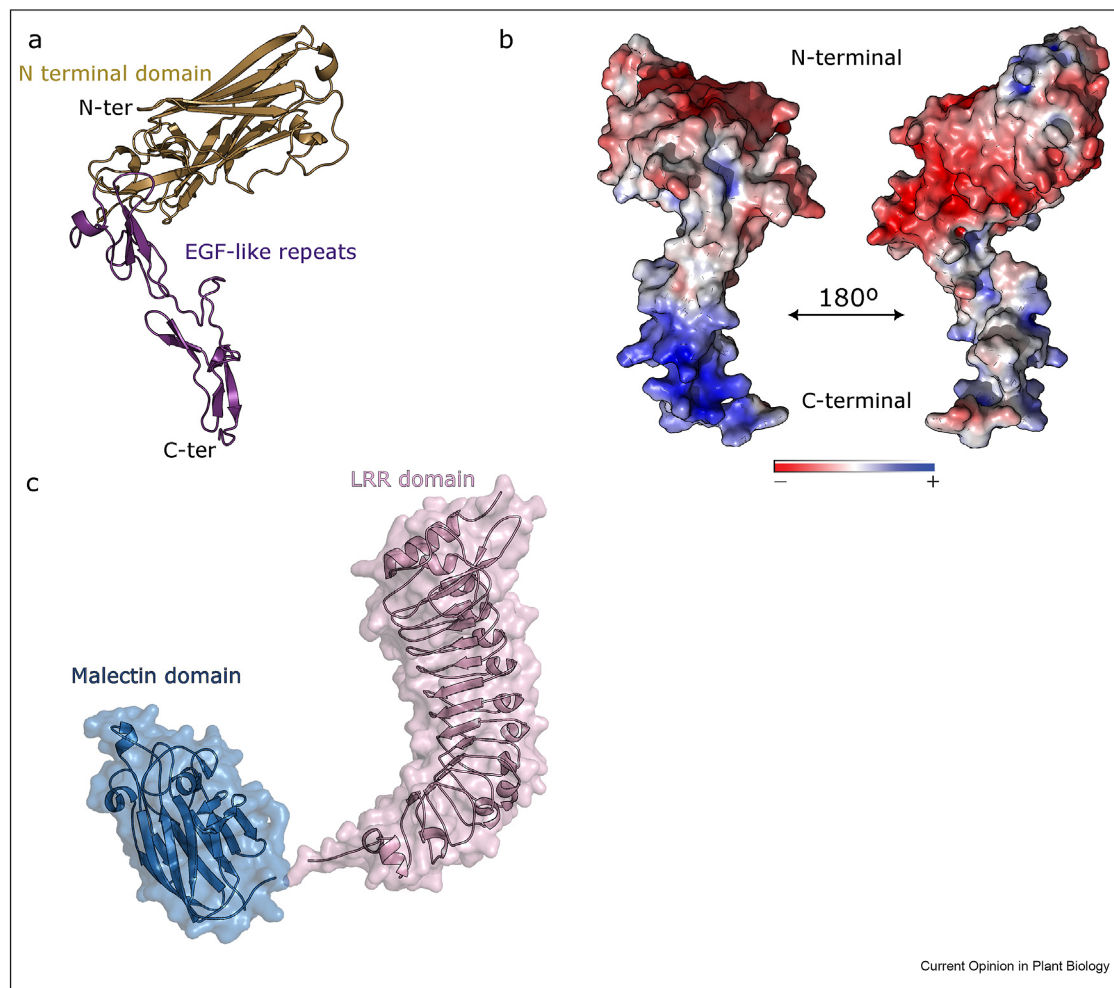
the XEG1 enzyme to trigger immunity [47•] and supporting a ligand-specificity mechanism.

Binding of the enzyme to the receptor induces allosteric changes in RXEG1, allowing for the recruitment of the co-receptor BAK1 and the activation of the signaling complex (Figure 2d–f). Structural comparison of the RXEG1 with the XEG1- and XEG1-BAK1 complexes reveals that the receptor undergoes substantial conformational changes in its N-loop-out region, the island domain (ID), and the last four LRRs (Figure 2e–f), creating a *de novo* binding site for the co-receptor BAK1. Binding of XEG1 to the receptor promotes the stabilization of the N-loop-out and generates striking conformational changes in the receptor's ID. The N-terminal antiparallel β -sheet of the ID rotates outwards ∼25°, and a structural remodeling happens in the C-terminal, converting the α-helix in the apo-RXEG1(ID) into an antiparallel β-sheet in the XEG1-BAK1 ternary complex (Figure 2f). This new β-sheet structure makes direct contact with the N-terminal of the shape-complementary BAK1 protein. Moreover, the last four conserved LRRs, disordered in the XEG1-RXEG1 binary complex, become well defined upon binding of the co-receptor (Figure 2e), indicating ligand-induced conformational flexibility of the receptor C-terminal to recruit BAK1 and trigger receptor activation. The last piece of the puzzle would be to uncover how the SOBIR completes the RXEG1-RLP signaling module and how this module integrates with downstream signaling components.

Architecture of cell-wall-derived DAMPs receptors

Cell-wall-derived polysaccharides have been shown to act as damage-associated molecular patterns (DAMPs) [61]. Pectin fragments (OGs = oligogalacturonides), mixed-linked β-1,3/1,4-glucans (MLGs), and cellulose-

Figure 3



Model structures of cell-wall-derived DAMP receptors. (a) Electrostatic surface representation of the WAK1 AlphaFold model. Solvent-accessible surface electrostatic potential has been calculated using the APBS plugin (PyMOL) at pH 5. The potential is given with the negative (red) and positive (blue) contour levels in the range from −5.0 to +5.0 kBT. (b) AlphaFold model representation of the two domains of the LRR-Malectin receptor, CORK1/IGP1.

derived oligosaccharides are host-derived DAMPs that trigger different immunity-related responses such as calcium influx, reactive oxygen species (ROS) production, mitogen-activated protein kinase (MAPK) activation, and defense-related gene expression, leading to enhanced pathogen resistance [62]. To date, not many receptors have been identified to sense cell-wall-derived DAMPs.

The wall-associated kinase 1 (WAK1) has been reported to sense oligogalacturonides (OGs) [63,64], but no structural details on the ligand-binding recognition and receptor activation mechanisms have been elucidated. The extracellular domain contains two epidermal growth factor-like (EGF-like) repeats and a N-terminal domain named the "polygalacturonic acid-binding" region [65] (Figure 3a). Electrostatic analysis on the predicted model from AlphaFold [66], shows that at apoplastic acidic pH, the N-terminal of the receptor is mainly negatively charged (Figure 3b). That contradicts the suggested electrostatic binding with polyanionic de-esterified pectins through the N-terminal domain of the receptor. However, the epidermal growth factor (EGF)-like domains uncover a basic patch enriched in Lys and Arg residues exposed to the solvent (Figure 3b) with an unknown function.

Recently, two parallel studies have identified an LRR-malectin receptor kinase named cello -oligomer receptor kinase 1(CORK1)/impaired in glycan perception1 (IGP1) to directly perceive cellotriose (CEL3) and cellopentaose (CEL5) in isothermal titration assays and to trigger immune responses upon the CEL-derived oligosaccharide binding [67•'68••]. These receptor families are composed of a N-terminal LRR domain with 12 LRRs, followed by a malectin domain (Figure 3c), and they have been involved in plant immunity and the control of plant reproduction [69,70]. Previous biochemical and structural studies [27] favor the malectin domain to be the carbohydrate sensor; however, a potential binding pocket comprising the two domains could also be possible, inducing a receptor conformational change and activation.

Perspectives

In this review, we describe and analyze the latest mechanistic advances in cell wall architecture and communication. A mechanistic understanding of cell wall assembly and signaling is an exciting new frontier that will open new avenues to uncover how cells expand, differentiate, and react to different stimuli. The compelling physiological, biochemical, and structural evidence on the *Cr*RLK1Ls membrane receptors and LRX proteins suggests that they function as cell-wall sensors and structural proteins, respectively [12,14,15••,21,29••]. However, a detailed mechanistic understanding of how these proteins perceive cell-wall changes, shape the cell wall, and integrate with potential mechano-sensing would be the next question to address. A promising step to better elucidate their functional role would be to combine structural and biochemical approaches with single-molecule super-resolution imaging to reveal their in vivo distribution and arrangement at the wall in relation to the cell wall components [6]. Moreover, the latest structure of the LRR-RLP receptor RXEG1 in complex with the xyloglucanase XEG1 and BAK1 reveals a novel ligand-perception and receptor-activation mechanism where the enzyme-ligand does not act as a molecular glue but induces a series of allosteric changes to recruit the co-receptor. Future cryogenic electron microscopy (cryo-EM) structures with the full-length proteins, including SOBIR, will uncover how that multimeric complex is assembled at the membrane and transfers the signal to cytoplasmic signaling components. Key mechanistic outstanding questions in plant membrane signaling, common to most described pathways, are how big sensosome complexes are assembled and regulated at the membrane and how specific signals at the cell surface are translated to distinct outputs using common downstream components, such as mitogen-activated protein kinases (MAPKs). The identification of novel cell wall-derived ligands represents a technical challenge. Therefore, novel biochemical and screening strategies will play a key role in identifying new cell-surface receptors that can sense cell-wall-derived carbohydrates. Mechanistic and physiological characterization of these new polysaccharide receptors and signaling pathways will help to uncover how the cell wall communicates with the cell and to understand how the cell differentiates between developmental [71] and pathogen-induced DAMPS, integrating the information to reshape the wall during growth and other developmental processes and to adapt to specific biotic and abiotic cues.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

We apologize for not being able to cite all related references due to space limitations.

The plant signaling mechanism laboratory is supported by the University of Lausanne, the European Research Council (ERC) grant agreement no. 716358 (J.S.) and the Swiss National Science Foundation grant no. 310030_204526 (J.S.).

References

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Swaminathan S, Lionetti V, Zabotina OA: **Plant cell wall integrity perturbations and priming for defense**. *Plants (Basel)* 2022, **11**: 3539.

2. Zhou K: **The regulation of the cell wall by glycosylphosphatidylinositol-anchored proteins in Arabidopsis**. *Front Cell Dev Biol* 2022, **10**:904714.

3. Vaahtera L, Schulz J, Hamann T: **Cell wall integrity maintenance during plant development and interaction with the environment**. *Nat Plants* 2019, **5**:924–932.

4. Rui Y, Dinneny JR: **A wall with integrity: surveillance and maintenance of the plant cell wall under stress**. *New Phytol* 2020, **225**:1428–1439.

5. Bacete L, Mélida H, Miedes E, Molina A: **Plant cell wall-mediated immunity: cell wall changes trigger disease resistance responses**. *Plant J* 2018, **93**:614–636.

6. Haas KT, Wightman R, Meyerowitz EM, Peaucelle A: **Pectin homogalacturonan nanofilament expansion drives morphogenesis in plant epidermal cells**. *Science* 2020, **367**: 1003–1007.

7. Palacio-Lopez K, Sun L, Reed R, Kang E, Sørensen I, Rose JKC, Domozych DS: **Experimental manipulation of pectin architecture in the cell wall of the unicellular charophyte, penium margaritaceum**. *Front Plant Sci* 2020, **11**.

8. Herger A, Dünser K, Kleine-Vehn J, Ringli C: **Leucine-rich repeat extensin proteins and their role in cell wall sensing**. *Curr Biol* 2019, **29**:R851–R858.

9. Somoza SC, Sede AR, Boccardo NA, Muschietti JP: **Keeping up with the RALFs: how these small peptides control pollen–pistil interactions in Arabidopsis**. *New Phytol* 2021, **229**:14–18.

10. •• Moussu S, Broyart C, Santos-Fernandez G, Augustin S, Wehrle S, Grossniklaus U, Santiago J: **Structural basis for recognition of RALF peptides by LRX proteins during pollen tube growth**. *Proc Natl Acad Sci USA* 2020, **117**:7494–7503.

This study elucidates the crystal structure of dimeric LRX receptors in complex with RALF peptides and decodes the RALF's dual distinct perception mechanisms to activate two different receptor families.

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

This study uncovers the 3D structure of the ternary complex composed by the CrRLK1L FER in complex with LLG proteins allosterically activated by RAFL peptides.

12. Feng W, Kita D, Peaucelle A, Cartwright HN, Doan V, Duan Q, Liu M-C, Maman J, Steinhorst L, Schmitz-Thom I, *et al.*: **The FERONIA receptor kinase maintains cell-wall integrity during salt stress through Ca2+ signaling**. *Curr Biol* 2018, **28**: 666–675. .e5.

13. • Gronnier J, Franck CM, Stegmann M, DeFalco TA, Abarca A, von Arx M, Dünser K, Lin W, Yang Z, Kleine-Vehn J, *et al.*: **Regulation of immune receptor kinase plasma membrane nanoscale organization by a plant peptide hormone and its receptors**. *Elife* 2022, **11**, e74162.

This study shows how the activity of an immunity membrane complex is affected by nanoscale mobility behaviors mediated by RALF signaling and the receptor kinase FERONIA.

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

15. •• 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.*: **RALF4/19 peptides interact with LRX proteins to control pollen tube growth in Arabidopsis**. *Science* 2017, **358**:1600–1603.

This study uncovers the functional role of RALF peptides in the regulation of cell wall integrity via LRX proteins during pollen tube growth.

16. Duan Q, Liu M-CJ, Kita D, Jordan SS, Yeh F-LJ, Yvon R, Carpenter H, Federico AN, Garcia-Valencia LE, Eyles SJ, *et al.*: **FERONIA controls pectin- and nitric oxide-mediated male–female interaction**. *Nature* 2020, **579**:561–566.

17. Draeger C, Ndinyanka Fabrice T, Gineau E, Mouille G, Kuhn BM, Moller I, Abdou M-T, Frey B, Pauly M, Bacic A, *et al.*: **Arabidopsis leucine-rich repeat extensin (LRX) proteins modify cell wall composition and influence plant growth**. *BMC Plant Biol* 2015, **15**:155.

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

19. Schoenaers S, Balcerowicz D, Breen G, Hill K, Zdanio M, Mouille G, Holman TJ, Oh J, Wilson MH, Nikonorova N, *et al.*: **The auxin-regulated CrRLK1L kinase ERULUS controls cell wall composition during root hair tip growth**. *Curr Biol* 2018, **28**: 722–732. .e6.

20. Gigli-Bisceglia N, van Zelm E, Huo W, Lamers J, Testerink C: **Arabidopsis root responses to salinity depend on pectin modification and cell wall sensing**. *Development* 2022, **149**, dev200363.

21. Hématy K, Sado P-E, Van Tuinen A, Rochange S, Desnos T, Balzergue S, Pelletier S, Renou J-P, Höfte H: **A receptor-like kinase mediates the response of Arabidopsis cells to the inhibition of cellulose synthesis**. *Curr Biol* 2007, **17**:922–931.

22. Gonneau M, Desprez T, Martin M, Doblas VG, Bacete L, Miart F, Sormani R, Hématy K, Renou J, Landrein B, *et al.*: **Receptor kinase THESEUS1 is a Rapid alkalinization factor 34 receptor in arabidopsis**. *Curr Biol* 2018, **28**:2452–2458. .e4.

23. Valentin R, Cerclier C, Geneix N, Aguié-Béghin V, Gaillard C, Ralet M-C, Cathala B: **Elaboration of extensin-pectin thin film model of primary plant cell wall**. *Langmuir* 2010, **26**: 9891–9898.

24. Lamport DTA, Kieliszewski MJ, Chen Y, Cannon MC: **Role of the extensin superfamily in primary cell wall architecture**. *Plant Physiol* 2011, **156**:11–19.

25. • Moussu S, Augustin S, Roman A-O, Broyart C, Santiago J: **Crystal structures of two tandem malectin-like receptor kinases involved in plant reproduction**. *Acta Crystallogr D* 2018, **74**:671–680.

This study uncovers the 3D structure of the dual malectinreceptors ANX1/2 analyzes their potential sugar binding sites, and tests their capacity to recognize an array of known cell wall polysaccharides.

26. Du S, Qu L-J, Xiao J: **Crystal structures of the extracellular domains of the CrRLK1L receptor-like kinases ANXUR1 and ANXUR2**. *Protein Sci* 2018, **27**:886–892.

27. Schallus T, Jaeckh C, Fehér K, Palma AS, Liu Y, Simpson JC, Mackeen M, Stier G, Gibson TJ, Feizi T, *et al.*: **Malectin: a novel carbohydrate-binding protein of the endoplasmic reticulum and a candidate player in the early steps of protein N-glycosylation**. *Mol Biol Cell* 2008, **19**:3404–3414.

28. Lin W, Tang W, Pan X, Huang A, Gao X, Anderson CT, Yang Z: **Arabidopsis pavement cell morphogenesis requires FERONIA binding to pectin for activation of ROP GTPase signaling**. *Curr Biol* 2022, **32**:497–507. .e4.

29. •• Zhou X, Lu J, Zhang Y, Guo J, Lin W, Van Norman JM, Qin Y, Zhu X, Yang Z: **Membrane receptor-mediated mechanotransduction maintains cell integrity during pollen tube growth within the pistil**. *Dev Cell* 2021, **56**:1030–1042. .e6.

This study implicates the pollen-expressed CrRLK1Ls in the transduction of mechanical signals to maintain cell wall integrity during cell expansion.

30. Luo N, Yan A, Liu G, Guo J, Rong D, Kanaoka MM, Xiao Z, Xu G, Higashiyama T, Cui X, *et al.*: **Exocytosis-coordinated mechanisms for tip growth underlie pollen tube growth guidance**. *Nat Commun* 2017, **8**:1687.

31. Cannon MC, Terneus K, Hall Q, Tan L, Wang Y, Wegenhart BL, Chen L, Lamport DTA, Chen Y, Kieliszewski MJ: **Self-assembly of the plant cell wall requires an extensin scaffold**. *Proc Natl Acad Sci USA* 2008, **105**:2226–2231.

32. Moussu S, Ingram G: **The EXTENSIN enigma**. *The Cell Surface* 2023, **9**:100094.

33. Marzol E, Borassi C, Bringas M, Sede A: **Rodríguez garcia DR, capece L, estevez JM: filling the gaps to solve the extensin puzzle**. *Mol Plant* 2018, **11**:645–658.

34. Zhao C, Zayed O, Yu Z, Jiang W, Zhu P, Hsu C-C, Zhang L, Tao WA, Lozano-Durán R, Zhu J-K: **Leucine-rich repeat extensin proteins regulate plant salt tolerance in Arabidopsis**. *Proc Natl Acad Sci USA* 2018, **115**:13123–13128.

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

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

37. Nicolas WJ, Fäßler F, Dutka P, Schur FKM, Jensen G, Meyerowitz E: **Cryo-electron tomography of the onion cell wall shows bimodally oriented cellulose fibers and reticulated homogalacturonan networks**. *Curr Biol* 2022, **32**:2375–2389. .e6.

38. Cosgrove DJ: **Growth of the plant cell wall**. *Nat Rev Mol Cell Biol* 2005, **6**:850–861.

39. Kubicek CP, Starr TL, Glass NL: **Plant cell wall-degrading enzymes and their secretion in plant-pathogenic fungi**. *Annu Rev Phytopathol* 2014, **52**:427–451.

40. Charkowski A, Blanco C, Condemine G, Expert D, Franza T, Hayes C, Hugouvieux-Cotte-Pattat N, López Solanilla E, Low D, Moleleki L, *et al.*: **The role of secretion systems and small molecules in soft-rot Enterobacteriaceae pathogenicity**. *Annu Rev Phytopathol* 2012, **50**:425–449.

41. Song W, Forderer A, Yu D, Chai J: **Structural biology of plant defence**. *New Phytol* 2021, **229**:692–711.

42. Jamieson PA, Shan L, He P: **Plant cell surface molecular cypher: receptor-like proteins and their roles in immunity and development**. *Plant Sci* 2018, **274**:242–251.

43. Wan W-L, Fröhlich K, Pruitt RN, Nürnberger T, Zhang L: **Plant cell surface immune receptor complex signaling**. *Curr Opin Plant Biol* 2019, **50**:18–28.

44. Liang X, Zhou J-M: **Receptor-like cytoplasmic kinases: central players in plant receptor kinase-mediated signaling**. *Annu Rev Plant Biol* 2018, **69**:267–299.

45. van der Burgh AM, Joosten MHAJ: **Plant immunity: thinking outside and inside the box**. *Trends Plant Sci* 2019, **24**:587–601.

46. Lk F-L, N K, M T, Kv S, Jd J: **Phylogenomic analysis of the receptor-like proteins of rice and Arabidopsis**. *Plant Physiol* 2005, **138**.

47. • Wang Y, Xu Y, Sun Y, Wang H, Qi J, Wan B, Ye W, Lin Y, Shao Y, Dong S, *et al.*: **Leucine-rich repeat receptor-like gene screen reveals that Nicotiana RXEG1 regulates glycoside hydrolase 12 MAMP detection**. *Nat Commun* 2018, **9**:594.

This study uses an elegant screening method based on gene silencing to identify receptor–ligand pairs. Here they identify the RLP receptor RXEG1 to bind its ligand, the xyloglucanase XEG1, forming a complex with the co-receptors BAK1 and SOBIR1 to transduce the signal inside the cell.

48. • Gust AA, Felix G: **Receptor like proteins associate with SOBIR1-type of adaptors to form bimolecular receptor kinases**. *Curr Opin Plant Biol* 2014, **21**:104–111.

This review gives a perspective on the functioning of RLPs and how proteins such as SOBIR1 act as adaptors, providing a kinase domain to the complex that can be activated upon specific ligand interaction and recruitment of shape-complementary co-receptors.

49. Gao M, Wang X, Wang D, Xu F, Ding X, Zhang Z, Bi D, Cheng YT, Chen S, Li X, *et al.*: **Regulation of cell death and innate immunity by two receptor-like kinases in Arabidopsis**. *Cell Host Microbe* 2009, **6**:34–44.

50. Liebrand TWH, van den Berg GCM, Zhang Z, Smit P, Cordewener JHG, America AHP, Sklenar J, Jones AME, Tameling WIL, Robatzek S, *et al.*: **Receptor-like kinase SOBIR1/EVR interacts with receptor-like proteins in plant immunity against fungal infection**. *Proc Natl Acad Sci USA* 2013, **110**: 10010–10015.

51. Albert I, Böhm H, Albert M, Feiler CE, Imkampe J, Wallmeroth N, Brancato C, Raaymakers TM, Oome S, Zhang H, *et al.*: **An RLP23-SOBIR1-BAK1 complex mediates NLP-triggered immunity**. *Nat Plants* 2015, **1**:15140.

52. Postma J, Liebrand TWH, Bi G, Evrard A, Bye RR, Mbengue M, Kuhn H, Joosten MHAJ, Robatzek S: **Avr4 promotes Cf-4 receptor-like protein association with the BAK1/SERK3 receptor-like kinase to initiate receptor endocytosis and plant immunity**. *New Phytol* 2016, **210**:627–642.

53. Jehle AK, Lipschis M, Albert M, Fallahzadeh-Mamaghani V, Fürst U, Mueller K, Felix G: **The receptor-like protein ReMAX of Arabidopsis detects the microbe-associated molecular pattern eMax from Xanthomonas**. *Plant Cell* 2013, **25**:2330–2340.

54. Zhang L, Kars I, Essenstam B, Liebrand TWH, Wagemakers L, Elberse J, Tagkalaki P, Tjoitang D, van den Ackerveken G, van Kan JAL: **Fungal endopolygalacturonases are recognized as microbe-associated molecular patterns by the Arabidopsis receptor-like protein RESPONSIVENESS TO BOTRYTIS POLYGALACTURONASES1**. *Plant Physiol* 2014, **164**:352–364.

55. Böhm H, Albert I, Oome S, Raaymakers TM: **Ackerveken GV den, nürnberger T: a conserved peptide pattern from a widespread microbial virulence factor triggers pattern-induced immunity in arabidopsis**. *PLoS Pathog* 2014, **10**, e1004491.

56. Du J, Verzaux E, Chaparro-Garcia A, Bijsterbosch G, Keizer LCP, Zhou J, Liebrand TWH, Xie C, Govers F, Robatzek S, *et al.*: **Elicitin recognition confers enhanced resistance to Phytophthora infestans in potato**. *Nat Plants* 2015, **1**:15034.

57. Ma Z, Song T, Zhu L, Ye W, Wang Y, Shao Y, Dong S, Zhang Z, Dou D, Zheng X, *et al.*: **A Phytophthora sojae glycoside hydrolase 12 protein is a major virulence factor during soybean infection and is recognized as a PAMP**. *Plant Cell* 2015, **27**: 2057–2072.

58. •• Sun Y, Wang Y, Zhang X, Chen Z, Xia Y, Wang L, Sun Y, Zhang M, Xiao Y, Han Z, *et al.*: **Plant receptor-like protein activation by a microbial glycoside hydrolase**. *Nature* 2022, **610**:335–342.

This study uncovers, for the first time, the allosteric activation mechanism of LRR-RLP receptors upon ligand binding.

59. Roman A-O, Jimenez-Sandoval P, Augustin S, Broyart C, Hothorn LA, Santiago J: **HSL1 and BAM1/2 impact epidermal cell development by sensing distinct signaling peptides**. *Nat Commun* 2022, **13**:876.

60. Huang J-W, Liu W, Lai H-L, Cheng Y-S, Zheng Y, Li Q, Sun H, Kuo C-J, Guo R-T, Chen C-C: **Crystal structure and genetic modifications of FI-CMCase from Aspergillus aculeatus F-50**. *Biochem Biophys Res Commun* 2016, **478**:565–572.

61. Gust AA, Pruitt R, Nürnberger T: **Sensing danger: key to activating plant immunity**. *Trends Plant Sci* 2017, **22**:779–791.

62. Locci F, Benedetti M, Pontiggia D, Citterico M, Caprari C, Mattei B, Cervone F, De Lorenzo G: **An Arabidopsis berberine bridge enzyme-like protein specifically oxidizes cellulose oligomers and plays a role in immunity**. *Plant J* 2019, **98**: 540–554.

63. He Z-H, Fujiki M, Kohorn BD: **A cell wall-associated, receptor-like protein kinase ***. *J Biol Chem* 1996, **271**:19789–19793.

64. Brutus A, Sicilia F, Macone A, Cervone F, De Lorenzo G: **A domain swap approach reveals a role of the plant wall-**

associated kinase 1 (WAK1) as a receptor of oligogalacturonides. *Proc Natl Acad Sci USA* 2010, **107**:9452–9457.

65. Decreux A, Messiaen J: **Wall-associated kinase WAK1 interacts with cell wall pectins in a calcium-induced conformation**. *Plant Cell Physiol* 2005, **46**:268–278.

66. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Žídek A, Potapenko A, *et al.*: **Highly accurate protein structure prediction with AlphaFold**. *Nature* 2021, **596**:583–589.

67. • Tseng Y-H, Scholz SS, Fliegmann J, Krüger T, Gandhi A, Furch ACU, Kniemeyer O, Brakhage AA, Oelmüller R: **CORK1, A LRR-malectin receptor kinase, is required for cellooligomer-induced responses in Arabidopsis thaliana**. *Cells* 2022, **11**: 2960.

This article, along with Martín-Dacal et al., identifies the first plant membrane receptor capable of sensing cellooligomers.

68. •• Martín-Dacal M, Fernández-Calvo P, Jiménez-Sandoval P, López G, Garrido-Arandía M, Rebaque D, del Hierro I, Berlanga DJ, Torres Má Kumar V, *et al.*: **Arabidopsis immune responses triggered by cellulose- and mixed-linked glucan-derived oligosaccharides require a group of leucine-rich repeat malectin receptor kinases**. *Plant J* 2023, **113**:833–850.

This article, along with Tseng et al., reports the first plant membrane receptor to perceive β-1,4 glucans using quantitative biochemistry and physiological assays.

69. Rajaraman J, Douchkov D, Hensel G, Stefanato FL, Gordon A, Ereful N, Caldararu OF, Petrescu A-J, Kumlehn J, Boyd LA, *et al.*: **An LRR/malectin receptor-like kinase mediates resistance to non-adapted and adapted powdery mildew fungi in barley and wheat**. *Front Plant Sci* 2016, **7**.

70. Lee HK, Goring DR: **Two subgroups of receptor-like kinases promote early compatible pollen responses in the Arabidopsis thaliana pistil**. *J Exp Bot* 2021, **72**:1198–1211.

71. Hayashi T, Yoshida K, Woo Park Y, konishi T, Baba K: **cellulose metabolism in plants**. In *International review of cytology*. Academic Press; 2005:1–34.