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Review

Molecular mechanisms of early plant pattern-triggered immune signaling

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SUMMARY

All eukaryotic organisms have evolved sophisticated immune systems to appropriately respond to biotic stresses. In plants and animals, a key part of this immune system is pattern recognition receptors (PRRs). Plant PRRs are cell-surface-localized receptor kinases (RKs) or receptor proteins (RPs) that sense microbe- or self-derived molecular patterns to regulate pattern-triggered immunity (PTI), a robust form of antimicrobial immunity. Remarkable progress has been made in understanding how PRRs perceive their ligands, form active protein complexes, initiate cell signaling, and ultimately coordinate the cellular reprogramming that leads to PTI. Here, we discuss the critical roles of PRR complex formation and phosphorylation in activating PTI signaling, as well as the emerging paradigm in which receptor-like cytoplasmic kinases (RLCKs) act as executors of signaling downstream of PRR activation.

INTRODUCTION

All eukaryotes deploy cell-surface receptors to perceive extracellular signals. In plants, the largest families of such receptors are the receptor kinases (RKs) and receptor proteins (RPs). Many plant RKs and RPs have been identified as pattern-recognition receptors (PRRs), which perceive self- or non-self-derived elicitors to regulate antimicrobial immunity (Lee et al., 2021). Such elicitors include non-self-microbe- or pathogen-associated molecular patterns (MAMPs or PAMPs) and endogenous damage-associated molecular patterns (DAMPs) or immune-modulating peptides that act as phytocytokines (Gust et al., 2017). Numerous PRRs have been identified that regulate pattern-triggered immunity (PTI) in response to bacterial, fungal, oomycete, nematode, or insect pathogens, and are well documented in recent reviews (Albert et al., 2020; Boutrot and Zipfel, 2017; Lee et al., 2021; Schellenberger et al., 2019).

Upon elicitor perception by cognate PRRs, numerous cell signaling events are initiated, including production of apoplastic reactive oxygen species (ROS), altered ion fluxes, mitogen-activated protein kinase (MAPK) cascades, callose deposition, and large-scale transcriptional programming, together culminating in PTI (Boller and Felix, 2009; Macho and Zipfel, 2014; Yu et al., 2017). PTI constitutes one of the two perception systems of the plant immune system along with intracellular immunity mediated by nucleotide-binding leucine-rich repeat receptors (NLRs) that induce what is typically referred to as effector-triggered immunity (ETI) (Jones and Dangl, 2006).

PTI signaling is a topic of intensive research, and much progress has been made in understanding its pathways, from the mechanisms of ligand perception to the regulation of specific downstream outputs. Here, we focus on the key, interrelated roles of large-scale protein complexes and dynamic phosphorylation in mediating the molecular activation of immune signaling, and suggest directions for future research to expand our understanding of these important pathways.

Plant PRRs are cell surface immune receptors

In mammals, PAMPs and DAMPs are recognized by both extracellular and intracellular receptors (Kagan et al., 2014). In plants, by contrast, all known PRRs are cell-surface localized RKs or RPs (Albert et al., 2020). Plant RKs share structural similarity to metazoan receptor tyrosine kinases (RTKs), featuring an extracellular domain (ECD), a single-pass transmembrane helix, and a cytosolic protein kinase domain, while RPs are similar but lack a cytosolic kinase domain, having instead a short cytosolic tail (Dievart et al., 2020; Wang et al., 2008). These families are enormously expanded within plant lineages, where the receptor-like kinase (RLK) superfamily, which comprises both RKs and related receptor-like cytoplasmic kinases (RLCKs), represents one of the largest gene families in all of the species examined to date (Dievart et al., 2020), including >600 members in the model plant *Arabidopsis thaliana* (hereafter *Arabidopsis*) (Shiu and Bleecker, 2003).

The majority of characterized PRRs possess leucine-rich repeat (LRR) ECDs, which are the most common form of plant RKs and RPs (Shiu and Bleecker, 2001). Two well-studied PRRs in *Arabidopsis* are the LRR-RKs FLAGELLIN-SENSING 2 (FLS2) and ELONGATION FACTOR-TU RECEPTOR (EFR), which perceive the bacterial peptide epitopes flg22 (from flagellin) and elf18 (from Elongation Factor Tu), respectively, to regulate antibacterial immunity (Gómez-Gómez and Boller, 2000; Kunze et al., 2004; Zipfel et al., 2004, 2006). Work with FLS2 and EFR has significantly contributed to the emerging paradigm of plant RK signaling, wherein ligand-binding bridges extracellular interaction between receptor and co-receptor

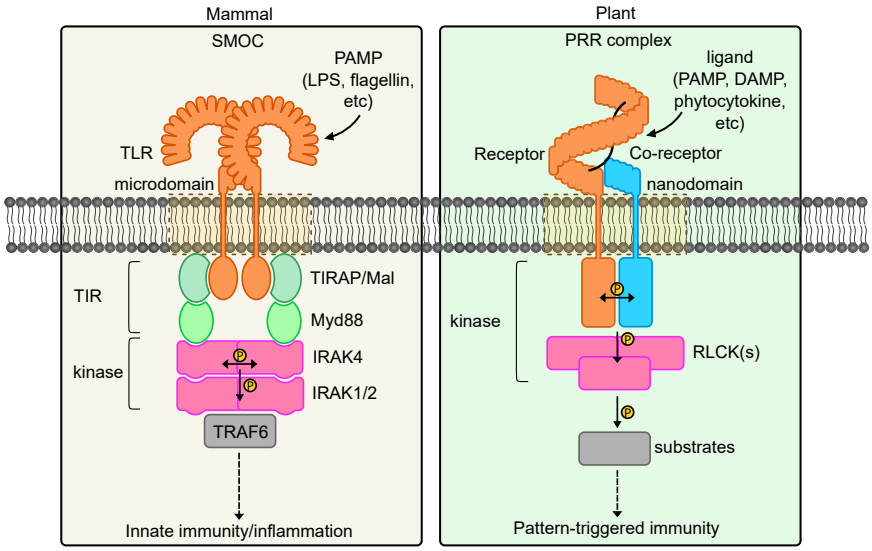

Figure 1. Comparison of mammalian and plant PRR signaling complexes

A simplified model wherein PAMP perception by TLR complexes stimulates the formation of SMOCs is displayed from mammals (left). A generic LRR-RK PRR complex is shown for plants (right). In both models, receptor complex formation recruits and/or activates cytoplasmic kinases of the IRAK/Pelle/RLK superfamily, which then trigger appropriate downstream (immune) responses. In humans, IRAK phosphorylation is thought to primarily occur within the myddosome, while in plants, RLCKs have been found to directly transphosphorylate numerous downstream substrate proteins

(Hohmann et al., 2017). This extracellular interaction in turn brings cytosolic kinase domains into close proximity, and thus allows auto- and/or trans-phosphorylation within the receptor complex. All LRR-RK-type PRRs characterized to date use small, shape-complementary LRR-RKs of the SOMATIC EMBRYOGENESIS RECEPTOR KINASE (SERK) family as co-receptors (Hohmann et al., 2017; Ma et al., 2016). Both EFR and FLS2 specifically form ligand-dependent associations with the SERK isoform BRI1-ASSOCIATED KINASE 1 (BAK1, also called SERK3), and this complex formation is essential for all downstream signaling (Chinchilla et al., 2007; Heese et al., 2007; Roux et al., 2011). LRR-RPs function similarly to LRR-RKs despite their lack of a cytosolic domain, wherein the LRR-RP interacts with an adaptor LRR-RK, SUPPRESSOR OF BIR1 (SOBIR1), to form a bipartite receptor (Albert et al., 2015; Liebrand et al., 2013; Postma et al., 2016); this complex can thereby form a ligand-induced interaction with a SERK co-receptor in a manner similar to LRR-RKs (Gust and Felix, 2014).

While the majority of characterized PRRs feature an LRR-type ECD, numerous others have been identified in plants. The LysM-RKs CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1) and LYSM-CONTAINING RECEPTOR-LIKE KINASE 5 (LYK5), for example, form a chitin receptor complex in *Arabidopsis* to regulate antifungal immunity (Cao et al., 2014). Interestingly, while chitin perception is broadly conserved across plant species, there are variations in the exact nature of ligand-binding and receptor composition. In rice (*Oryza sativa*), for example, OsCERK1 does not actually participate in chitin binding, which is instead achieved by the glycosylphosphatidylinositol (GPI)-anchored LysM RP OsCEPiB, with OsCERK1 acting as a co-receptor to initiate signaling via its cytosolic kinase domain (Gong et al., 2017). Other non-LRR type PRRs include the *Arabidopsis* G-lectin RK LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION (LORE), which recognizes bacterial 3-OH-FAs (Kutschera et al., 2019; Ranf et al., 2015), the L-lectin RKs DOES NOT RESPOND TO NUCLEOTIDES 1 (DORN1), and LecRK-I.8, which recognize extracellular ATP and NAD^+, respectively, as DAMPs (Choi et al., 2014; Wang et al., 2017a), and EGF-type RKs of the WALL-ASSOCIATED KINASE (WAK) family, which are proposed to recognize plant cell wall-derived oligogalacturonides (OGs) (Brutus et al., 2010; Kohorn and Kohorn, 2012).

Aside from receptor and co-receptor(s), most PRR complexes examined to date have been found to involve one or more RLCK. RLCKs share homology with RKs but lack an ECD. Some RLCKs possess a transmembrane helix, while others are associated with the plasma membrane via N-terminal acylation (Liang and Zhou, 2018). RLCKs are hypothesized to act as executors of downstream cytosolic signaling events, and members of the large RLCK-VII family (also named PBS1-LIKE KINASES, PBLs), in particular, have been identified as key components of immune signaling (Couto and Zipfel, 2016; Rao et al., 2018; Zhang et al., 2010). Thus, while the specific composition of individual PRR complexes vary and are not completely resolved, a model has emerged in which such complexes contain at minimum a ligand-binding RK/RP, co-RK(s), and RLCK(s), with RLCK(s) propagating signal transduction via phosphorylation of downstream substrate proteins (Figure 1).

Supramolecular PRR signaling platforms

Plant PRRs share key conceptual similarities to both RTKs and TOLL-LIKE RECEPTORs (TLRs) in metazoans (Figure 1). RTKs share similar topology with plant RKs, although neither the ECDs nor kinase domains of RTKs and RKs are homologous (Shiu and Bleecker, 2001), and in the case of RTKs, downstream signaling is achieved via the recruitment of phosphotyrosine-binding proteins, for which little evidence is available in plants (Lim and Pawson, 2010; Macho et al., 2015).

TLRs, however, like many plant PRRs, use LRR-type ECDs to perceive PAMPs, such as bacterial flagellin or lipopolysaccharide (LPS) (Fitzgerald and Kagan, 2020). Interestingly, the mechanism of flagellin perception by human TLR5 is distinct from that of plant PRRs (Fliegmann and Felix, 2016), which include the widely conserved, aforementioned FLS2 (recognizing flg22) and the Solanaceae-specific FLS3 (recognizing the flgII-28 epitope) (Hind et al., 2016). However, a key difference between

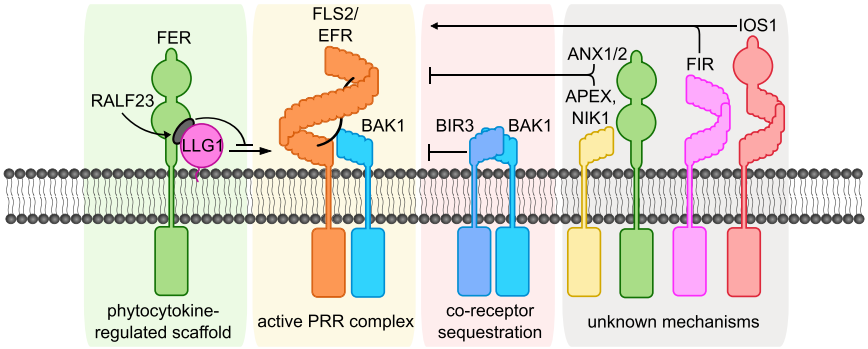

Figure 2. Regulatory RKs modulate PRR complex formation
Examples of regulatory *Arabidopsis* RKs that modulate FLS2 and/or EFR complex formation with BAK1 are displayed. BIR3 acts as a key negative regulator of complex formation by binding and sequestering BAK1 in the absence of ligand. FER and its co-receptor LLG1 act as positive regulators of complex formation, which is in turn inhibited by the perception of RALF23 by the FER-LLG1 receptor complex. The molecular mechanisms by which additional regulatory RKs influence PRR complex formation are to date unknown.

TLRs and plant PRRs is the intracellular domain involved, which in the case of TLRs is a Toll/interleukin-1 (IL-1) receptor (TIR) domain as opposed to the kinase domain of plant RKs. TLRs form so-called supramolecular organizing centers (SMOCs), such as myddosomes, which are specialized protein complex-derived cell signaling organelles that are dynamically formed upon infection or wounding in mammals (Balka and De Nardo, 2019; Kagan et al., 2014; Latty et al., 2018). TLRs form homo- or heterodimers upon ligand perception, which leads to the recruitment of adaptor proteins such as MYELOID DIFFERENTIATION PRIMARY RESPONSE 88 (MYD88) and TIR DOMAIN CONTAINING ADAPTOR PROTEIN (TIRAP/Mal) to the cytosolic TIR domain of the TLR dimer (Kagan et al., 2014; Latty et al., 2018). Adaptor proteins in turn nucleate polymerization of the SMOC, recruiting kinases of the IL-1 RECEPTOR ASSOCIATED KINASE (IRAK) family of tyrosine kinase-like kinases to form an active myddosome, which recruits the E3-ligase TRAF6 to trigger activate downstream inflammation/immune signaling.

In contrast to TLRs, plant PRRs do not require TIR-TIR domain interaction-mediated polymerization and instead rely on direct interaction between the homologous kinase domains of RKs and RLCKs (Figure 1). Interestingly, the kinase domain of the plant RLK superfamily is homologous to the metazoan IRAK/Pelle family (Dievart et al., 2020; Shiu and Bleecker, 2001), which is indicative of a convergent role for these distantly related RLCKs in mediating signaling downstream of transmembrane receptors across kingdoms (Liang and Zhou, 2018), although with key differences. In the case of TLR signaling, IRAK kinase activity is thought to primarily mediate auto- and trans-phosphorylation within the myddosome, which allows the recruitment of TRAF6 (Balka and De Nardo, 2019; Fitzgerald and Kagan, 2020), although several other IRAK substrates have been identified (Smith et al., 2009; Uematsu et al., 2005; Zhang and Ghosh, 2002). In contrast to the induced polymerization within SMOCs, RLCKs are pre-associated with PRRs in plants, while ligand perception leads to co-receptor recruitment and RLCK activation via phosphorylation (discussed below). Importantly, RLCKs have been found to subsequently activate downstream signaling via direct trans-phosphorylation of substrate proteins (discussed in detail below).

While we now have extensive data on how ligand-induced receptor and co-receptor PRR complexes form in plants, there is no direct evidence regarding the stoichiometry of RLCKs and/or other cytosolic components within active PRR complexes. Furthermore, while LRR-RKs and their co-RKs form 1:1 complexes *in vitro* (Hohmann et al., 2017), it has not yet been resolved whether such heterodimers can associate to form larger complexes *in vivo*. Cell-surface receptors are also spatially regulated within the plasma membrane (Figure 1), and TLRs have been documented in detergent-resistant membrane fractions and localize within distinct microdomains (Katagiri et al., 2001; Triantafilou et al., 2004), which are thought to facilitate ligand-induced interaction (Fessler and Parks, 2011). Mammalian RTKs also localize to specific plasma membrane nanodomains, which can be arranged in a polar manner across the cell (Gao et al., 2015). Cell biological work has now shown that plant RKs also localize to distinct nanodomains within the plasma membrane, which may facilitate pathway-specific signaling (Bücherl et al., 2017; Gronnier et al., 2020). However, the nature and stoichiometry of these nanodomains remains to be definitively resolved.

Regulatory RKs and PRR complex formation

Another layer of complexity in plant PRR complex formation is the role of so-called regulatory or accessory RKs, which can modulate the ligand-induced interaction between PRRs and their co-receptors (Figure 2). For instance, members of the BAK1-INTERACTING RECEPTOR-LIKE KINASE (BIR) family such as BIR3 interact with BAK1 in the resting state and are thought to sequester it to prevent the ectopic activation of immune signaling (Hohmann et al., 2020; Imkampe et al., 2017; Ma et al., 2017). FERONIA (FER), however, is a malectin-like RK of the CrRLK1L family, which together with the GPI-anchored protein LORELEI-like-GPI-anchored protein 1 (LLG1) can promote FLS2-BAK1 complex formation (Stegmann et al., 2017; Xiao et al., 2019). FER and LLG1 both associate with components of the PRR complex *in vivo*, suggesting that they can function as scaffolds and/or chaperones (Shen et al., 2017; Stegmann et al., 2017). This promotion of complex formation is inhibited by the perception of peptides of the RAPID ALKALINZATION FACTOR (RALF) family, such as RALF23, which here acts as an inhibitory phytocytokine (Stegmann et al., 2017). RALF23 is perceived by the FER-LLG1 receptor complex (Stegmann et al., 2017; Xiao et al., 2019), which presumably allows for the fine-tuning of immune responses. This modulation of FLS2-BAK1 complex formation by FER and RALF23 is likely due to the altered mobility of FLS2 within the

Table 1. Phosphosites within PRR complexes

Species	PRR complex component	Phosphosite region	Phosphosite position	Responsible kinase	Method	Molecular function	Reference
Arabidopsis	BAK1	kinase domain (VIa)	Y403	N.D. (BAK1?)	MS (*in vitro*), SDM, immunoblotting	activation of Tyr-VIa-type LRR-RK signaling	Perraki et al., 2018
Arabidopsis	BAK1	C-tail	S612	N.D. (BAK1?)	MS (*in vivo*), SDM, immunoblotting	activation of Tyr-VIa-type LRR-RK signaling	Perraki et al., 2018
Arabidopsis	BAK1	C-tail	S602/T603/S604	N.D.	MS (*in vivo*), SDM	activation of Tyr-VIa-type LRR-RK signaling	Perraki et al., 2018
Arabidopsis	BIK1	N-terminal β-loop	S89/T90	EFR	MS (*in vitro*), SDM	activation of BIK1, immunity	Lal et al., 2018
Arabidopsis	BIK1	activation loop	S236/T237	BAK1	SDM	activation and stability of BIK1, immunity	Lu et al., 2010; Feng et al., 2012; Wang et al., 2018
Arabidopsis	BIK1	activation loop	S236	SIK1/MAP4K3	MS (*in vitro*)	BIK1 stability, activation	Zhang et al., 2018
Arabidopsis	BIK1	activation loop	S233, S236, T242	MAP4K4	MS (*in vitro*)	BIK1 stability, activation	Jiang et al., 2019
Arabidopsis	BSK1	kinase domain	S230	RLK902	MS (*in vivo*), SDM + *in vitro* assay	full resistance to *Pseudomonas syringae*	Zhao et al., 2019
Arabidopsis	BSK5	kinase domain	S209/T210	EFR, PEPR1	SDM + *in vitro* assay	full PAMP-induced resistance	Majhi et al., 2019
Arabidopsis	CERK1	cytosolic juxtamembrane domain	S268, S282/T283	BAK1	MS (*in vivo*), SDM	priming of antifungal immune responses	Gong et al., 2019
Arabidopsis	CERK1	cytosolic juxtamembrane domain	S266, S268, S270, S270	N.D.	MS (*in vivo*)	chitin induced; unknown	Petutschnig et al., 2010
Arabidopsis	DORN1	kinase domain	S391, S440, S451	DORN1	MS (*in vivo*), SDM	activation of immune signaling	Chen et al., 2017
Arabidopsis	EFR	kinase domain (VIa)	Y836	N.D.	MS (*in vivo*), SDM	activation of immune signaling	Macho et al., 2014
Arabidopsis	EFR	αC-helix	S753	N.D.	MS (*in vivo*), SDM	inhibition of immune signaling	Bender et al., 2021
Arabidopsis	EFR	activation loop	S887/S888	N.D.	MS (*in vivo*), SDM	activation of immune signaling	Bender et al., 2021
Arabidopsis	FLS2	kinase domain (IV)	S938	FLS2?	MS (*in vitro*), SDM	activation of immune signaling	Cao et al., 2013
Arabidopsis	PBL27	N.D.	N.D.	CERK1	*in vitro* assay	full chitin-induced MAPK activation (?)	Shinya et al., 2014
Arabidopsis	CERK1	kinase domain (VIa)	Y428	CERK1	MS (*in vitro*), SDM, immunoblotting	activation of immune signaling	Suzuki et al., 2016; Liu et al., 2018
Arabidopsis	CERK1	kinase domain	T479, T573	CERK1	MS (*in vitro*), SDM	activation of immune signaling	Suzuki et al., 2016

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Table 1. *Continued*

Species	PRR complex component	Phosphosite region	Phosphosite position	Responsible kinase	Method	Molecular function	Reference
Arabidopsis	CERK1	kinase domain	S493	CERK1	MS (*in vitro*), SDM	transphosphorylation of substrates (PBL27)	Suzuki et al., 2019
Arabidopsis	FLS2	juxtamembrane domain	T867	N.D.	SDM	receptor activation and endocytosis	Robatzek et al., 2006
Arabidopsis	LORE	kinase domain (VIa)	Y600	N.D.	MS (*in vitro*), SDM, immunoblotting	activation of immune signaling	Luo et al., 2020
Rice	OsRLCK185	activation loop	S240A, T241A, T246A	OsCERK1	SDM + *in vitro* assay	activation of OsRLCK185, immunity	Yamaguchi et al., 2013
Rice	XA21	juxtamembrane domain	S686, T688, S689	XA21	Tryptic phosphopeptide mapping, SDM	XA21 stability	Xu et al., 2006
Rice	XA21	juxtamembrane domain	T705	XA21	SDM	autophosphorylation and immune signaling	Chen et al., 2010

MS, mass spectrometry; N.D., not determined; SDM, site-directed mutagenesis.

plasma membrane (Gronnier et al., 2020). In contrast to FER, the related *Cr*RLK1Ls ANXUR1 (ANX1) and ANX2 act as negative regulators of FLS2-BAK1 complex formation (Mang et al., 2017), although whether this is also RALF regulated is unknown.

Additional regulatory RKs can also promote (e.g., INDUCED OOMYCETE SUSCEPTIBILITY 1 [IOS1], an LRR-malectin-RK [Yeh et al., 2016] or FLS2-INTERACTING RECEPTOR [FIR], an LRR-RK [Smakowska-Luzan et al., 2018]) or inhibit (e.g., APEX [Smakowska-Luzan et al., 2018] or NSP-INTERACTING KINASE 1 [NIK1] [Li et al., 2019], both short ECD LRR-RKs) the formation of active *Arabidopsis* PRR complexes, although the mechanisms by which these RKs regulate PRR signaling remains undetermined. Members of the WAK family have also been identified as positive regulators of PRR complexes in tomato (Zhang et al., 2020) and cotton (Wang et al., 2020), and *Arabidopsis* WAKs have also recently been shown to regulate positively responses triggered by multiple elicitors (Kohorn et al., 2021). Given the contrasting roles of various accessory RKs, important questions remain regarding the exact composition and dynamics of PRR complexes in the absence and presence of ligand. Accessory RKs can themselves also associate with cytosolic proteins, such as the RLCK-VII isoform RIPK/PBL14 in the case of FER (Du et al., 2016), raising the intriguing possibility that accessory RKs may facilitate polymerization of cytosolic complexes as well.

RK phosphorylation within PRR complexes

Given that plant RKs are characterized by their dual-specificity Tyr and Ser/Thr kinase domain (Macho et al., 2015; Shiu and Bleecker, 2001), the analysis of phosphorylation events in plant PRR-mediated immune signaling has been an area of intense research for decades, and, as such, numerous RK phosphosites were shown to regulate PTI (Table 1).

BAK1 is the primary co-receptor for numerous LRR-RKs regulating both immunity (such as FLS2 and EFR) and growth and development, such as the brassinosteroid (BR) receptor BRASSINOSTEROID INSENSITIVE 1 (BRI1) (Li et al., 2002; Nam and Li, 2002). Given the antagonism between BR and immune signaling (Albrecht et al., 2012; Belkhadir et al., 2012), the shared role of BAK1 raises the critical question of how pathway-specific signaling is achieved at the molecular level. BRI1 and FLS2 are spatially separated through their localization to distinct nanodomains (Bücherl et al., 2017), which may partially underlie how these pathways can be differentially activated by their distinct ligands. However, the isolation of the *bak1-5* allele, which is caused by a C408Y mutation in the BAK1 cytosolic domain and disrupts BAK1 function in immunity but not BR signaling, suggested that differential phosphorylation may support pathway-specific functions for BAK1 (Schwessinger et al., 2011). This has been further resolved through the identification of a suite of BAK1 phosphosites that together constitute a phosphocode required for PTI but not BR signaling (Perraki et al., 2018). These BAK1 phosphocode sites include several Ser/Thr sites within the BAK1 C-terminal tail, which was previously suggested to be specifically essential for BAK1 immune function (Wu et al., 2018). This phosphocode is not, however, indicative of growth versus

immunity signaling per se, as several non-immune LRR-RKs are also sensitive to BAK1 phosphocode disruption, such as ERECTA (ER) or PHYTOSULFOKINE (PSK) RECEPTOR 1 and 2 (PSKR1/2) (Perraki et al., 2018), which regulate stomatal patterning or cell expansion and root growth, respectively (Ma et al., 2016).

Aside from sites within the C-tail, the BAK1 phosphocode also includes a conserved phosphotyrosine site, Y403, within kinase subdomain VIa (Perraki et al., 2018). Intriguingly, the homologous Tyr-VIa residue is required for immune activation by EFR (Macho et al., 2014) as well as the developmental LRR-RK ER (Perraki et al., 2018). Thus, it appears to be the presence or absence of this Tyr-VIa site that determines whether a given LRR-RK is sensitive to the BAK1 phosphocode, as BRI1 possesses a Y-to-F substitution at this position. Interestingly, the Tyr-VIa residue is also critical for functioning of the non-LRR-RK PRRs CERK1 (Liu et al., 2018; Suzuki et al., 2016) and LORE (Luo et al., 2020), suggesting a conserved mechanism for this phosphosite in activating RK signaling.

Other regulatory phosphosites within the cytosolic domain of plant PRRs have been identified that regulate the activation, catalytic activity, and/or turnover and endocytosis. In addition to Tyr-VIa, a number of regulatory phosphosites were recently characterized on EFR, including sites that both negatively (S753, within the αC-helix) and positively (S887/S888, in the activation loop) regulate elf18-induced immune signaling (Bender et al., 2021). Several autophosphorylation sites within the CERK1 kinase domain (including the Tyr-VIa residue, Y428), have been identified as critical for downstream signaling by CERK1 (Liu et al., 2018; Suzuki et al., 2016), which may regulate its overall kinase activity. A screen for potential FLS2 autophosphorylation sites identified S938 within kinase subdomain IV as required for downstream signaling *in vivo* (Cao et al., 2013). XA21, a rice LRR-RK PRR that mediates antibacterial immunity (Song et al., 1995), also requires phosphorylation at several cytosolic juxtamembrane domain residues for its activity and/or stability (Chen et al., 2010; Xu et al., 2006). Interestingly, several chitin-induced autophosphorylation sites have also been identified in the juxtamembrane domain of CERK1 (Petutschnig et al., 2010), although to date the role of these sites remains unknown. FLS2 juxtamembrane phosphorylation also affects its function, possibly by regulating endocytosis following activation (Robatzek et al., 2006). Similarly, phosphorylation of LYK5 by CERK1 is postulated to regulate chitin-induced LYK5 endocytosis, although specific phosphosite(s) involved remain to be identified (Erwig et al., 2017). Finally, phosphorylation of S493 in the CERK1 kinase domain was identified as being important for chitin-induced signaling. Interestingly, while this site did not regulate *in vitro* CERK1 autophosphorylation activity, it did affect the transphosphorylation of substrates, including the RLCK PBL27 (Suzuki et al., 2019), while Y557 phosphorylation also regulates interactions with RLCKs (Liu et al., 2018), suggesting that RK phosphorylation may regulate the activation of RLCK(s) within the PRR complex.

The endogenous kinase activity of EFR was recently found to be dispensable for elf18-induced BAK1-EFR phosphorylation and immune signaling (Bender et al., 2021), suggesting that EFR does not activate its co-receptor via trans-phosphorylation. Interestingly, regulatory phosphosites on EFR did modulate BAK1 autophosphorylation/activation, suggesting an allosteric mechanism of PRR complex activation. Further work will be critical to resolving the molecular mechanisms of phosphorylation-dependent RK activation within PRR complexes, especially those involving non-RD kinases, such as EFR, as such kinases play particularly important roles in innate immunity across kingdoms (Dardick et al., 2012; Schwessinger et al., 2011).

Consistent with the importance of phosphorylation in activating RK-mediated immune signaling, phosphatases have been identified that directly interact with several such RKs. The PP2C isoforms XB15 and POLTERGEIST-LIKE 4 and 5 (PLL4 and 5) are homologs from rice and *Arabidopsis* that were identified as interactors of XA21 and *Arabidopsis* EFR, respectively, where they act as negative regulators of immunity (DeFalco et al., 2021; Holton et al., 2015; Park et al., 2008). Other PP2Cs have been identified that regulate CERK1 (Liu et al., 2018), while a PP2A complex was found to negatively regulate the activation of BAK1 (Segonzac et al., 2014).

Activation of RLCKs by PRRs

Members of several RLCK families have been found to function downstream of PRRs (Table 2), including most prominently RLCK-VII/PBL and RLCK-XII/BRASSINOSTEROID SIGNALING KINASE (BSK) isoforms (Liang and Zhou, 2018). BSKs were first identified as functioning downstream of BRI1 to positively regulate BR signaling (Tang et al., 2008); however, *Arabidopsis* BSK1 and its rice homolog were also found to function in PTI (Shi et al., 2013; Wang et al., 2017c). There have been conflicting reports of BSK kinase activities (Grütter et al., 2013; Tang et al., 2008), and some isoforms have been proposed to function as pseudokinase scaffolds (Ren et al., 2019). However, phosphorylation of the BSK1 kinase domain is critical for its function in BR signaling (Tang et al., 2008) and for full resistance to bacterial infection (Zhao et al., 2019). BSK5 has also been identified as a positive regulator of PTI, and can be phosphorylated at regulatory sites by PRRs such as EFR and AtPEP RECEPTOR 1 (PEPR1) (Majhi et al., 2019), although the role of BSK5 in downstream signaling is not fully resolved.

RLCK-VII/PBLs, however, are highly active kinases and have been found to directly phosphorylate numerous substrate proteins to initiate PTI signaling (discussed below). Among RLCK-VII isoforms, BOTRYTIS-INDUCED KINASE 1 (BIK1) (Veronese et al., 2006) and its close homolog PBL1 have emerged as key convergent signaling hubs downstream of diverse PRR complexes (DeFalco et al., 2021; Kadota et al., 2014; Li et al., 2014; Liu et al., 2013; Lu et al., 2010; Monaghan et al., 2015; Ranf et al., 2014; Zhang et al., 2010). Activation of BIK1 relies on its phosphorylation, which can be rapidly detected upon PAMP perception (Lu et al., 2010; Zhang et al., 2010). Several activating phosphosites have been mapped on BIK1, including the activation loop residues S236/T237 (Lu et al., 2010; Zhang et al., 2010), which can be phosphorylated by BAK1 (Feng et al., 2012). These activation loop sites are broadly conserved across related kinases (Wang et al., 2018), indicating a conserved mechanism by which these kinases are activated within PRR complexes. Similarly, CERK1 was shown to directly phosphorylate PBL27 (Shinya et al., 2014), although specific

Table 2. RLCKs functioning downstream of PRRs

Ligand	Type	Source	Receptor type	Receptor	Co-receptor	Species	RLCK	RLCK family	References
elf18	PAMP	bacteria	LRR-RK	EFR	BAK1	*Arabidopsis*	BIK1, PBL1 (RLCK-VII-8)	VII	Lu et al., 2010; Zhang et al., 2010; Liu et al., 2013; Kadota et al., 2014, Li et al., 2014; Ranf et al., 2014; Rao et al., 2018
elf18	PAMP	bacteria	LRR-RK	EFR	BAK1	*Arabidopsis*	BSK5	XII	Majhi et al., 2019
elf18	PAMP	bacteria	LRR-RK	EFR	BAK1	*Arabidopsis*	PBL13	VII	Lin et al., 2015; Lee et al., 2020
elf18	PAMP	bacteria	LRR-RK	EFR	BAK1	*Arabidopsis*	PCRK1/PBL39, PCRK2/PBL40	VII	Sreekanta et al., 2015; Kong et al., 2016
elf18	PAMP	bacteria	LRR-RK	EFR	BAK1	*Arabidopsis*	RLCK-VII-5	VII	Rao et al., 2018
elf18	PAMP	bacteria	LRR-RK	EFR	BAK1	*Arabidopsis*	RLCK-VII-7	VII	Rao et al., 2018
elf18	PAMP	bacteria	LRR-RK	EFR	BAK1	*Arabidopsis*	PBL19 (RLCK-VII-4)	VII	Bi et al., 2018; Rao et al., 2018
flg22	PAMP	bacteria	LRR-RK	FLS2	BAK1	*Arabidopsis*	BIK1, PBL1 (RLCK-VII-8)	VII	Lu et al., 2010; Zhang et al., 2010; Liu et al., 2013; Kadota et al., 2014, Li et al., 2014; Ranf et al., 2014; Rao et al., 2018
flg22	PAMP	bacteria	LRR-RK	FLS2	BAK1	*Arabidopsis*	BSK1	XII	Shi et al., 2013
flg22	PAMP	bacteria	LRR-RK	FLS2	BAK1	*Arabidopsis*	PCRK1/PBL39, PCRK2/PBL40	VII	Sreekanta et al., 2015; Kong et al., 2016
flg22	PAMP	bacteria	LRR-RK	FLS2	BAK1	*Arabidopsis*	PBL13	VII	Lin et al., 2015; Lee et al., 2020
flg22	PAMP	bacteria	LRR-RK	FLS2	BAK1	*Arabidopsis*	RLCK-VII-5	VII	Rao et al., 2018
flg22	PAMP	bacteria	LRR-RK	FLS2	BAK1	*Arabidopsis*	RLCK-VII-7	VII	Rao et al., 2018
flg22	PAMP	bacteria	LRR-RK	FLS2	BAK1	*Arabidopsis*	PBL19 (RLCK-VII-4)	VII	Bi et al., 2018; Rao et al., 2018
Atpeps	phyocytokine	self	LRR-RK	PEPR1/2	BAK1	*Arabidopsis*	BIK1, PBL1 (RLCK-VII-8)	VII	Lu et al., 2010; Zhang et al., 2010; Liu et al., 2013; Kadota et al., 2014, Li et al., 2014; Ranf et al., 2014; Rao et al., 2018
Atpeps	phyocytokine	self	LRR-RK	PEPR1/2	BAK1	*Arabidopsis*	BSK5	XII	Majhi et al., 2019
Atpeps	phyocytokine	self	LRR-RK	PEPR1/2	BAK1	*Arabidopsis*	PBL19 (RLCK-VII-4)	VII	Bi et al., 2018
nlp20	PAMP	bacteria	LRR-RP	RLP23	SOBIR/SERKs	*Arabidopsis*	PBL31 (RLCK-VII-7)	VII	Pruitt et al., 2020
3-OH-FAs	PAMP	bacteria	G-lectin-RK	LORE	N.D.	*Arabidopsis*	PBL34 (RLCK-VII-5)	VII	Luo et al., 2020

(*Continued on next page*)

Table 2. *Continued*

Ligand	Type	Source	Receptor type	Receptor	Co-receptor	Species	RLCK	RLCK family	References
RALF23	Phytocytokine	self	GPI-AP	LLG1	FER	*Arabidopsis*	PBL14/RIPK	VII	Du et al., 2016
Chitin	PAMP	fungi	LysM-RK	LYK4/5	CERK1	*Arabidopsis*	BIK1, PBL1	VII	Lu et al., 2010; Zhang et al., 2010; Liu et al., 2013; Kadota et al., 2014; Li et al., 2014; Ranf et al., 2014
Chitin	PAMP	fungi	LysM-RK	LYK4/5	CERK1	*Arabidopsis*	PBL27	VII	Yamada et al., 2016a
Chitin	PAMP	fungi	LysM-RK	LYK4/5	CERK1	*Arabidopsis*	RLCK-VII-5	VII	Rao et al., 2018
Chitin	PAMP	fungi	LysM-RK	LYK4/5	CERK1	*Arabidopsis*	RLCK-VII-7	VII	Rao et al., 2018
Chitin	PAMP	fungi	LysM-RK	LYK4/5	CERK1	*Arabidopsis*	PBL19 (RLCK-VII-4)	VII	Bi et al., 2018; Rao et al., 2018
flg22	PAMP	bacteria	LRR-RK	SlFLS2	SERKs	tomato	PTI1	VIII	Schwizer et al., 2017
flgII-28	PAMP	bacteria	LRR-RK	SlFLS3	SERKs	tomato	PTI1	VIII	Schwizer et al., 2017
Avr4	PAMP	fungi	LRR-RP	Cf-4	SOBIR/SERKs	tomato	ACIK1	VII	Rowland et al., 2005
Avr9	PAMP	fungi	LRR-RP	Cf-9	SOBIR/SERKs	tomato	ACIK1	VII	Rowland et al., 2005
RaxX	PAMP	bacteria	LRR-RK	XA21	SERKs	rice	OsRLCK107, OsRLCK57, OsRLCK118, OsRLCK176	VII	Zhou et al., 2016
RaxX	PAMP	bacteria	LRR-RK	XA21	SERKs	rice	OsRLCK102	VII	Wang et al., 2016
Chitin	PAMP	fungi	LysM-RP	OsCEBiP	OsCERK1	rice	OsRLCK185	VII	Wang et al., 2017b; Xu et al., 2017; Yamada et al., 2017
Chitin	PAMP	fungi	LysM-RP	OsCEBiP	OsCERK1	rice	OsRLCK176	VII	Ao et al., 2014
Chitin	PAMP	fungi	LysM-RP	OsCEBiP	OsCERK1	rice	OsBSR1	VII	Kanda et al., 2017
Chitin	PAMP	fungi	LysM-RP	OsCEBiP	OsCERK1	rice	OsRLCK107, OsRLCK57, OsRLCK118	VII	Li et al., 2017
Chitin	PAMP	fungi	LysM-RP	OsCEBiP	OsCERK1	rice	OsBSK1-2	XII	Wang et al., 2017c
peptidoglycan	PAMP	bacteria	LysM-RK	OsLYP4/OsLYP6	OsCERK1	rice	OsRLCK176	VII	Ao et al., 2014
peptidoglycan	PAMP	bacteria	LysM-RK	OsLYP4/OsLYP6	OsCERK1	rice	OsRLCK107, OsRLCK57, OsRLCK118	VII	Li et al., 2017
lipochitooligsaccharides	Nod factors	bacteria	LysM-RK	LjNFR1/LjNFR5	SymRK?	lotus	NiCK4	N.D.	Wong et al., 2019

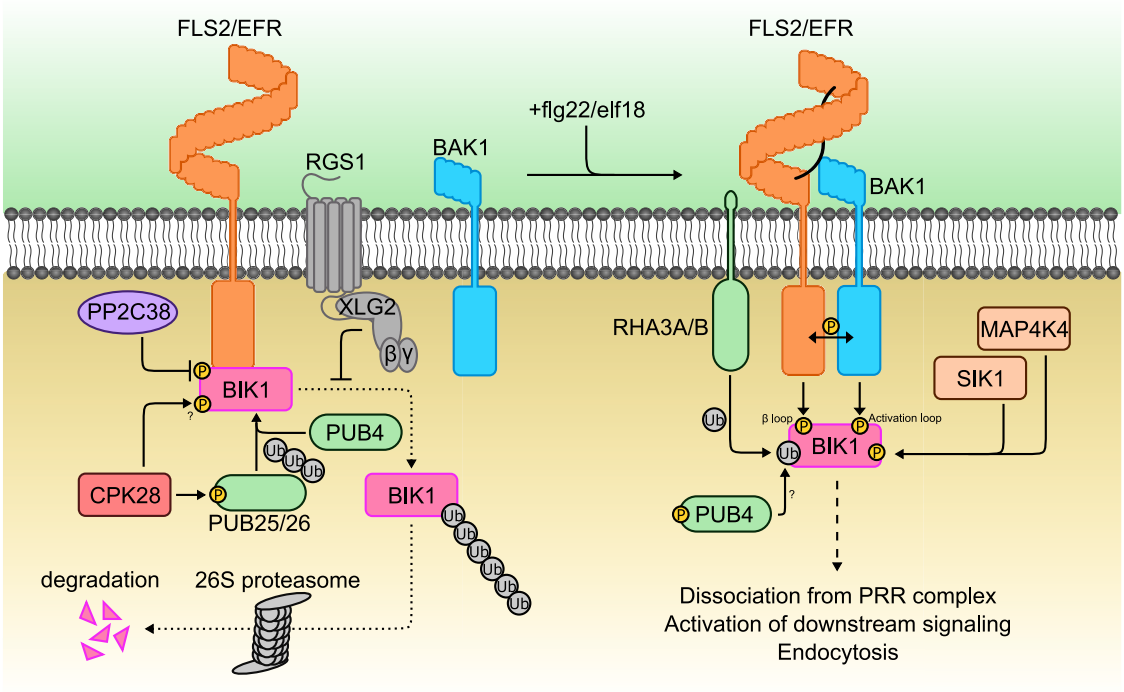

Figure 3. Regulation of BIK1 activation and turnover

In the resting/non-activated state, BIK1 is kept dephosphorylated and inactive by PP2C38, and is degraded upon poly-ubiquitination by PUB25/26 and/or PUB4. BIK1 degradation is promoted by CPK28 and inhibited by G proteins. Upon ligand perception, BIK1 is activated via phosphorylation and is mono-ubiquitinated by RHA3A/B, which promotes its dissociation from the complex. Active BIK1 is further stabilized by PUB4 via an unknown mechanism and the MAP4Ks SIK1 and MAP4K4 via phosphorylation.

site(s) have not been mapped. Interestingly, the BIK1 kinase domain has an extended N-terminal β loop, which contains two additional EFR-targeted phosphosites, Ser89/Thr90, that are also important for the activation of EFR-dependent immune signaling (Lal et al., 2018). These sites are partially conserved across other RLCK-VII/PBL isoforms, and thus may also represent a conserved mechanism for activation, although given the dispensability of EFR catalytic activity for immune activation (Bender et al., 2021), it remains to be determined which kinase(s) may phosphorylate these sites in other PRR complexes. The importance of RLCK-VIIs in PTI signaling is further underscored by their serving as a convergent target for several bacterial effector proteins that suppress immune responses (Feng et al., 2012; Zhang et al., 2010).

BIK1 is a key component of PTI signaling and is subject to complex regulation in both its activation and turnover (Figure 3). Numerous regulatory BIK1-interacting proteins have been identified, including negative regulators such as CALCIUM-DEPENDENT PROTEIN KINASE 28 (CPK28) (Monaghan et al., 2014), the protein phosphatase PP2C38 (Couto et al., 2016), and the E3-ligases PLANT UBOX PROTEIN 25 and 26 (PUB25/26) (Wang et al., 2018) and PUB4 (Derkacheva et al., 2020). Along with heteromeric G proteins, which stabilize BIK1 (Liang et al., 2016), these interactors form a regulatory module controlling the turnover and activation of BIK1 in immune signaling (Wang et al., 2018). Furthermore, the MAP4Ks SERINE/THREONINE KINASE 1 (SIK1)/MAP4K3 and MAP4K4 have been found to also positively regulate BIK1 abundance and PTI signaling via direct interaction and phosphorylation (Jiang et al., 2019; Zhang et al., 2018), although exactly how this relates to the PUB25/26 and/or PUB4 homeostatic mechanisms is unexplored. Finally, while PUB25/26-mediated polyubiquitination leads to BIK1 degradation (Wang et al., 2018), BIK1 is also monoubiquitinated upon PAMP perception in a phosphorylation-dependent manner (Ma et al., 2020). This monoubiquitination is mediated by the E3-ligases RING-H2 FINGER A3A (RHA3A) and RHA3B and regulates dissociation of BIK1 from the PRR complex and endocytosis (Ma et al., 2020). Interestingly, monoubiquitination was also observed in at least one other RLCK-VII isoform (Ma et al., 2020), suggesting a potentially widespread mechanism.

Execution of immune signaling via substrate phosphorylation

Aside from its role in the activation of RLCKs, BAK1 has also been found to directly target several important substrate proteins. Upon flg22 treatment, BAK1 was found to directly phosphorylate PUB12 and PUB13, which recruits and/or activates these E3-ligases to trigger FLS2 polyubiquitination and degradation, and thus attenuation of PTI signaling (Lu et al., 2011). BAK1 was also found to directly phosphorylate the monosaccharide transporter SUGAR TRANSPORT PROTEIN 13 (STP13) in its cytosolic C terminus following flg22 perception, which upregulates STP13 activity to decrease apoplastic free sugars that could otherwise be used by pathogens (Yamada

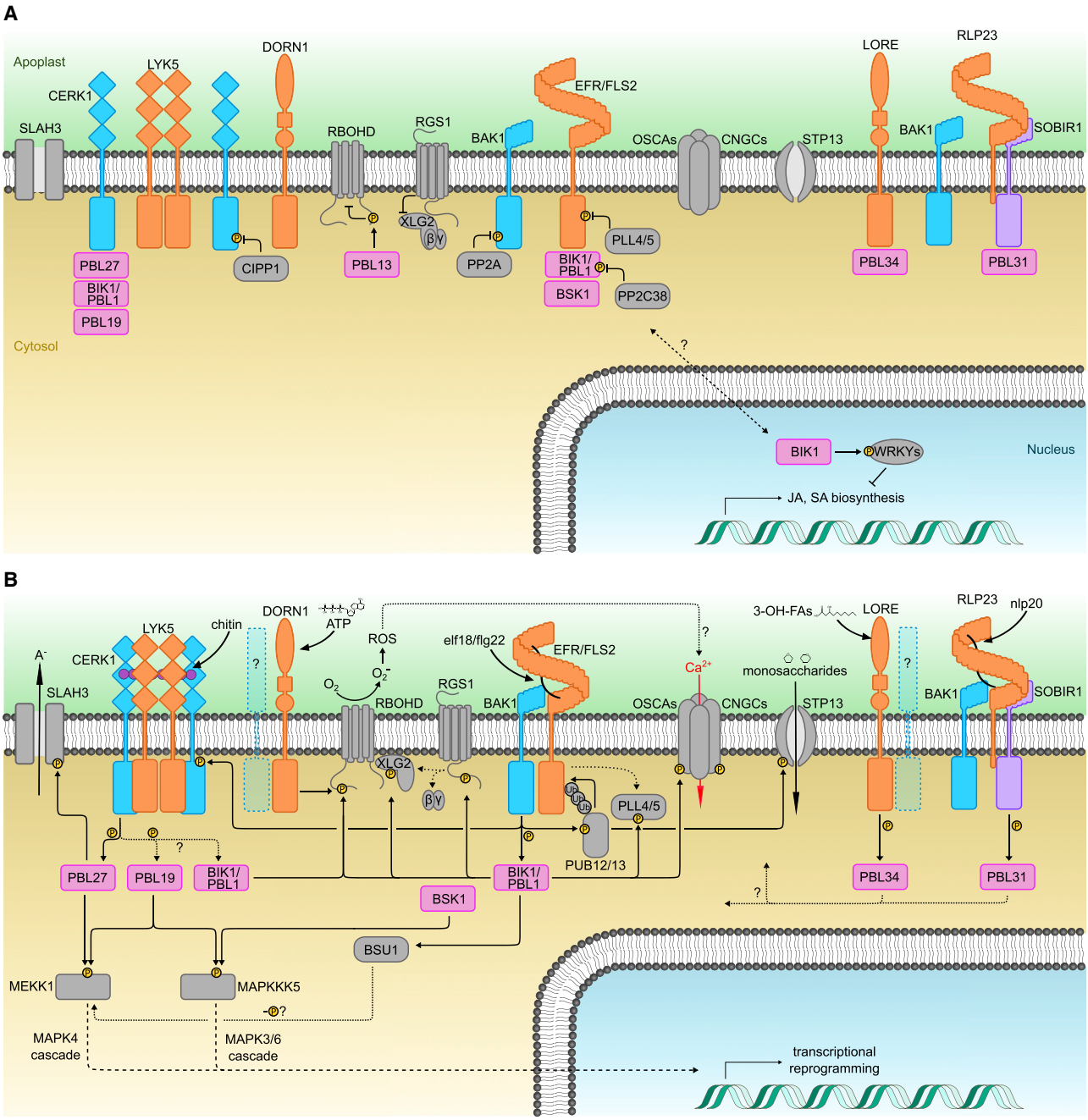

Figure 4. Execution of *Arabidopsis* PTI signaling via phosphorylation

Known substrates of *Arabidopsis* PRRs, co-receptors, and/or downstream RLCKs are shown in both the absence (A) and presence (B) of ligand. In some cases, specific RLCK(s) have been identified as signaling components downstream of PRRs, but for which no direct substrates have yet been found. Ligand-binding receptors are shown in orange, co-receptors in cyan, the adaptor RK SOBIR1 in purple, RLCKs in magenta, and regulatory/substrate proteins in gray.

et al., 2016a). There is also emerging evidence that PRR complexes can phosphorylate other RKs to cross-prime responses to additional PAMPs, as BAK1 was recently found to phosphorylate CERK1 within its cytosolic juxtamembrane domain upon flg22 perception, which primes the chitin receptor complex and boosts antifungal responses after bacterial infection (Gong et al., 2019). Aside from such BAK1 activity, however, transphosphorylation of substrate proteins by RLCK(s) is thought to be the primary mechanism by which active PRR complexes transduce downstream signaling (Liang and Zhou, 2018). Over the past decade, much work has pursued the identification and characterization of such substrate proteins, and many have been identified that regulate several key aspects of PTI signaling (Figure 4; Table 3).

Table 3. PRR complex substrate proteins

Species	Substrate protein	Phosphosite	Responsible kinase	Method	Molecular function	References
Arabidopsis	CERK1	S268, S282/T283	BAK1	MS (*in vivo*), SDM	priming of chitin responses (see Table 1)	Gong et al., 2019
Arabidopsis	STP13	T485	BAK1	*in vitro* kinase assay, SDM	promoting monosaccharide import	Yamada et al., 2016a
Arabidopsis	PUB12/13	N.D.	BAK1	*in vitro* and *in vivo* kinase assays	polyubiquitination of FLS2; attenuation of signaling	Lu et al., 2011
Arabidopsis	BSU1	S251	BIK1	MS (*in vivo*), SDM, *in vitro* kinase assay	activation of immune signaling; MEKK1 regulation	Park et al., 2019
Arabidopsis	CNGC4	Y484, S514, T518, S543, S544, T613, T652, S653, S655 (C terminus)	BIK1	MS (*in vitro*)	CNGC2/4 channel regulation; full responses at high $[Ca^{2+}]_{ext}$	Tian et al., 2019
Arabidopsis	WRKY33, 50, 57	N.D.	BIK1	*in vitro* kinase assay	JA and SA transcriptional responses	Lal et al., 2018
Arabidopsis	PLL4	S124, S140, S164	BIK1	*in vitro* kinase assay, SDM	regulation of dissociation from RKs	DeFalco et al., 2021
Arabidopsis	XLG2	S148, S150	BIK1 (?)	MS (*in vivo*), SDM	unknown; promotes immunity	Liang et al., 2016
Arabidopsis	OSCA1.3	S54	BIK1/PBL1	MS (*in vivo*), SRM, SDM, *in vitro* kinase assay	Ca^{2+} channel activity; stomatal immunity	Thor et al., 2020
Arabidopsis	RBOHD	S39, S343, S347	BIK1/PBL1	MS (*in vivo*), SDM, *in vitro* kinase assay	activation of ROS production	Kadota et al., 2014; Li et al., 2014
Arabidopsis	RGS1	S428, Ser431	BIK1/PBL1	MS (*in vivo*), *in vitro* kinase assay	de-repression of G-proteins, dissociation from FLS2 complex	Liang et al., 2018
Arabidopsis	MAPKKK5	S289	BSK1	*in vitro* kinase assay, SDM	full disease resistance	Yan et al., 2018
Arabidopsis	RBOHD	S22, S24	DORN1	kinase client assay, *in vitro* kinase assay, SDM	ROS production, stomatal closure	Chen et al., 2017
Arabidopsis	PP2C38	S77	N.D. (BAK1 or BIK1)	MS (*in vivo*), SDM, *in vitro* kinase assay	downregulation of phosphatase activity	Couto et al., 2016
Rice	OsCNGC9	N.D. (C terminus)	OsRLCK185	*in vitro* kinase assay	Ca^{2+} channel activity	Wang et al., 2019
Rice	OsMAPKKK18	N.D. (N and C termini)	OsRLCK185	*in vitro* kinase assay	activation of OsMPK3/6 cascade	Yamada et al., 2017
Rice	OsMAPKKKε	N.D. (C terminus)	OsRLCK185	*in vitro* kinase assay	OsMPK3/6 cascade activation	Wang et al., 2017b
Arabidopsis	RBOHD	S862	PBL13	MS (*in vitro*), SDM, *in vitro* kinase assay, immunoblotting	inhibition of RBOHD activity	Lee et al., 2020
Arabidopsis	RBOHD	T912	PBL13	MS (*in vitro*), SDM, *in vitro* kinase assay	promotion of RBOHD ubiquitination/ degradation	Lee et al., 2020
Arabidopsis	MAPKKK5	S599	PBL19	MS (*in vivo*), *in vitro* kinase assay, immunoblotting	MPK3/6 cascade activation	Bi et al., 2018

(*Continued on next page*)

Table 3. *Continued*

Species	Substrate protein	Phosphosite	Responsible kinase	Method	Molecular function	References
Arabidopsis	MEKK1	S603	PBL19	MS (*in vivo*), *in vitro* kinase assay, immunoblotting	MPK4 cascade activation	Bi et al., 2018
Arabidopsis	MAPKKK5	S617, S622, S658, S660, T677, S685 (C terminus)	PBL27	*In vitro* kinase assay	MPK3/6 cascade activation	Yamada et al., 2016a
Arabidopsis	SLAH3	S127, S189	PBL27	MS (*in vitro*), SDM, *in vitro* kinase assay	channel activation; stomatal closure in response to chitin	Liu et al., 2019

The first identified BIK1 substrate was the NADPH oxidase RESPIRATORY BURST ORTHOLOG D (RBOHD) (Kadota et al., 2014; Li et al., 2014). RBOHD is responsible for the production of ROS upon PAMP/DAMP perception (Nühse et al., 2007), which is one of the hallmarks of PTI signaling. RBOHD is a plasma membrane-localized, six-transmembrane enzyme with cytosolic N and C termini, and is subject to complex regulation by calcium and post-translational modification (Kadota et al., 2015). Upon PAMP perception, BIK1 and its close homolog PBL1 directly phosphorylate several key residues on the RBOHD N terminus, including S39 and S343/347 (Kadota et al., 2014; Li et al., 2014), thus providing a direct molecular link from PRR complex activation to ROS production. Given their strict requirement for RBOHD function, this suggests that these BIK1/PBL1-targeted sites are required for the subsequent regulation of RBOHD by Ca^{2+} binding and/or CPKs (Kadota et al., 2015). The MAP4K SIK1, which phosphorylates BIK1 to positively regulate accumulation, can also directly phosphorylate the RBOHD N terminus at overlapping sites with BIK1 (Zhang et al., 2018), suggesting a semiredundant mechanism by which this key substrate can be activated in immune signaling. Accordingly, it has now been found that the PRR DORN1 can also directly phosphorylate distinct N-terminal sites on RBOHD in a ligand-dependent manner to regulate ROS production and subsequent stomatal closure upon ATP perception (Chen et al., 2017), further indicating that RBOHD phosphorylation serves as a major regulatory point of convergence in PTI signaling. It is unclear whether this mechanism is specific to DORN1, as well as what RLCK(s) may function downstream of ATP perception.

The role of RLCK-VIIs in regulating PAMP-induced ROS burst is conserved across angiosperms, and in rice several isoforms, including OsRLCK185 (Yamaguchi et al., 2013) and OsRLCK176 (Ao et al., 2014), also genetically regulate OsRBOH-dependent ROS production. While this has yet to be demonstrated, these OsRLCK-VIIs presumably directly phosphorylate OsRBOH(s) in a manner similar to that in *Arabidopsis*. As in *Arabidopsis* (Rao et al., 2018), several additional OsRLCK-VIIs are genetically involved in PTI signaling (see Table 2) (Li et al., 2017), indicative of partial redundancy between isoforms.

Like ROS production, Ca^{2+} influx upon PAMP perception is an early hallmark of PTI signaling that is dependent on BIK1/PBL1 (Li et al., 2016; Lu et al., 2010; Monaghan et al., 2015; Ranf et al., 2014); however, decades of intensive work had yielded no clear candidates for the calcium channel(s) involved in PTI. Recently, it was found that the CYCLIC NUCLEOTIDE-GATED CHANNEL (CNGC)-IVb isoforms CNGC2 and CNGC4 form a functional channel *in vivo* that is required for full flg22-induced Ca^{2+} flux in plants grown under high external [Ca^{2+}] (Tian et al., 2019). CNGC2/4 were found to interact with BIK1, with BIK1 proposed to phosphorylate the C terminus of CNGC4 to counteract inhibition by the calcium sensor CALMODULIN (CaM) (Tian et al., 2019), a key regulator of CNGCs (DeFalco et al., 2016). Given the role of CNGC2 in regulating Ca^{2+} movement from the vasculature under high external [Ca^{2+}] conditions (Wang et al., 2017d) and the conditional immune phenotypes of *cngc2* or *cngc4* (Tian et al., 2019), the exact role of CNGC2/4 in PTI signaling remains to be fully resolved. BIK1 was also

suggested to phosphorylate the C terminus of the CNGC-IVa isoform CNGC20, which could promote the stability of the channel (Zhao et al., 2021), although the details and specificity of such regulation are not yet clear. Other work has also linked the close CNGC20 homolog, CNGC19, to immune signaling downstream of Atpep1 perception (Meena et al., 2019); however, a clear role for CNGC19 and/or CNGC20 in PRR pathways could not be independently confirmed (Yu et al., 2019). Interestingly, the C terminus of a phylogenetically distinct rice CNGC isoform, OsCNGC9, was identified as a substrate of the RLCK-VII OsRLCK185, and OsCNGC9 was found to be required for Ca^{2+} influx after chitin perception and antifungal immunity (Wang et al., 2019). Such results indicate that multiple CNGCs may function in immune signaling and under different conditions, and, as such, the roles and regulation of Ca^{2+} channels in PTI remain to be fully elucidated.

Other families of Ca^{2+} channels are also involved in PTI signaling, as the REDUCED HYPEROSMOLALITY-INDUCED Ca^{2+} INCREASE (OSCA) family member OSCA1.3 was recently identified as a BIK1/PBL1 substrate (Thor et al., 2020). BIK1/PBL1 rapidly phosphorylates OSCA1.3 at a single site in its N-terminal cytosolic loop upon flg22 treatment, which increases its Ca^{2+} channel activity. OSCA1.3 (along with OSCA1.7) are highly expressed in guard cells, where they mediate PAMP-induced Ca^{2+} influx to promote stomatal closure in response to various elicitors but not abscisic acid or abiotic stress (Thor et al., 2020). Intriguingly, members of the GLUTAMATE RECEPTOR-LIKE (GLR) family of Ca^{2+} channels were also recently implicated in PTI. GLR2.7, 2.8, and 2.9 were identified based on their transcriptional response to elicitor treatment, and loss of these three channels compromised PAMP- and DAMP-induced calcium signaling (Bjornson et al., 2021), although the mechanisms governing their activation remain unknown.

Interestingly, the heteromeric G proteins that regulate PTI and BIK1 homeostasis are in turn regulated by phosphorylation. Dissociation of G proteins from the FLS2 complex is regulated by phosphorylation of the GTPase accelerating protein, REGULATOR OF G PROTEIN SIGNALING 1 (RGS1), at conserved sites in its C terminus by RLCK-VIIs such as BIK1 (Liang et al., 2018). EXTRA LARGE G PROTEIN 2 (XLG2) is also phosphorylated at its N terminus; however, the role of this phosphorylation remains uncharacterized (Liang et al., 2016).

The PLL-type phosphatases PLL4 and PLL5 are negative regulators of EFR-mediated immune signaling and dissociate from the EFR complex upon elf18 perception (Holton et al., 2015). Recently, BIK1 was found to directly phosphorylate PLL4 at a cluster of conserved sites in its N terminus, which in turn decreases interaction with EFR and FLS2, providing a regulatory circuit that governs immune RK activation (DeFalco et al., 2021). Interestingly, this molecular circuitry is conserved in LRR-RK pathways that regulate growth and development, suggesting extensive overlap between the signaling pathways of immune and non-immune RKs, as also suggested for the SHENGHEN pathway regulating endodermal development (Fujita et al., 2020).

Like many RLCKs, BIK1 is a plasma membrane-associated protein due to N-terminal myristoylation (Lu et al., 2010). Recently, however, partial nuclear localization of BIK1 was also observed, and BIK1 was found to directly phosphorylate several WRKY family transcription factors, which are proposed to transcriptionally regulate jasmonic acid (JA) and salicylic acid (SA) synthesis in the absence of PRR activation (Lal et al., 2018). It is not yet determined whether this represents two distinct "pools" of BIK1, or whether BIK1 can re-localize between the membrane and nucleus. Furthermore, it is not clear how dynamic mono-ubiquitination may affect such localization (Ma et al., 2020), and thus the exact nature of BIK1 nuclear signaling and its contribution to PTI is not resolved.

While BIK1 is the most genetically important RLCK-VII downstream of several PRR complexes, it is now clear that multiple RLCK-VII isoforms and/or subfamilies participate in immune signaling (Rao et al., 2018). Furthermore, BIK1 actually plays a genetically negative role in LRR-RP signaling (Wan et al., 2019), whereas the RLCK-VII-7 subfamily, specifically PBL31, promotes immune signaling downstream of RLP23 (Pruitt et al., 2020). Recent work has demonstrated that PBL34, a member of the RLCK-VII-5 subfamily, is the primary RLCK-VII functioning downstream of LORE, and is required for full 3-OH-FA-induced immunity (Luo et al., 2020); however, substrates for PBL31 or PBL34 in these immune pathways remain to be identified.

In addition to Ca^{2+}, other ion fluxes are also rapidly modulated by PAMP perception, which lead to a rapid depolarization of the PM (Jabs et al., 1997). Guard cell anion efflux, in particular, is involved in PAMP-induced stomatal closure and therefore immunity (Guzel Deger et al., 2015; Jeworutzki et al., 2010). PBL27 was recently found to directly phosphorylate the cytosolic N terminus of the S-type anion channel SLAC1 HOMOLOG 3 (SLAH3), activating the channel to promote chitin-induced stomatal closure (Liu et al., 2019). It remains to be determined whether additional RLCK(s) target anion channel(s) to regulate responses to additional PAMPs in guard cells and/or other tissues, although preliminary data suggest this may be the case (Liu et al., 2019).

The activation of MAPK cascades is another critical component of PTI signaling and ultimately regulates large-scale transcriptional reprogramming of the cell and subsequent immunity (Boller and Felix, 2009; Meng and Zhang, 2013). While it was initially unclear whether MAPK activation was downstream of RLCK(s), likely due to genetic redundancy (Feng et al., 2012), this has been resolved by the identification of RLCKs that directly regulate MAPKKK activation downstream of PAMP perception. Members of the RLCK-VII-4 subfamily were identified as genetically required for chitin-induced MAPK activation in *Arabidopsis*, and the RLCK-VII-4 isoform PBL19 was found to directly phosphorylate the C termini of MAPKKK isoforms MAPKKK5 and MEKK1, thereby activating MPK3/6 and MPK4 cascades, respectively (Bi et al., 2018). An additional RLCK-VII isoform, PBL27, was also previously found to directly phosphorylate MAPKKK5 (Yamada et al., 2016b). The protein phosphatase BRI1-SUPRESSOR 1 (BSU1) was also recently proposed as a BIK1 substrate that can also positively regulate MEKK1; however, the mechanism of such regulation is currently unknown (Park et al., 2019). Finally, the RLCK-XII isoform BSK1 has also been suggested to phosphorylate MAPKKK5 (Yan et al., 2018), indicating convergent targeting of these MAPKKKs. The mechanism

of MAPK activation by RLCK-VIIs is also conserved in rice, where OsRLCK185 directly phosphorylates both OsMAPKKKε (Wang et al., 2017b) and OsMAPKKK18 (Yamada et al., 2017) to regulate the rice MPK3/6 cascade in a manner similar to *Arabidopsis* MAPKKK3/5 (Yamada et al., 2017).

Aside from the specific negative genetic role of BIK1 in LRR-RP signaling (Wan et al., 2019), several other RLCK-VII isoforms have been identified as negative regulators of PTI. PBL13 was initially identified as a negative regulator of PAMP-induced ROS production (Lin et al., 2015), and recent work showed that PBL13 phosphorylates the RBOHD C terminus at regulatory sites that inhibit activity or promote E3-ligase-dependent degradation of RBOHD in the basal state (Lee et al., 2020). PBL13 is not broadly conserved across plant taxa and possesses unique C-terminal repeats that are required for its targeting of RBOHD (Lee et al., 2020); thus, it remains to be determined whether other RLCK(s) can also negatively regulate RBOHD via C-terminal phosphorylation. Notably, RIPK/PBL14 is a close homolog of PBL13 and also proposed to negatively regulate PTI (Liu et al., 2011), although recent work indicates that RIPK also plays a positive role in the regulation of elicitor-induced ROS production (Li et al., 2021). The divergent RLCK-VII isoform CONSTITUTIVE DIFFERENTIAL GROWTH 1 (CDG1), which positively regulates BR signaling (Kim et al., 2011), was recently also shown to inhibit the accumulation of PRRs such as FLS2 and CERK1, thereby blocking immune signaling (Yang et al., 2021). In the same study, CDG1 was also found to phosphorylate the RPM1-INTERACTING PROTEIN4 (RIN4) upon perception of the bacterial effector AvrRpm1. Interestingly, previous work also found that RIN4 was phosphorylated upon flg22 perception in a BIK1/PBL1-dependent manner (Chung et al., 2014), although a specific responsible kinase was not identified. Deciphering the molecular, genetic, and biochemical bases of these differential RLCK-VII/PBL functions will be key to understanding how this essential family of kinases activates and regulates signaling downstream of the enormous array of plant RKs.

Concluding remarks

While there are increasingly comprehensive large-scale datasets of *in planta* phosphosites (Mergner et al., 2020), including some that are PAMP inducible (Benschop et al., 2007; Kadota et al., 2019; Nühse et al., 2007), such efforts still lag behind those in mammalian research (Needham et al., 2019). Furthermore, even as high-quality phosphoproteomic datasets are developed, a major remaining challenge will be to (1) determine the functional relevance of sites in PTI signaling and (2) assign specific kinases to specific sites on substrate proteins. In this regard, plant signaling research has made important progress in recent years, and thus is poised for many exciting discoveries in the future. It will be particularly informative to examine PTI signaling at the network level, and map protein-protein interactions and kinase-substrate phosphorylation at a larger scale, as well as determine potential cell- or tissue-specific interactions/responses (Emonet et al., 2021). Recent progress has been made in identifying kinase substrates (Figure 4); however, the bulk of substrates for even well-characterized RLCKs are likely unknown. In addition, many RLCKs continue to be identified downstream of PRRs (Table 2). Comprehensive mapping of pathway-specific RLCKs and their substrates thus remains a critical task to further our understanding of plant immunity and RK signaling. Finally, while traditionally described as two distinct pathways, it is increasingly clear that there is extensive overlap and interplay between PRR- and NLR-mediated immune signaling (Ngou et al., 2021; Pruitt et al., 2020; Yuan et al., 2021). Further work will be needed to resolve the molecular mechanisms underpinning this interplay, as well as clarify how these pathways together generate robust immunity to diverse pathogens.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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Update

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Correction

Molecular mechanisms of early plant pattern-triggered immune signaling

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The source of RaxX was mislabeled in the original version of Table 2 as ''fungi,'' which we have corrected to ''bacteria.'' We have also corrected the omission of ACIK1 as an RLCK-VII acting downstream of the tomato LRR-RPs Cf-4 and Cf-9, also in Table 2. The authors regret this error.

