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Plant Cell Surface Molecular Cypher: Receptor-Like Proteins and Their Roles in Immunity and Development

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Abstract

Plant receptor-like proteins (RLPs) are a family of transmembrane receptors which are distinguished from receptor-like kinases (RLKs) by their lack of a cytoplasmic kinase domain. RLPs continue to be implicated in a broad range of plant immunological and developmental processes as critical sensors or participants in receptor complexes on the plasma membrane. RLPs often associate with RLKs to activate or attenuate signal perception and relay. Some RLPs also physically cluster with RLKs and bear similar expression patterns. Here, we discuss the characteristics, function, and expression of characterized RLPs in the context of their associated RLKs in plant immunity and development.

Keywords

Receptor-like protein; Leucine-rich repeat; Receptor-like kinase; Pattern recognition receptor; Plant Immunity; Plant development

1. Introduction

Perception of extracellular signals by transmembrane receptors is a nearly ubiquitous feature of cellular life. The term *phytosemiotics* was coined to describe the study of the signal processes used by plants to sense and respond to stimuli at the cellular level [1]. In plants, a continuous and coordinated communication with the environment must be uniquely mandated by their general sessility. Further, their lack of a circulatory system with specialized immune cells likely compounds this challenge, and may account for some of the differences in immune signaling mechanisms between plants and animals [2].

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Plant immunity and development often rely on similar or overlapping cellular mechanisms for signal recognition and transduction. Frequently, transmembrane receptors are the first actors in play. While receptor tyrosine kinases (RTK) are the most common class of transmembrane receptor protein kinases (RPKs) in animals, they are comparatively rare in plants. Instead, plant RPKs most often use serine/threonine kinases (STKs) for immediate downstream signal transduction, although several plant RPKs have tyrosine kinase activity [3–5]. The term receptor-like kinase (RLK) refers to plant receptor and nonreceptor protein kinases [6]. Phylogenetic analysis suggests that an ancient gene duplication event prior to the divergence of plants and animals led to the independent evolution of plant RLKs and animal RTKs [4]. RLKs comprise more than 60% of all kinases in *Arabidopsis thaliana* [4, 7]. The proportion of homologous RLKs found in tandem clusters and segmentally duplicated regions in the *A. thaliana* genome suggests a selective pressure towards the expansion of this gene family [8]. RLKs with various extracellular ligand-recognition domains (e.g. leucine-rich repeats, lectin/lectin-like, epidermal growth factor-like, etc.) have been identified. However, the leucine-rich repeat containing RLKs (LRR-RLKs) are not only the most common, but also the most studied RLKs in plants [6, 9].

Receptor-like proteins (RLPs) share structural similarity with RLKs but lack a cytoplasmic kinase domain [10, 11]. This likens them to the Toll-like receptors (TLRs) involved in mammalian immunity, which also contain an extracellular LRR domain and a short cytoplasmic tail [12]. It should come as no surprise, then, that several RLPs and RLKs have been identified as pattern recognition receptors (PRRs) critical in plant innate immunity [13, 14]. Certain RLPs and RLKs also have crucial functions in plant development [15, 16]. This is consistent with the originally observed dorsal-ventral patterning function of the *Drosophila* Toll gene for which human TLRs were named [17]. The combined immune and developmental functions of RLPs and RLKs suggest they may play a broad and critical role in the detection of both self and non-self.

Far more is currently known about RLKs relative to RLPs, but a growing body of evidence suggests that the role of RLPs is as important as RLKs. By examining the character, function, and expression of RLPs in the context of their associated RLKs, we aim to summarize that body of evidence, and to discuss the state of knowledge on RLPs in plant immunity and development.

2. RLP Characteristics and Expression

In simplest terms, RLPs can be thought of as transmembrane receptors with an extracellular ligand binding domain and a short cytoplasmic tail (Figure 1). It can be generally assumed, then, that RLPs are single-pass (type I) transmembrane proteins [4, 10, 11]. However, some proteins without transmembrane domains might be considered as RLPs by virtue of their attachment to the extracellular face of the plasma membrane using a glycosylphosphatidylinositol (GPI) anchor [18, 19]. In addition, RLPs traditionally contain an N-terminal signal peptide.

The most common extracellular ligand-binding domain found in RLPs is LRRs (Figure 1) [8]. There are about 223 LRR-RLKs and 57 LRR-RLPs in *Arabidopsis* [4, 7, 10, 11]. In

LRR-RLKs, the intracellular kinase domain exhibits more conservation than the extracellular LRR domain [20]. A quantification of the number of LRRs in *Arabidopsis* LRR-RLKs reveals a bimodal distribution where RLKs containing three to six and 21 to 24 repeats are frequent, but proteins with three to nine LRRs are the most common. By contrast, most *Arabidopsis* LRR-RLPs have a high number LRRs ranging from 16 to 28 repeats [15]. Plant-derived extracellular LRRs are 22–23 amino acids long, and each repeat forms a LxxLxxLxLxxNxLSGxIPxxLGx consensus [21]. In contrast to bacterial and animal LRR proteins which form a horseshoe-shaped structure, plant LRRs usually form a twisted or superhelical assembly because of an additional β-sheet forming the inner surface of the solenoid [21]. Likely due to their variability, LRRs have been observed to recognize a range of ligands, including sterols, lipids, sugars, peptides, lipopeptides, nucleic acids, and others.

RLP and/or RLK ectodomains have also been found to contain legume-like (L-type) lectins, G-type lectins, calcium-dependent (C-type) lectins, and the lectin-like Lysin-motifs (LysM). Plant lectin or lectin-like domains are usually involved in the perception of carbohydrates and glycans [22]. Among them, LysM RLKs or RLPs are well studied, while C-type lectins are rare in plants. There are 42–45 L-type lectin RLKs and six L-type lectin RLPs in *Arabidopsis* [22–24]. Compared to 39 G-type lectin-containing RLKs, only three G-type lectin-containing RLPs are predicted in *Arabidopsis* [23]. Further, of these predicted G-type lectin-containing RLPs in *A. thaliana*, one (AT4G21370) is an ortholog of the S-locus receptor kinase (SRK) in *A. lyrata.* In *A. lyrata* and other crucifers, this gene is a critical regulator of self-incompatibility which is expressed in the stigma. In *A. thaliana*, this gene is spliced differently, introducing an early stop codon before the cytoplasmic kinase domain [25]. The role of this gene in the evolution of self-fertility in *A. thaliana* remains to be determined. By contrast, only one C-type lectin-containing RLK (AT1G52310) has been predicted, and no C-type lectin-containing RLPs were found in *Arabidopsis* [23].

LysMs, named for their similarity to bacterial autolysins, are considered a distinct plant lectin subfamily and found in both plant RLKs and RLPs [8, 26] (Figure 1). In bacteria, autolysins catalyze the hydrolysis of the glycosidic bonds in peptidoglycan (PGN) to facilitate cell wall restructuring processes in growth and cell division [27]. LysMs in eukaryotes are likely the result of a horizontal gene transfer event, and in plants they are often adapted to recognize polysaccharide ligands such as PGN and fungal chitin [28, 29]. There are 5 LysM RLKs and 3 LysM RLPs that are characterized in *Arabidopsis* [28].

Other motifs, such as malectin-like domains, cysteine rich domains of unknown function (DUF26), and thaumatin domains have been found as ectodomains of RLKs and RLPs [8]. Except for malectin-like domain containing RLKs, their functions have not been well characterized. Malectin-like ectodomains were originally found in the *Catharanthus roseus* RLK1-like (*Cr*RLK1L) subfamily of RLKs [30–32]. Malectin domains bear similarity to the animal carbohydrate-binding malectin proteins involved in the endoplasmic reticulum quality control [33]. Several *Arabidopsis* malectin-like domain RLKs, such as FERONIA and ANXURs, have a broad role in plant development, hormone signaling and immunity [30–32, 34]. There is one malectin-like domain RLP (At4g00300) in *Arabidopsis* with uncharacterized function [8].

Interestingly, some RLKs such as IMPAIRED OOMYCETE SUSCEPTIBILITY 1 (IOS1) contain both LRR and malectin-like domains and are named malectin-like/LRR-RLKs [4, 35]. The FLG22-INDUCED RECEPTOR-LIKE KINASE (FRK1), whose expression level is often used as an indicator of early defense [36], also belongs to the malectin-like/LRR-RLK subfamily. We have identified putative malectin-like/LRR-RLPs in *Physcomitrella patens* (XP_001756784.1) and *Oryza sativa* (XP_015645303.1), suggesting that malectin-like/LRR ectodomains might be a common feature in RLKs and RLPs in different plant species.

An often-overlooked dimension of RLP functions is their tissue-specific expression. When and where each RLP is expressed may modify its function specificity, e. g. by interacting with specific RLKs. In this way, characterization of tissue-specific expression pattern of individual RLKs and RLPs is a critical step in understanding their functions. The expression pattern of all 223 LRR-RLKs at various developmental stages have been systematically characterized with *promoter::GUS* transgenic plants [37]. The tissue-specific expression patterns of LRR-RLKs may provide insights into their functions in certain developmental stages. Analysis of available microarray datasets indicates that *Arabidopsis* LRR-RLPs are differentially expressed under a panoply of biotic, abiotic, light, and hormone treatments (Figure 2) [38]. In general, the expression pattern of LRR-RLPs during abiotic and biotic stress is similar, but distinct from the pattern observed during light stress (Figure 2). Biotic stresses, including treatments with *Pseudomonas syringae, Phytopthora infestans, Botrytis cineria, Erysiphe orontii,* or pathogen elicitors, caused an expression change in more *Arabidopsis* LRR-RLPs (~90% of all LRR-RLPs tested) than abiotic stresses (~80% of all LRR-RLPs tested) or hormone treatments (~40% of all LRR-RLPs tested) (Figure 2) [32]. The virulent *P. syringae* pv. *tomato* DC3000 suppressed the expression of more LRR-RLPs than it induced (15 vs. 9), whereas its type III secretion mutant DC3000 *hrcC,* or a non-adapted bacterium *P. syringae* pv. *phaseolicola,* induced far more LRR-RLPs than it suppressed LRR-RLPs (~20 vs. ~2) [32], suggesting the importance of LRR-RLPs in the arms race of plant immunity and bacterial pathogenicity (see below details for plant immune system).

3. RLPs in Plant Immunity

3.1. The plant immune system

The first line of plant immunity is triggered by the recognition of conserved microbe-associated molecular patterns (MAMPs) [39]. Canonically, MAMPs are small, highly conserved molecules that are important for microbial survival or growth. MAMPs are perceived by PRRs and activate pattern-triggered immunity (PTI). PTI is characterized by the rapid deployment of a series of cellular responses including the activation of mitogen-activated protein kinase (MAPK) cascades, calcium-dependent protein kinases (CDPKs), the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), ion flux, callose deposition, phytohormone production, transcriptional reprogramming, production of phytoalexins, and likely other as-yet undiscovered responses [14, 40, 41].

Accordingly, pathogens have developed various virulence strategies to subvert PTI and promote pathogenicity. For example, pathogenic bacteria inject type III effectors (T3Es) and fungal pathogens haustorially deliver effectors to interfere with PTI signaling and responses

[42]. These effectors are sometimes recognized, directly or indirectly, by host intracellular proteins. These proteins are predominantly encoded by nucleotide-binding site leucine-rich repeat (NBS-LRR or NLR) proteins, also called resistance (R) proteins, and trigger the second tier of defense known as effector-triggered immunity (ETI) [39]. ETI is characterized by a more drastic and robust activation of cellular defenses relative to PTI. Often, this activation is accompanied by a cell death process local to the infection site known as the “hypersensitive response” (HR) [39].

3.2. RLPs involved in responses triggered by apoplastic fungal effectors

The first characterized RLP Cf-9, encodes a LRR-RLP in tomato [43]. Cf-9 confers resistance specific to races of *Cladosporium fulvum* carrying the Avr9 effector gene, which was characterized in a gene-for-gene resistance now referred as ETI. Later, a second tomato LRR-RLP designated Cf-4 was shown to be required for resistance to *C. fulvum* expressing Avr4 [44]. While MAMP perception by RLKs or RLPs canonically leads only to the activation of PTI, both Avr4 and Avr9 recognition results in the activation of HR, a response more commonly associated with ETI [43, 44]. In addition, Cf-9 does not seem to directly perceive Avr9, as there is no direct binding between them [45]. Thus, as their designation (Avr for “avirulence”) would suggest, this classifies Avr4/Avr9 as apoplastic effectors, rather than MAMPs [46]. This example confounds the traditional R-gene/Avr-gene interaction scheme, as Cf-4 and Cf-9 are structurally similar to PRRs, but functionally defined as R genes. Consequently, apoplastic effector recognition by RLPs is distinctly considered to activate “effector-triggered defense” (ETD). ETD is distinguished from ETI not only by the location of effector recognition, but also by its longer response time, propensity for heterodimer-mediated activation, and rate of cell death [46].

The tomato resistance gene *Ve*, which confers race-specific resistance to *Verticillium dahliae* or *V. albo-atrum* carrying Ave1, was identified as two closely linked LRR-RLPs, Ve1 and Ve2 [47]. Although Ve1 and Ve2 share 84% amino acid identity, subsequent analysis indicated that Ve1, not Ve2, confers Ave1-mediated resistance [48]. Ave1 is an apoplastic effector, thus, Ve1 also induces ETD. The *Brassica napus* blackleg resistance gene LepR3 is an LRR-RLP, which confers resistance to *Leptosphaeria maculans* carrying the effector AvrLm1 in a gene-for-gene manner [49]. AvrLm1 is also important in resistance triggered by an independent R gene Rlm1, whose identity is still unknown [49].

3.3. RLPs involved in responses triggered by fungal and oomycete MAMPs

Chitin is a well-studied fungal MAMP with distinct perception and signaling systems in *Arabidopsis* and rice [14, 28, 40]. In *Arabidopsis*, chitin is perceived by two LYM-RLKs, AtLYK5 and AtLYK1, also called CHITIN ELICITOR RECEPTOR KINASE 1 (AtCERK1) [50, 51]. In rice, OsCERK1 does not have apparent chitin binding activity, instead, the LysM-RLP *Os*CEBiP directly binds to chitin and homodimerizes upon chitin binding [52] [53]. *Os*CERK1 cooperates with OsCEBiP to mediate chitin-triggered responses [54]. Two additional rice LysM-RLPs designated *Os*LYP4 and *Os*LYP6 have also been shown to bind to chitin and are required for chitin-triggered responses in rice [55]. Unlike classical LRR-RLPs which are assumed to be inserted into the plasma membrane through the transmembrane domain, OsCEBiP likely associates with the membrane through a GPI anchor [19]. *Arabidopsis* LysM-RLPs, by contrast, do not appear to be required for *At*CERK1 function in chitin signaling [56].

Tomato LRR-RLPs ETHYLENE INDUCING XYLANASE 1 (*Sl*EIX1) and *Sl*EIX2 both bind to fungal xylanases, although only *Sl*EIX2 is important in xylanase-mediated responses [57]. Interestingly, *Sl*EIX1 could suppress *Sl*EIX2-activated defense, suggesting that *Sl*EIX1 may act as a functional decoy receptor [58]. *Arabidopsis* LRR-RLP *At*RLP23 specifically binds to the 20 amino acid peptide "NECROSIS AND ETHYLENE-INDUCING PEPTIDE1 (NEP1)-LIKE PROTEINS" (NLPs), designated nlp20, and is required for nlp20-mediated responses [59]. Nep1, was originally identified in *Fusarium oxysporum* f. sp *erythroxyli*. This family of proteins was subsequently identified in several microbial clades, from bacteria to oomycetes [60]. Despite that NLPs are highly conserved in several plant pathogenic microbes, the corresponding receptor RLP23 does not have an obvious ortholog in other plant species (Figure 3). However, heterologous expression of *At*RLP23 in potato conferred resistance to the oomycete *Phytophthora infestans* and the fungus *Sclerotinia sclerotiorum* [59]. The LRR-RLP ELICITIN RESPONSE (*Sm*ELR) from wild potato *Solanum microdontum* recognizes elicitin INF1, a conserved MAMP in several species of *Phytophthora,* and transfer of *Sm*ELR into cultivated potato enhanced its resistance to late blight [61]. The function of both *At*RLP23 and *Sm*ELR outside of their original genetic context points to the possibility that interspecies transfer of RLP genes may sometimes represent a means to improve plant resistance to fungal and oomycete pathogens. *Arabidopsis* RESPONSIVENESS TO BOTRYTIS POLYGALACTURONASES 1 (*At*RBPG1), which encodes LRR-RLP AtRLP42, forms a complex with polygalacturonases (PGs) derived from *Botrytis cinerea*, and is important for plant necrotic response to PGs [62]. Thus, *At*RBPG1 is a putative receptor of PGs.

Several other LRR-RLPs have been shown to be involved in fungal resistance, but their corresponding MAMPs or apoplastic effectors have not been identified. *Arabidopsis* LRR-RLP *At*RLP30 is involved in the response triggered by an unknown proteinaceous elicitor from *Sclerotinia sclerotium* designated *SCLEROTINIA CULTURE FILTRATE ELICITOR 1* (SCFE1). AtRLP30 was further demonstrated to be important for *Arabidopsis* resistance to *S. sclerotium* and *B. cinerea*. [63]. *Arabidopsis RESISTANCE TO FUSARIUM OXYSPORUM 2* (RFO2) is an LRR-RLP encoded by *At*RLP3 and confers resistance to the fungal pathogen *Fusarium oxysporum.* Although *At*RLP3/RFO2 and *At*RLP2 are physically adjacent and highly similar, *At*RLP2 does not contribute to resistance against *F.* oxysporum. Despite this, the extracellular LRR domains of *At*RLP2 and *At*RLP3/RFO2 are exchangeable for resistance [64]. Interestingly, the extracellular LRR domain of *At*RLP3/RFO2 is also highly similar to that of the LRR-RLK PSY1R, which recognizes tyrosine-sulfated peptide PSY1, whose signaling negatively regulates plant immunity. Thus, the authors propose that *At*RLP3/RFO2 acts as a decoy receptor for a *F. oxysporum* effector, which is otherwise recognized by PSY1R to promote pathogenicity [64]. The importance of *Arabidopsis* LRR-RLP *At*RLP52 in resistance against the fungal powdery mildew pathogen *Erysiphe cichoracearum* was demonstrated after it was first characterized for its responsiveness upon treatment with chito-octamers [65]. It remains unknown whether *At*RLP52 participates in chitin perception through interaction with *At*LYK5/*At*CERK1 complex. In wheat, RLP1.1 was shown to play a role in defense against the rust pathogen

Puccinia striiformis f. sp. tritici [66]. Additionally, apple LRR-RLP HcrVf2, located in a cluster of LRR-RLP genes, mediates resistance to apple scab caused by the ascomycete *Venturia inaequalis* (Cke.) Wint. [67].

3.4. RLPs involved in resistance to bacteria

Although several RLPs have been implicated in plant bacterial resistance, their exact role in PTI or ETI is not entirely clear. In one case, it has been shown that *Arabidopsis* LysM-RLPs LYM1 and LYM3 are required for bacterial PGN-mediated responses through direct binding to PGNs [68]. The *lym1, lym3*, and *lym1 lym3* mutants were all compromised in defense against *P. syringae* pv. *tomato* DC3000, and its type III secretion mutant strain *hrcC-* [68]. Similarly, rice LysM-RLPs, *Os*LYP4 and *Os*LYP6, also bind to PGN, and mediate PGN-triggered responses [55]. Thus, *Os*LYP4 and *Os*LYP6 could bind to both chitin and PGN and mediate resistance to fungal and bacterial pathogens.

Arabidopsis RECEPTOR OF ENIGMATIC MAMP OF XANTHOMONAS (ReMAX) is an LRR-RLP (*At*RLP1) implicated in the perception of eMAX, a MAMP found in several *Xanthomonas* species [69]. Further, *At*RLP30 was found to contribute to resistance against the non-adapted bacterial pathogen *P. syringae* pv. *phaseolicola* [10]. *Arabidopsis SUPPRESSOR OF NPR1, CONSTITUTIVE 2* (SNC2) is an LRR-RLP (*At*RLP51) [70]. The *snc2–1D* mutant, which bears a glycine to arginine mutation in the second glycine of the conserved GXXG motif of the transmembrane domain, exhibits a constitutive defense response, and increased pathogen resistance. Similarly, the corresponding mutation in *At*RLP55/SNC3, a close homolog of *At*RLP51/SNC2, also caused a constitutive activation of defense [70]. The GXXG motif is known to be important for the interaction of transmembrane helices [71], suggesting that *At*RLP51/SNC2 and *At*RLP55/SNC3 might interact with each other or another membrane proteins. Using a suppressor screen, it was shown that *At*RLP51/SNC2 signal transduction is likely mediated by *BIAN DA 1* (BDA1) (Chinese for “becoming big”), an ankyrin-repeat-containing protein with four transmembrane domains, to trigger defense through the activation of the transcription factor WRKY70 [72]. Both *At*RLP51/SNC2 and BDA1 are important for plant defense to *P. syringae* pv. *tomato* DC3000. It has been proposed that *At*RLP51/SNC2 may perceive an unknown MAMP from bacterium to activate immunity through BDA1 [72]. Interestingly, a mutation in one transmembrane domain of BDA1 also caused constitutive activation of defense [72], raising the possibility that transmembrane domains of AtRLP51/SNC2 and BDA1 interact with each other and suppress the defense response in the absence of pathogen infection.

3.5. RLPs involved in resistance to parasitic weeds

Parasitic plants also produce MAMP-like elicitors that induce classical defense responses. Tomato LRR-RLP *Cuscuta* Receptor 1 (*Sl*CuRe1) is involved in the response triggered by a small, potentially O-glycosylated peptide from the parasitic plant *Cuscuta reflexa* [73]. It remains unknown whether *Sl*CuRe1 is a genuine receptor of this peptide, and whether it shares the same signaling pathway as PRRs perceiving classical MAMPs. Nevertheless, ectopic expression of *Sl*CuRe1 in susceptible hosts increased resistance to *C. reflexa* [73], further substantiating the potential of RLPs in applied crop biotechnology.

4. RLPs in Plant Development

Several LRR-RLPs have been shown to function as key regulators in different plant developmental processes including stomatal patterning, meristematic tissue regulation, and hormone signaling. *Arabidopsis TOO MANY MOUTHS* (TMM) encoded by LRR-RLP *At*RLP17 expresses most highly in dividing postprotodermal cells and differentially modulates stomatal development in different organs with a negative role in cotyledons and positive role in hypocotyls and stems [74]. *Arabidopsis* CLAVATA 2 (CLV2) encoded by the LRR-RLP *At*RLP10 was implicated in maintaining shoot apical meristem (SAM), root apical meristem (RAM), and floral organ size—a function consistent with its broader expression pattern [75, 76]. Interestingly, expression of *AtRLP2* or *AtRLP12* under the *AtRLP10/CLV2* promoter in *clv2* mutant plants compensated for the mutant phenotype, emphasizing the importance of expression specificity in determining RLP functions [77]. In addition, overexpression of *AtRLP11* or *AtRLP3* rescued the *clv2–1* mutant phenotype [38], suggesting that CLV2 may regulate the protein stability of other RLPs. This is consistent with the observation that CLV2 regulates the stability of the associated LRR-RLK CLV1 [76]. The maize CLV2 ortholog *FASCIATED EAR 2* (FEA2) was also found to maintain normal shoot and floral meristematic tissue proliferation [78].

*Arabidopsis At*RLP41 is likely involved in abscisic acid (ABA) signaling or perception, as the T-DNA insertion lines exhibited a hyper-sensitivity to exogenous ABA treatment [10]. Since *At*RLP41 is highly induced during senescence, it may regulate ABA-mediated leaf senescence. *Arabidopsis* LRR-RLP *At*RLP44 is important for normal growth, and mediates the activation of brassinosteroid (BR) signaling [79]. BR regulates the expression of many cell wall-related genes, which likely modulate the output of the BR signaling through a feedback regulation. *At*RLP44 is involved in this feedback regulation by sensing cell wall perturbation, specifically pectin modification [79]. This is conceptually similar with plant immune responses triggered by damage-associated molecular patterns (DAMPs), some of which are also cell wall-related components [80]. Some DAMPs have been shown to be transcriptionally induced during plant PTI and are thought to amplify plant PTI signaling [81].

5. RLPs and RLKs come in pairs

5.1. RLPs and RLKs pair in functions

Without a kinase domain, RLPs likely act as receptors or regulators, and transduce signals by complexing with RLKs and other transmembrane/membrane-associated proteins [82, 83]. *Arabidopsis* LRR-RLK SUPPRESSOR OF *BIR1–1* (SOBIR1) and its orthologs have been found in complex with multiple LRR-RLPs, and the function of several of these RLPs requires the formation of this complex, including tomato Ve1 and Cf proteins, and *Arabidopsis At*RLP23 [59, 84]. In addition, these LRR-RLPs associate with and functionally require *Arabidopsis* LRR-RLK, BRI1-ASSOCIATED RECEPTOR KINASE 1 (AtBAK1), also called SOMATIC EMBRYOGENESIS RECEPTOR KINASE 3 (AtSERK3), or its orthologs. SERK family LRR-RLKs are shared co-receptors of multiple LRR-RLKs and usually dimerize with LRR-RLKs upon corresponding ligand perception [85]. Similarly, LRR-RLPs associate with BAK1/SERK complexes upon ligand perception, whereas they constitutively associate with SOBIR1 [59, 61, 86]. It has been shown that SOBIR1 can stabilize LRR-RLPs, which may lead to enhanced kinase activity of SOBIR1 and/or BAK1/SERK3 for signal transduction [84, 86]. BAK1 also associates with AtRLP44, which is involved in the feedback regulation of cell wall integrity through BR signaling [79]. Similarly, rice LysM-RLP *Os*CEBiP interacts with LysM-RLK *Os*CERK1 to activate chitin signaling [54]. *Arabidopsis* AtCERK1 is required for LYM1/LYM3-mediated PGN signaling although it remains unknown whether *At*CERK1 associates with LYM1/LYM3 [68].

Development-related LRR-RLPs also functionally associate with LRR-RLKs. Stomatal development regulator TMM associates with ERECTA (ER) family LRR-RLKs and their coreceptors in the SERK family to perceive EPIDERMAL PATTERNING FACTOR (EPF) peptide signals to regulate stomatal patterning [87, 88]. There are 11 EPF family members in *Arabidopsis* [89]. Although many EPFs could be perceived by ERs, they exhibit different or even opposite functions in stomatal patterning, likely due to the competitive binding between ligands and receptors [90, 91]. The crystal structure of ER and TMM ectodomains with different EPF peptides indicates that TMM determines the specificity of ER in the recognition of different EPFs [92]. To recognize certain EPFs, such as EPF1 and EPF2, TMM must interact with ER. Conversely, the recognition to other EPFs, such as EPFL4 and EPFL6, is inhibited by the interaction of TMM with ER. This represents an example of the role of an RLP in determining ligand-receptor recognition specificity. The function of RLPs in plant immunity might be different from TMM since PRR RLKs usually recognize specific MAMPs. In addition, the SAM regulator CLV2 forms a functional receptor complex with LRR-RLK CLV1 or a transmembrane kinase CORYNE (CRN) in perceiving secreted peptide ligand CLV3 [93, 94].

5.2. RLPs and RLKs pair on chromosome and in expression

It has been hypothesized that the expansion of the LRR-RLK gene family was mediated by a repetitive duplication through some mechanism like tandem or whole genome duplication [8, 11]. Like RLKs, many LRR-RLPs are also physically clustered on chromosomes. Among the 57 LRR-RLPs in *Arabidopsis*, RLP11-RLP16, RLP22-RLP28, RLP30-RLP33, RLP36-RLP43, and RLP47-RLP50 appear to form distinct clusters of more than three genes adjacent to each other (Figure 4).

Many RLPs and RLKs are found in large homogeneous gene clusters (i.e. clusters containing either RLK or RLP genes), and generally these clusters physically alternate between RLKs and RLPs on the chromosomes. Heterogeneous clusters (i.e. clusters containing both RLK and RLP genes) are rarer, and often have a single gene of one type among several of the other type in the cluster. For example, two RLPs, AtRLP2 (AT1G17240) and AtRLP3/RFO2 (AT1G17250), cluster with RLK AT1G17230 on chromosome 1. AtRLP55 (AT5G45770) clusters with RLKs AT5G45780, AT5G45800 and AT5G45840 on chromosome 5. Strikingly, in some cases, physically clustering LRR-RLPs and LRR-RLKs contain highly homologous ectodomains. For instance, the ectodomains of *At*RLP52 (At5g25910) and the RLK AT5G25930, which are separated by only 10 kb on chromosome 5 (Figure 4), share 86% identity in amino acid sequence. Based on these observations, it seems likely that some RLKs and RLPs may share a common ancestor. One or more RLKs may have originated from a fusion event between an RLP and a kinase; similarly, one or more RLPs may be a truncation product of a former RLK.

Several other physically clustering LRR-RLPs and LRR-RLKs are correlated in expression (Figure 4). For example, the RLP SNC2 (At4g18760) and the RLK MORPHOGENESIS OF ROOT HAIR 1 (MRH1) (At4g18640) are correlated in mean-normalized expression during development [95]. Despite their physical proximity and co-expression, SNC2 and MRH1 share little sequence identity. These observations of physical distance and expression beg exciting new questions about the functional roles of these genes with each other and in the broader cellular context.

5.3. RLPs are more divergent than RLKs in different plant species

The phylogenetic analyses indicate that there are subsets of RLPs and RLKs which are conserved across species (Figure 3). This conservation is apparent in several well-studied LRR-RLKs, such as FLS2, BRI1, and BAK1 in *Arabidopsis*, rice, moss, and poplar, suggesting their structural and functional conservation across those species (Figure 3B). However, most RLPs are clustered by plant species, not individual genes (Figure 3A). It is possible that their lack of an intracellular kinase makes RLPs relatively freer of selective pressure, and therefore more predisposed to neofunctionalization. This is consistent with the hypothesis that poplar and *Arabidopsis* LRR-RLPs were evolved independently [96]. One explanation is that the potential fitness cost of an RLP duplication event may be less than that of an RLK duplication event, as RLK redundancy could be more likely to produce maladapted functional changes in cellular perception. It appears that LRR-RLPs involved in plant development, such as TMM and CLV2, are more conserved than LRR-RLPs implicated in plant immunity, such as RFO2 and RBPG1, across plant species (Figure 3A). The phylogenetic analyses also suggest that each lineage likely expanded and diversified its own subset of RLPs. Interestingly, the moss *Physcomitrella patens*, a bryophyte, contains fourteen LRR-RLPs, all of which are in the subset that interleave among species (Figure 3A). This might suggest that a small subset of LRR-RLPs are basally conserved, and that the conditions which favored a major expansion of this core group occurred in a slightly different way for each subsequent lineage after the divergence of polysporangiophytes.

6. Concluding remarks and future perspectives

While RLPs remain the less-examined counterpart to RLKs, their roles in plant immunity and development are increasingly appreciated. The functions of several RLPs with LRR ectodomain have been well characterized. In particular, LRR-RLPs often complex with LRR-RLKs for ligand perception and/or signaling relay. LRR-RLPs may regulate LRR-RLK protein stability or function as a specificity switch for ligand-receptor recognition. Similarly, LYM-RLPs can complex with LYM-RLKs in signal sensing and transduction. However, the functions of RLPs with other ectodomains remain to be elucidated. Interestingly, several LRR-RLPs and LRR-RLKs are physically clustered on chromosomes, and sometimes they correlate in expression pattern. It remains to be determined whether these LRR-RLPs and LRR-RLKs share functional similarity.

Crystal structures of RLK receptor-coreceptor complexes have revealed insights into the potential activation mechanisms of these complexes [21, 97]. The structure of a recent plant RLK-RLP complex revealed a surprising role of the RLP TMM in regulating the ligand recognition specificity by its associated RLK [92]. It will be interesting to determine the structure of other RLPs, in particular immunity-related RLPs, in complex with RLKs upon ligand perception. In several cases, the shared RLK co-receptor BAK1 family of RLKs also participate in RLP-RLK receptor complexing [85]. The structure of these complexes will vastly improve attempts to elucidate the complexities of receptorsome activation and regulation. A recent investigation of 40,000 interactions between 200 *Arabidopsis* LRR-RLK ectodomains was used to construct an “extracellular LRR network”. This identified some previously uncharacterized LRR-RLKs that function in plant growth and immunity [98]. Further analysis revealed that some LRR-RLKs function as articulation points in this network to stabilize the LRR-RLK signaling pathways. A similar approach could be used to study the contribution of LRR-RLPs to these networks. The aforementioned crystal structure and interaction networks of RLKs were based only on their LRR ectodomains. If future studies are able to observe full length protein while retaining the integrity of the receptor complex in its biochemical context at the plasma membrane, this would help avoid perturbations from the native conformation, steric effects, and orientation relative to the bilayer normal of these complexes.

An improvement of the available tools for the continued examination of RLPs will likely be required to reveal the functional roles of RLPs in an expeditious fashion. It remains to be technically changeling to examine the function and interaction of transmembrane receptors. Some techniques, such as split-ubiquitin membrane yeast-two hybrid assays, or membrane anchored ligand and receptor (MALAR) yeast-two hybrid approaches show some promise in identifying interactions between membrane proteins—and even ligands and receptors [99, 100]. However, these techniques are still laborious and tend to produce false positives. Other assays, such as co-immunoprecipitation, gel filtration, and quartz crystal microbalance (QCM) biosensor assays would be required to verify interactions of candidates.

Despite that efforts to improve the experimental design underlying our investigation of RLPs are critical, that which has already been elucidated can be applied to crop improvement efforts. As previously mentioned, simple ectopic expression of functionally important RLPs has proven somewhat effective in conferring favorable resistance traits. It remains to be determined whether it could provide a durable resistance. Elegant solutions to plant-disease can only be obtained through a more complete understanding of the governing biomolecular dynamics.

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Highlights:

- Receptor-like proteins (RLPs) are transmembrane proteins with an extracellular domain and a short cytoplasmic tail functioning in diverse biological processes, including plant development and immunity.
- While different RLP subfamilies contain distinct extracellular domains, the leucine-rich repeat domain RLP subfamily is the most common and well-studied.
- Due to their lack of an intracellular kinase domain, RLPs often complex with receptor-like kinases (RLKs) for ligand perception and/or signaling relay.
- RLPs may regulate RLK protein stability or function as a specificity switch for ligand-receptor recognition.
- Some RLPs and RLKs are physically clustered, and sometimes they correlate in expression and physical proximity—although the biological function is not clear.
- The relatedness of LRR-RLPs across plant species reveals that the apparent diversity of RLPs is likely the result of an arbitrary expansion and neofunctionalization from one or more subsets of these RLPs by each plant species.

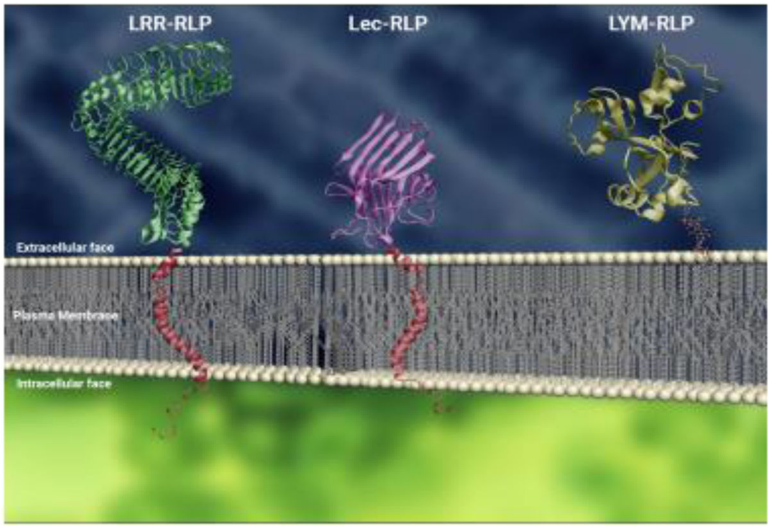

Figure 1: Conceptual representations of RLPs with different ectodomains on the plasma membrane.

From left: LRR-RLP (green), Lec-RLP (purple) and LYM-RLP (gold); transmembrane helices are depicted in red. The transmembrane helices were added to depict a representation of the structure, but do not represent the actual structure of the transmembrane domains of these proteins. The protein structure of OsCEBiP (PDB: 5JCD) represents the LYM in this figure, anchored to the membrane with a GPI anchor. The intracellular area is further indicated by the depiction of short cytoplasmic tails (red). Molecular structures were visualized using Visual Molecular Dynamics (VMD) software [101]. PM and GPI anchor were modeled using Autodesk 3DS Max (2017). Proteins containing domains with similar structure were used to represent RLPs: LRR-RLP (BRI1; PDB: 3RGX4), LecRLP (PHA-E; PDB: 3WCR5) [102, 103].

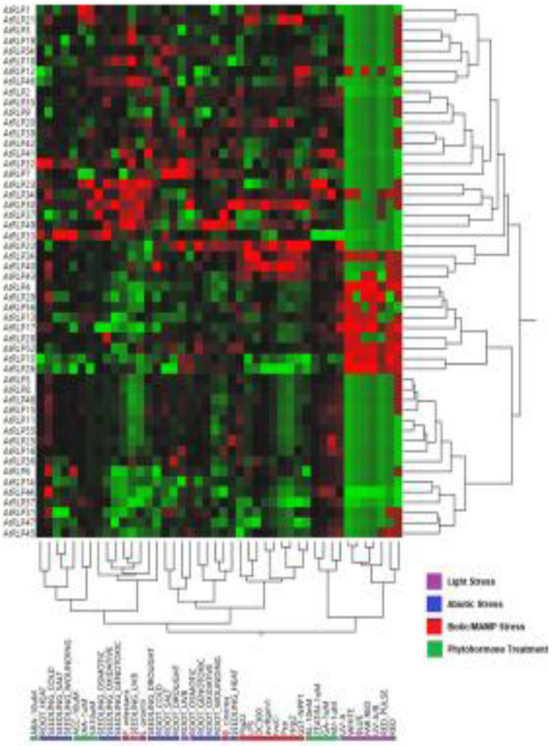

Figure 2: Expression heatmap of LRR-RLPs in *A. thaliana.*

RLPs (Y-axis), and treatments (X-axis) are depicted after hierarchical two-way clustering. Relative transcriptional induction (red) and suppression (green) represent visualization of fold change in expression from the global median using values acquired from the AtGenExpress Visualization Tool [104]. This heatmap was generated using the JMP statistical software.

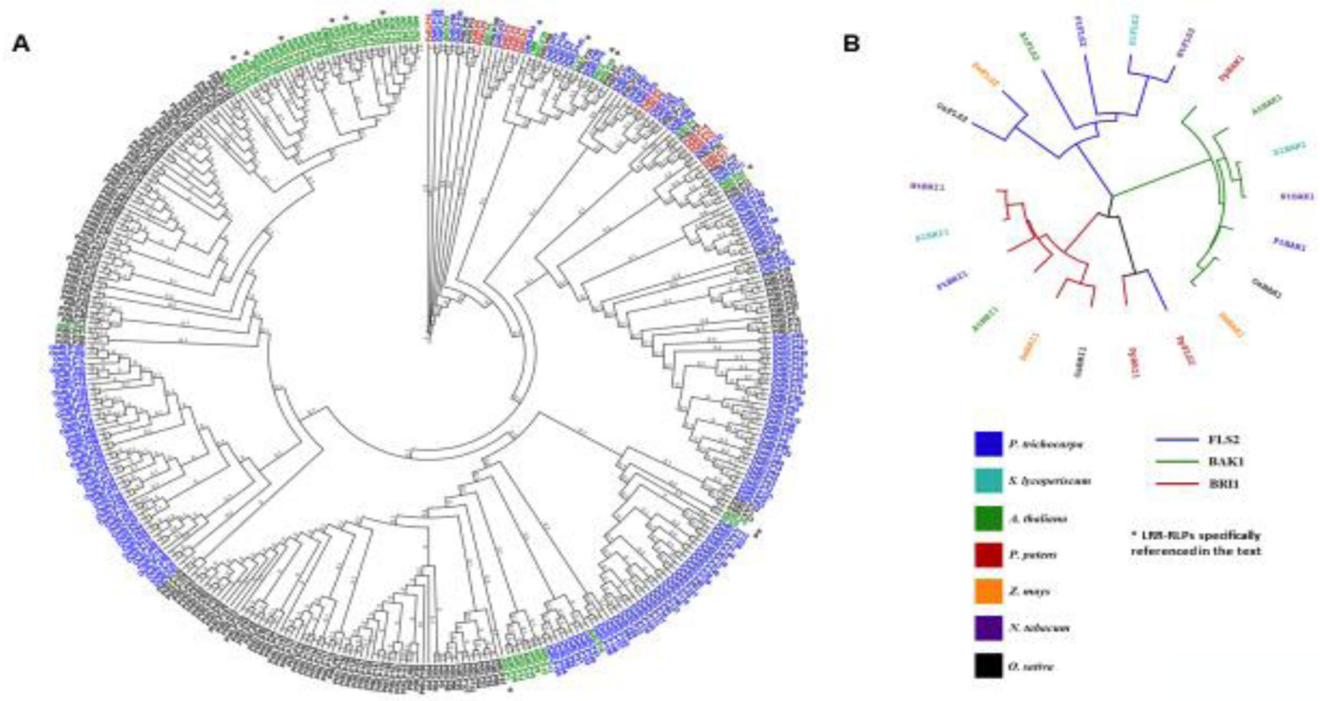

Figure 3. Phylogenetic analysis of RLPs and several well-studied RLKs.

(A) Phylogenetic tree of LRR-RLPs from *A. thaliana* (AtRLPs, green), *P. patens* (PpRLPs, red), *P. trichocarpa* (PtRLPs, black), and *O. sativa* (OsRLPs, blue). LRR-RLPs were identified for each species using the following bioinformatic pipeline: 1.) All Ref-seq proteins were scanned for matches to known protein domains using a client-side instance of EMBL-EBI InterPro Scan [105]. 2.) The dataset was filtered for proteins with predicted LRR domains, but without predicted kinase domains. 3.) The resulting proteins were manually screened for the expected C-terminal TM domain using TMHMM [106]. 4.) Datasets were further screened for the presence of a predicted signal peptide using SignalP 4.1 and PrediSi [107, 108].

(B) Phylogenetic tree containing LRR-RLK FLS2, BRI1 and BAK1 homologs in multiple plant species. The relatedness of RLKs FLS2, BAK1, and BRI1 are indicated with blue, green, and red leaves respectively. The species were chosen to represent the breadth of plant evolution, including bryophytes (*Physcomitrella patens;* red), monocots (*Oryza sativa;* black, *Zea mays;* orange), dicots (*Arabidopsis thaliana;* green, *Nicotiana tabacum*; purple, *Solanum lyoperiscum;* teal), and woody dicots (*Populus trichocarpa;* blue). Tree files were generated using the MEGA software [109], and visualized with iTOL [110]. Protein sequences were aligned using ClustalW [111]. Phylogenetic relationships were inferred with a maximum-likelihood method based on the JTT matrix-based model.

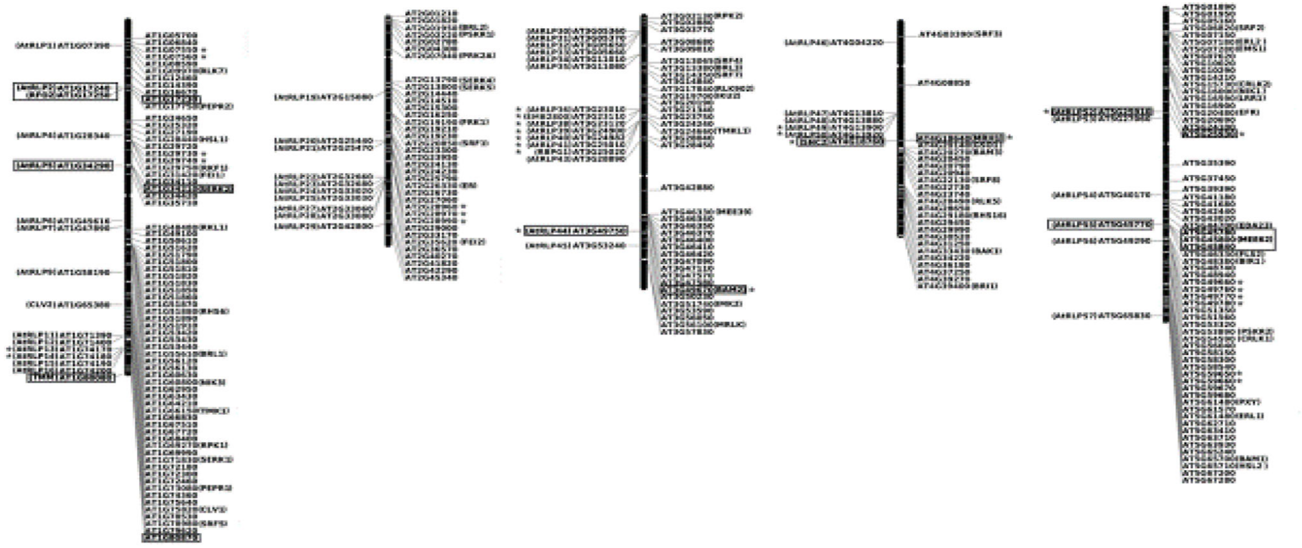

Figure 4. Physical map of *A. thaliana* LRR-RLPs and LRR-RLKs on chromosomes.

RLPs and RLKs (depicted on the left and right of each chromosome, respectively) were positioned using the TAIR chromosome map tool and further arranged for ease visualization. RLPs physically close to RLKs were indicated using boxes. Asterisks indicate proximal RLPs or RLKs with similar expression pattern. Physically clustered RLPs and RLKs were tested for correlations in expression using absolute expression values acquired from the *Arabidopsis* eFP browser (datasets “Developmental Map” and “Biotic Stress” http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi). A Spearman’s rho value with magnitude greater than |ρ| = 0.5 with a p-value of <0.05 was used to determine positive and negative correlations.