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# More than an on-and-off switch: Post-translational modifications of plant pattern recognition receptor complexes

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**Abstract**

Sensing microbe-associated molecular patterns (MAMPs) by cell surface–resident pattern recognition receptors (PRRs) constitutes a core process in launching a successful immune response. Over the last decade, remarkable progress has been made in delineating the mechanisms of PRR-mediated plant immunity. As the frontline of defense, the homeostasis, activities, and subcellular dynamics of PRR and associated regulators are subjected to tight regulations. The layered protein post-translational modifications, particularly the intertwined phosphorylation and ubiquitylation of PRR complexes, play a central role in regulating PRR signaling outputs and plant immune responses. This review provides an update about the PRR complex regulation by various post-translational modifications and discusses how protein phosphorylation and ubiquitylation act in concert to ensure a rapid, proper, and robust immune response.

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MAMPs, PRR complex, Plant immunity, Post-translational modifications, Phosphorylation, Ubiquitylation.

## Introduction

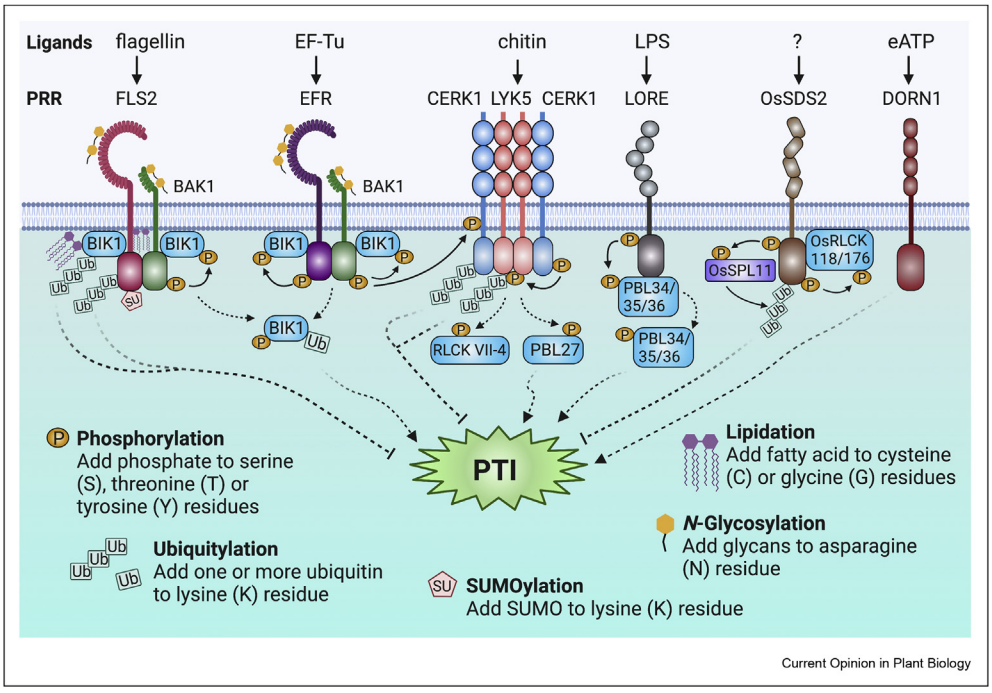
Plants have evolved the innate immune system to protect themselves against pathogen infections. The first line of inducible defense is mediated by plasma membrane (PM)–resident pattern recognition receptors (PRRs), which recognize microbe-associated molecular patterns (MAMPs) or host-derived damage-associated molecular patterns (DAMPs) and trigger a series of immune responses. This so-called pattern-triggered immunity (PTI) collectively confers resistance against a broad spectrum of pathogens and pests [1–3].

Protein post-translational modification (PTM) is a versatile regulatory process that expands the proteome functionality, regulates gene expression, regulates protein interaction and stability, and shapes the intensity and duration of signaling pathways [4,5]. Different types of PTMs could target the same protein substrate for a harmonic or opposing action-regulating protein subcellular localization, enzymatic activities, substrate recruitment, and interaction with other molecules. Diverse PTMs, including phosphorylation, ubiquitylation, SUMOylation, *N*-glycosylation, lipidation, ADP-ribosylation, and proteolysis, have been implicated in regulating PTI signaling (Figure 1) [4,6–8]. In particular, the versatility of PTMs regulating PRR complex activation and attenuation plays an important role in an effective immune response. Here, we review recent studies on layered PTMs with a focus on protein phosphorylation and ubiquitylation acting on PRR complexes and discuss their interplays for a harmonic PRR complex–mediated immune signaling.

## Border controls: PM-tethered PRR receptorsomes at the frontline of plant immunity

Plant PRRs are usually single-pass transmembrane receptor kinases (RKs) or receptor proteins [9,10]. The distinct extracellular domains, including leucine-rich repeats (LRRs), lysine motifs (LysM), lectin motifs, epidermal growth factor (EGF)-like repeats, and malectin-like motifs, are determinants for ligand recognition (Figure 1) [10,11]. LRR-RKs lie in the subfamily with the largest and most well-studied members. For instance, FLAGELLIN-SENSING2 (FLS2) and ELONGATION FACTOR-TU (EF-Tu) RECEPTOR (EFR) perceive bacterial flagellin and EF-

**Figure 1**

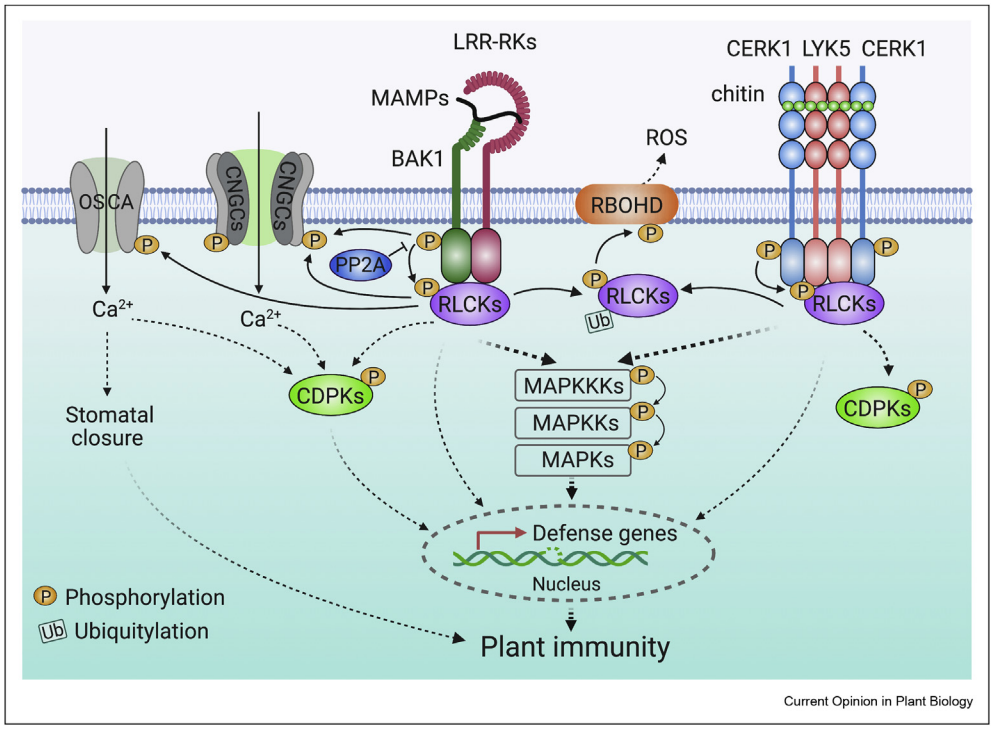



**Different post-translational modifications regulate PRR complexes in PTI signaling.** *Arabidopsis* FLS2–BAK1 and EFR–BAK1 are LRR-RK complexes, LYK5–CERK1 is a LysM-RK complex, LORE is a lectin S-domain RK, DORN1 is a lectin domain RK, and rice OsSDS2 is an S-domain RK. The cognate ligands of PRR complexes are shown. PRR complexes undergo autophosphorylation/transphosphorylation and further phosphorylate downstream RLCKs, including BIK1 and PBLs. In addition, several PRR complexes, including FLS2–BAK1–BIK1, LYK5–CERK1, and OsSDS2, were shown to be either polyubiquitylated or monoubiquitylated to regulate PRR complex stability or activation. OsSPL11 ubiquitylates OsSDS2 for degradation. FLS2 is also SUMOylated, which contributes to the release of BIK1 from the FLS2–BAK1 complex for activating PRR signaling. In addition, FLS2 is palmitoylated. BIK1 is tethered to the PM, likely through myristoylation modification. FLS2, BAK1, and EFR also undergo different *N*-glycosylations for regulating PRR maturation and secretion. BAK1, BRI1-ASSOCIATED RECEPTOR KINASE1; BIK1, BOTRYTIS-INDUCED KINASE1; CERK1, CHITIN ELICITOR RECEPTOR KINASE1; DORN1, DOES NOT RESPOND TO NUCLEOTIDES1; EFR, ELONGATION FACTOR-TU RECEPTOR; FLS2, FLAGELLIN-SENSING2; LORE, LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION; LPS, lipopolysaccharide; LRR, leucine-rich repeat; LysM, lysine motif; LYK5, LYSIN MOTIF RECEPTOR KINASE5; PBL, AVRPPHB SUSCEPTIBLE1–LIKE KINASE; PM, plasma membrane; PRR, pattern recognition receptor; PTI, pattern-triggered immunity; RK, receptor kinase; SUMO, small ubiquitin-like modifier.

Tu, respectively [12,13]. The LysM—containing RKs CHITIN ELICITOR RECEPTOR KINASE1 (CERK1) and LYSIN MOTIF RECEPTOR KINASE5 (LYK5) recognize chitin oligomers and initiate chitin-induced immune responses [14,15]. The EGF RK WALL-ASSOCIATED KINASE1 (WAK1) functions as a candidate receptor of oligogalacturonides (OGs) [16]. The lectin S-domain RK LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION (LORE) mediates lipopolysaccharide (LPS) sensing in *Arabidopsis* [17]. The lectin domain—containing RK DOES NOT RESPOND TO NUCLEOTIDES1 (DORN1) recognizes extracellular ATP (eATP) [18], and LecRK-VI.2 and LecRK-I.8 are potential receptors of extracellular $NAD^+$ ($eNAD^+$) and $NAD^+$ phosphate ($eNADP^+$) [19*].

Upon ligand perception, LRR-RKs often recruit somatic embryogenesis receptor kinases (SERKs) as coreceptors [20] and relay the signaling through associated receptor-like cytoplasmic kinases (RLCKs), such as BOTRYTIS-INDUCED KINASE1 (BIK1) and AVRPPHB SUSCEPTIBLE1 (PBS1)-LIKE KINASES (PBLs) [21,22]. As key regulators associated with multiple PRRs, RLCKs bifurcate immune signaling by activating mitogen-activated protein kinase (MAPK) cascades, NADPH oxidases for reactive oxygen species (ROS) production, cyclic nucleotide—gated channels (CNGCs) for calcium burst, and hyperosmolality-gated calcium-permeable channels (OSCA) for stomatal closure (Figure 2) [23—28]. SERK family coreceptors are usually not required for other types of RKs except LRR-RKs [17,29]. However, recent reports show that SERKs are necessary for the function of lectin RK LecRK-VI.2 [19*]. In addition, ligand-induced LRR-RK—SERK complex assembly is modulated by other types of RKs, such as the malectin-like RK FERONIA (FER) and ANXURs [30,31] and the malectin-like/LRR-RK IMPAIRED OOMYCETE SUSCEPTIBILITY1 (IOS1) [32]. Thus, PRRs, coreceptors, and

**Figure 2**




**PRR complexes relay signaling events via RLCKs.** BAK1 is a shared coreceptor of different LRR-RKs, and CERK1 is a coreceptor of LYK5. Upon ligand binding, PRR complexes phosphorylate different RLCKs to relay diverse downstream signaling events. RLCKs phosphorylate RBOHD, an NADPH oxidase, to activate ROS production, CNGC $Ca^{2+}$ channels to regulate $Ca^{2+}$ influx, OSCA $Ca^{2+}$ channels to regulate stomatal closure, and MAPK cascades (MAPKKKs–MAPKKs–MAPKs) to modulate immune gene expression, collectively contributing to plant immunity. The RLCKs downstream of LRR-RK–BAK1 complexes that activate MAPK cascades remain unknown. PBL27 and RLCKVII-4 act downstream of CERK1 and regulate MAPK activation. A fraction of RLCK BIK1 can be detected in the nucleus, where it interacts with transcription factors regulating gene transcription. PRR activation also activates CDPKs, which may function in parallel with MAPK cascades regulating gene transcription. BAK1, BRI1-ASSOCIATED RECEPTOR KINASE1; CERK1, CHITIN ELICITOR RECEPTOR KINASE1; CNGC, cyclic nucleotide–gated channel; LRR, leucine-rich repeat; LYK5, LYSIN MOTIF RECEPTOR KINASE5; MAMP, microbe-associated molecular pattern; MAPK, mitogen-activated protein kinase; PBL, AVRPPHB SUSCEPTIBLE1–LIKE KINASE; PRR, pattern recognition receptor; RK, receptor kinase; RLCK, receptor-like cytoplasmic kinase; ROS, reactive oxygen species; CDPKs, calcium-dependent protein kinases.

RLCKs often form multiprotein PRR complexes, called receptorsomes here, that are dynamically regulated upon MAMP perception.

## Phosphoregulation of PRR receptorsomes

### Autophosphorylation and transphosphorylation of PRRs and associated RKs

A common theme in activating early PRR signaling is the rapid and complex autophosphorylation and transphosphorylation within the PRR complexes, including receptors, coreceptors, scaffold proteins, associated RLCKs, and other regulators (Figure 1) [1,9]. Plant RKs can be divided into RD and non-RD kinases based on the presence or absence of an arginine (R) before the catalytic aspartate (D) residue [10]. Most PRR RKs are non-RD kinases lacking strong autophosphorylation activities compared with RD kinases [33]. The SERK coreceptors are RD kinases exhibiting the strong autophosphorylation and transphosphorylation activities required to regulate PRR signaling. Multiple serine/threonine (S/T) phosphosites of SERK3, also named BRI1-ASSOCIATED RECEPTOR KINASE1 (BAK1), were identified and required for autophosphorylation and transphosphorylation of BAK1 [34]. In particular, mutation of T455 at the catalytic domain eliminates the phosphorylation of BAK1 [34]. Moreover, the carboxyl-terminal (CT) tail of BAK1 promotes its autophosphorylation required for its function in plant immunity, but neither in development nor in cell death regulation [35]. Through a phosphoproteomics approach, four phosphosites of BAK1 at the carboxyl-terminal tail were identified to positively regulate BAK1-mediated immunity but not development [36**]. Thus, a phosphocode-based dichotomy of BAK1 was proposed to explain the functional specificity of the BAK1

coreceptor in multiple receptorsomes. Comparative quantitative phosphoproteomics of BAK1 phosphorylation dynamics upon different ligand stimulation may reveal the differential phosphocodes of BAK1 in association with different RK receptors. Another critical question is whether this phosphocode is universal to various SERK members because different SERKs have an unequal genetic contribution to a given biological process [37]. Equally important, BAK1 phosphorylation status is monitored by protein phosphatase type 2A holoenzyme, which negatively regulates PRR signaling [38].

Although few examples exist in PRR signaling, the sequential transphosphorylation between SERK coreceptors and RK receptors is assumed to augment signaling activation [34,39]. Interestingly, a recent report shows that BAK1 transphosphorylates chitin coreceptor CERK1 upon bacterial MAMP flagellin perception, which accelerates chitin responses and enhances plant resistance to fungal infections (Figure 1) [40**]. The authors hypothesized that initial bacterial infections could prime plant response to the subsequent fungal attacks and suggested crosstalk between bacterial and fungal infections through transphosphorylation of coreceptors. SERK3 also transphosphorylates the LRR-RK NUCLEAR SHUTTLE PROTEIN-INTERACTING KINASE1 (NIK1), an essential regulator in plant antiviral immunity [41]. BAK1-mediated NIK1 transphosphorylation is important for NIK1 function in antiviral immunity, whereas NIK1 suppresses the FLS2–BAK1 complex assembly and negatively regulates antibacterial immunity. It remains unknown how NIK1 phosphorylation confers the opposite actions in plant immunity against viral and bacterial infections.

LYK5, which has a higher chitin-binding affinity than CERK1, forms a chitin-inducible complex with CERK1 (Figure 1) [14]. Although without detectable kinase activity, LYK5 is required for chitin-induced CERK1 autophosphorylation and homodimerization [14]. Conversely, CERK1, an RD kinase, transphosphorylates LYK5 and regulates LYK5-mediated endocytosis in response to chitin [42]. Altogether, these studies imply that CERK1 bears a function similar to BAK1 in regulating PRR signaling through autophosphorylation and transphosphorylation. In contrast, cotton GhCERK1 does not transphosphorylate GhLYK5, whereas the wall-associated kinase GhWAK7A transphosphorylates GhLYK5 and promotes the GhCERK1–GhLYK5 complex assembly to activate chitin signaling [43**]. With increased sensitivity of phosphorylation detection instrumentations, the importance of phosphorylation and its dynamics of non-RD PRR RKs will likely be revealed.

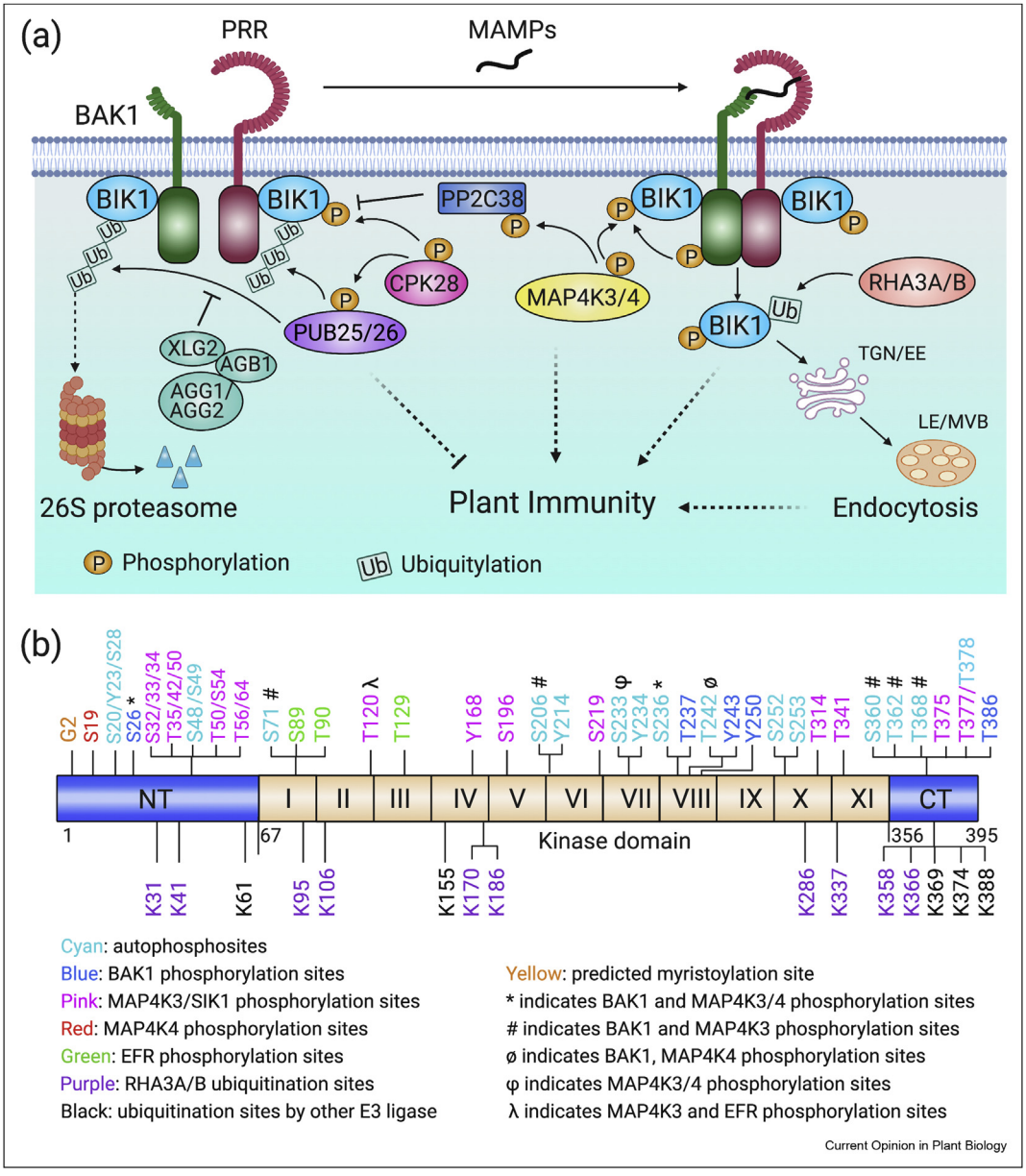
## Phosphoregulation of PRR-associated RLCKs

PRR-tethered RLCKs are convergent and central kinases relaying immune signaling from the PM to intracellular events (Figure 2) [21,22]. MAMP perception triggers rapid phosphorylation of BIK1 and PBL1 [44,45]. The BAK1 coreceptor directly phosphorylates BIK1, which is required for BIK1 release from the FLS2–BAK1 complex and its function in plant immunity (Figure 3a) [46,47]. In addition, the PRR EFR directly phosphorylates BIK1 at residues outside the kinase catalytic loop (Figure 3b) and regulates the plant defense hormone jasmonic acid (JA) and salicylic acid (SA) levels [48**]. The same study also shows that a fraction of BIK1 was detected in the nucleus, where BIK1 interacts with WRKY transcription factors. An interesting question is how BIK1 is released from the PRR-tethered PM and translocated to the nucleus. In addition, the RLCKs BR SIGNALING KINASE1 (BSK1), PATTERN-TRIGGERED IMMUNITY COMPROMISED RLCK1 (PCRK1), and PCRK2 also complex with FLS2 and are likely phosphorylated upon MAMP perception [49,50]. How these RLCKs are activated by PRR complexes and their relationship with BIK1/PBL1 await further exploration.

In chitin-triggered signaling, rice OsRLCK185 is directly phosphorylated by OsCERK1 and regulates chitin-induced immune response, including MAPK activation [51]. *Arabidopsis* PBL27, an ortholog of OsRLCK185, is phosphorylated by CERK1 and mediates chitin-induced defense (Figure 1) [52]. Interestingly, CERK1 preferentially phosphorylates PBL27, whereas BAK1 mainly phosphonates BIK1, suggesting the substrate and signaling specificity of different combinatory RKs–RLCKs. However, another study shows that a subfamily of the RLCK VII-4 is required for chitin-induced MAPK activation through regulating MAPKKK5 phosphorylation in *Arabidopsis* (Figure 1) [26]. Thus, it remains a debate which RLCKs directly regulate MAPK activation upon LYK5–CERK1 activation [26,27]. The rice OsRLCK118 and OsRLCK176 are phosphorylated by a monocot-specific S-domain RK, SPL11 CELL-DEATH SUPPRESSOR 2 (SDS2), that regulates cell death and immunity in rice (Figure 1) [53]. It is well established that BIK1 regulates ROS production by phosphorylating NADPH oxidases (Figure 2) [24,25]. Similarly, OsRLCK118/176 positively regulates plant immunity by phosphorylating NADPH oxidases for ROS production [53]. Thus, RLCKs connect PM-resident RKs to diverse intracellular signaling events by phosphorylating different substrates.

The homeostasis and activation of RLCKs are also subjected to phosphoregulation by additional kinases. CALCIUM-DEPENDENT PROTEIN KINASE 28 (CPK28) phosphorylates BIK1 and promotes 26S proteasome–dependent BIK1 degradation, thereby negatively regulating BIK1-mediated immune signaling (Figure 3a) [54]. Similarly, rice OsCPK4, an ortholog of *Arabidopsis* CPK28, phosphorylates and degrades OsRLCK176, an ortholog of BIK1 [55]. In contrast, two

**Figure 3**




**Layered post-translational modifications of BIK1. (a)** ,In the resting state, BIK1 homeostasis is modulated by CPK28, PUB25/26, heterotrimeric G proteins (XLG2, AGB1, and AGG1/AGG2), and PP2C38. CPK28 phosphorylates PUB25/26, which ubiquitylates BIK1 for degradation. Heterotrimeric G proteins stabilize BIK1 by inhibiting PUB25/26 activity. CPK28 phosphorylates BIK1 to promote its degradation, which is counter-regulated by phosphatase PP2C38. Upon MAMP perception by PRR complexes, BIK1 is phosphorylated by BAK1, monoubiquitylated by RHA3A/B, and released from the FLS2–BAK1 complex. Monoubiquitylation of BIK1 triggers its endocytosis and signal activation. In addition, BIK1 is phosphorylated by MAP4K3/4 for stabilization and enhancing PRR signaling. MPK4K4 also phosphorylates PP2C38, leading to PP2C38 release from BIK1. **(b)**, Different phosphorylation and ubiquitylation sites in BIK1. NT, N-terminal domain; I~XI, eleven kinase subdomains; CT, C-terminal domain. Cyan: autophosphorylation sites [46,47,62]; blue: BAK1 phosphorylation sites [46,62]; pink: MAP4K3/SIK1 phosphorylation sites [57**]; red: MAPK4K4 phosphorylation sites [56**]; green: EFR phosphorylation sites [48**]; purple: RHA3A/B ubiquitylation sites [74**]; black: ubiquitylation sites by other E3 ligases [65**]; yellow: predicted myristoylation site [88]. * indicates BAK1 and MAP4K3/4 phosphorylation sites; # indicates BAK1 and MAP4K3 phosphorylation sites; ø indicates BAK1, MAP4K4 phosphorylation sites; φ indicates MAP4K3/4 phosphorylation sites; λ indicates MAP4K3 and EFR phosphorylation sites. BAK1, BRI1-ASSOCIATED RECEPTOR KINASE1; BIK1, BOTRYTIS-INDUCED KINASE1; EFR, ELONGATION FACTOR-TU RECEPTOR; FLS2, FLAGELLIN-SENSING2; MAMP, microbe-associated molecular pattern; PRR, pattern recognition receptor; RHA3A, RING-H2 FINGER A3A.

plant MAPKKK KINASES MAP4K3 and MAP4K4 phosphorylate BIK1 at different residues and stabilize BIK1 proteins (Figure 3) [56**, 57**]]. Some MAP4K4-phosphorylated BIK1 residues are required for BIK1 autophosphorylation [56**]. Notably, MAP4K3/4 does not appear to act upstream of canonical MAMP-induced MAPK cascades [56**, 57**]]. The relationship between BIK1 phosphorylation and MAPK activation in PRR signaling remains elusive. Besides, BIK1 phosphorylation status is counter-regulated by protein phosphatase PP2C38 that functions as a negative regulator of BIK1-mediated signaling (Figure 3a) [58]. Nevertheless, differential phosphocodes likely exist for BIK1 stability and activation regulated by different upstream kinases and phosphatases.

### Tyrosine phosphorylation of PRR receptorsomes

Although plants lack classical metazoan-specific tyrosine kinases, the tyrosine (Y) phosphorylation of PRR complexes has been proven to be functionally important in plant immunity. Upon ligand activation, the PRR EFR is phosphorylated at a tyrosine residue (Y836) required for EFR-mediated immune responses [59]. A tyrosine phosphosite (Y403) of BAK1, conserved among some other RKs, including Y836 of EFR and Y428 of CERK1, is also required for flg22-induced immune responses [36**]. In addition, the tyrosine phosphorylation of CERK1 at Y428 is essential for chitin-triggered signaling [60*]. Upon chitin perception, CERK1 recruits CERK1-INTERACTING PROTEIN PHOSPHATASE 1 (CIPP1) to dephosphorylate Y428 of CERK1, thereby attenuating chitin-triggered signaling [60*]. LPS receptor LORE also undergoes tyrosine phosphorylation at Y600 upon ligand perception, which subsequently phosphorylates downstream RLCKs PBL34/35/36 (Figure 1) [61**]. Mass spectrometry (MS) analysis identified several tyrosine sites of BIK1 autophosphorylation and BAK1-mediated transphosphorylation (Figure 3b), which are essential for BIK1 function in plant immunity [46,62]. Thus, plant RKs and RLCKs are dual-specificity kinases with both serine/threonine and tyrosine kinase activities. The importance of plant PRR tyrosine phosphorylation is further supported by the observation that bacterial virulence effectors use tyrosine phosphatases to counter-regulate PRR tyrosine phosphorylation, thereby impeding MAMP-induced immunity [59,61**]. Apparently, plants and metazoans rely on the similar mechanism of tyrosine phosphorylation cascades to regulate membrane-resident receptor signaling.

## Ubiquitylation regulation of PRR receptorsomes

Mounting evidence indicates the essential role of protein ubiquitylation in regulating the homeostasis, subcellular dynamics, and activity of PRR and RLCK complexes (Figure 1). Profound and dynamic changes of protein ubiquitylation were detected *in planta* before and after MAMP treatment [63,64]. Multiple ubiquitylated lysine residues were identified in more than 70 RKs and 17 RLCKs, including FLS2, EFR, CERK1, LORE, FER, BIK1, and PBL1 [65**]. Of note, many protein ubiquitylation components and proteasome regulatory components were enriched as ubiquitylated proteins as early as 30 min after MAMP treatment, implicating ubiquitylation and the engagement of the proteasome machinery as an early active defense mechanism in plant immunity [64]. Upon flagellin perception, FLS2 is polyubiquitylated by two closely related PLANT U-BOX (PUB) E3 ubiquitin ligases, PUB12 and PUB13, which interact with and are phosphorylated by BAK1 in regulating FLS2 stability and signaling [66–68]. Moreover, FLS2 homeostasis is regulated by autophagy [69]. It remains unknown whether PUB12/13-mediated ubiquitylation facilitates autophagy-mediated FLS2 degradation and how FLS2 is specifically selected for autophagy. Besides, PUB12/13-mediated ubiquitylation modulates CERK1 or LYK5 protein abundance and negatively regulates chitin-triggered signaling [70,71]. Thus, the same E3 ligases could ubiquitylate multiple PRRs and regulate their homeostasis. It is of interest to investigate how the shared E3 ligases maintain the specificity in regulating different PRR-mediated immune signaling. In contrast to PUB12/13 that negatively regulates PRR signaling, PUB4 positively regulates MAMP signaling [72]. Although interacting with CERK1, it remains to be determined how PUB4 plays a positive role in chitin-triggered signaling and how it coordinates with PUB12/13-mediated ubiquitylation.

RLCK BIK1 homeostasis and activation are regulated by different types of ubiquitylation (Figure 3a). BIK1 is polyubiquitylated by two related E3 ligases PUB25 and PUB26, which regulate BIK1 protein stability before MAMP perception [73]. In response to MAMP treatments, BIK1 is rapidly monoubiquitylated by RING-type E3 ubiquitin ligases RING-H2 FINGER A3A (RHA3A) and RHA3B [74**]. In contrast to PUB25/26 that negatively regulates BIK1-dependent signaling, RHA3A/B-mediated BIK1 monoubiquitylation positively regulates BIK1 function and plant immunity [74**]. Interestingly, *in vivo* and *in vitro* MS and mutational analyses revealed that multiple lysine residues in BIK1 could accept ubiquitin (Figure 3b) [74**]. In the case that a single site is mutated, the alternative sites could still be ubiquitylated. In addition to nine lysine residues of BIK1 that are ubiquitylated by RHA3A/B [74**], five additional lysine residues were identified by MS analysis in BIK1 transgenic plants (Figure 3b) [65**]. It is possible that PUB25/26 and RHA3A/B may compete with these sites or target different sites to regulate BIK1 stability and activation. PUB25/26

appears only to regulate the steady-state, not activated, BIK1 proteins, whereas RHA3A/B mainly acts on the activated but not steady-state BIK1 proteins (Figure 3a) [73, 74**]]. Thus, the sequential and distinct ubiquitylation modifications of BIK1 by different E3 ligases may determine its different fates in plant immunity.

One known consequence of PRR complex ubiquitylation is to direct protein internalization through endocytic pathways [75]. Both FLS2 and BIK1 are internalized upon MAMP perception [76]. BIK1 internalization is mediated by RHA3A/B-mediated monoubiquitylation [74**]. Although awaiting additional experimental validation, PUB12/13-mediated FLS2 polyubiquitylation is likely to play a role in FLS2 endocytosis [77]. Importantly, FLS2 and BIK1 internalization displays distinct dynamics. Upon ligand perception, BIK1 is rapidly internalized into endocytic vesicles for signaling activation, whereas FLS2 is internalized at late time points for degradation and signaling attenuation [76]. It is critical to investigate how FLS2 and BIK1 internalization is spatiotemporally regulated. Do they share the same endocytic route and mechanism? What is the relationship between endocytosis and signaling activation/attenuation? Interestingly, exocytosis may also be involved in PRR regulation. The exocyst subunits EXO70B1 and EXO70B2 regulate the abundance of PM-resident FLS2 [78]. Although the detailed mechanism is unknown, EXO70B2 is ubiquitylated by PUB22, negatively regulating MAMP signaling [79].

## Intertwined phosphorylation and ubiquitylation regulation of PRR receptorsomes

Phosphorylation and ubiquitylation are two intertwined PTMs in regulating cellular signaling. The crosstalk of phosphorylation and ubiquitylation is also a common regulatory mechanism of PRR complex homeostasis and activation. Phosphorylation can regulate the E3 ligase activities, which in turn ubiquitylate the protein kinases. For instance, CPK28 phosphorylates PUB25/26 and enhances their E3 ligase activities for BIK1 polyubiquitylation and degradation (Figure 3a) [73]. Interestingly, heterotrimeric G proteins could inhibit PUB25/26 activity and stabilize BIK1 [73]. The rice RK OsSDS2 phosphorylates the E3 ubiquitin ligase OsSPL11, which in turn ubiquitylates OsSDS2 for degradation in a kinase-dependent manner (Figure 1), suggesting that phosphorylation of OsSPL11 may promote its E3 enzymatic activity [53]. The rice LRR-RK OsXA21 also phosphorylates E3 ligase OsXB3 that positively regulates OsXA21 signaling [80]. It remains unknown whether OsXB3 directly ubiquitylates OsXA21 for activation or degrades a negative regulator in XA21 signaling.

In addition, phosphorylation could promote protein–protein interactions, thereby regulating ubiquitylation. BAK1 phosphorylates PUB12/13, which enhances the PUB12/13 association with FLS2 to promote FLS2 ubiquitylation and degradation [66]. It remains unknown whether BAK1-mediated PUB12/13 phosphorylation affects their E3 ligase activities. However, BRASSINOSTEROID INSENSITIVE1 (BRI1)-mediated PUB12/13 phosphorylation not only promotes their interactions but also increases PUB12/13 activities [77]. Ligand-induced BIK1 phosphorylation is a prerequisite for RHA3A/B-mediated BIK1 monoubiquitylation, which triggers the release of BIK1 from PRR complexes and endocytic trafficking (Figure 3a) [74**]. The follow-up question is whether BIK1 phosphorylates RHA3A/B and regulates their activities. Notably, BIK1 monoubiquitylation does not affect its kinase activities [74**]. The BIK1 homolog PBL13 phosphorylates the NADPH oxidase RBOHD and regulates its stability and activity [81*]. PBL13-mediated RBOHD degradation is likely mediated by the PBL13 INTERACTING RING DOMAIN E3 LIGASE (PIRE) that ubiquitylates RBOHD [81*]. The phosphomimetic mutant of RBOHD displays enhanced ubiquitylation and reduced protein abundance. In this case, RBOHD is a substrate of both kinase and E3 ligase, and its stability is coordinately regulated by phosphorylation and ubiquitylation. Undoubtedly, additional elaborate regulations involving phosphorylation and ubiquitylation of PRR complexes will be continuously revealed in future.

## SUMOylation regulation of PRR complexes

Similar to ubiquitylation, SUMOylation, which attaches the small ubiquitin-like modifier (SUMO) to the target proteins, has been implicated in plant immunity. For instance, key SA regulator NONEXPRESSER OF PR GENES1 (NPR1) is SUMOylated, and its SUMOylation is required for proteasome-mediated degradation [82]. SUMOylation also plays a role in PRR signaling. Upon flagellin perception, FLS2 is SUMOylated by the SUMO E2 enzyme SUMO-CONJUGATING ENZYME1 (SCE1), and the SUMOylated FLS2 is deSUMOylated by the deSUMOylating protease (Desi3a) [83]. MAMP-induced SUMOylation of FLS2 enhances the release of BIK1 from the FLS2-BAK1 complex to trigger intracellular immune signaling (Figure 1). It remains interesting to determine whether PUB12/13-mediated FLS2 polyubiquitylation and SCE1-mediated FLS2 SUMOylation compete with the same lysine sites and the relationship of FLS2 SUMOylation and BIK1 monoubiquitylation in releasing BIK1 from the FLS2–BAK1 complex.

## Glycosylation regulation of PRR complexes

It has been well recognized that PRR RKs, including FLS2, BAK1, and EFR, are subjected to protein *N*-

glycosylation modification (Figure 1) [84]. *N*-glycosylation's function is to ensure protein's correct folding and secretion, and the misfolded proteins will be degraded through the endoplasmic reticulum (ER) quality control (ERQC) pathway. Thus, *N*-glycosylation of PRRs is vital for their maturation and abundance, rather than PRR ligand binding or activation [84]. Intriguingly, even for the closely related PRRs, *N*-glycosylation is only essential for the functions of some, but not others. For instance, protein abundance and signaling of EFR, not FLS2, are compromised in the mutants with defects in *N*-glycosylation or ERQC [84]. It will be interesting to know whether *N*-glycosylation of these PRRs undergoes dynamic changes upon ligand binding and whether this is the determinant of *N*-glycosylation importance for different PRRs. In addition, *bak1/serk4*-induced cell death depends on protein *N*-glycosylation and ERQC [85]. *N*-glycosylation—mediated maturation of the cysteine-rich RKs (CRKs), the expression of which is strongly induced in *bak1/serk4*, partially contributes to the cell death phenotype. Another two related *bak1/serk4* cell death regulators, CNGC19 and CNGC20, the transmembrane $Ca^{2+}$ channels, are also likely glycosylated [86*].

## Lipidation regulation of PRR complexes

Protein lipidation, including myristoylation and palmitoylation, covalently attaches fatty acids to the targets through acyl linkages and regulates protein turnover, trafficking, and microdomain partitioning [87]. Palmitoylation is a reversible process catalyzed by palmitoyl acyltransferases (PATs). Proteomics analysis revealed that many plant proteins, including the PRR complexes, undergo myristoylation and palmitoylation modifications [88,89]. BIK1 is likely myristoylated, and myristoylation is required for BIK1 PM localization and ligand-induced monoubiquitylation [74]. However, mutation of two predicted palmitoylation sites (C830 and C831) of FLS2 does not affect its PM localization and function in FLS2 signaling, hinting that FLS2 may be fortuitously palmitoylated or palmitoylation also occurs at additional sites [90].

Glycosylphosphatidylinositol (GPI) modification is another type of lipidation, through which a phosphoglyceride can be attached to the C-terminus of the target protein [91]. Bioinformatics and proteomic analyses have revealed more than 300 putative GPI-anchored proteins in *Arabidopsis*, including FER, LORELEI, LORELEI-LIKE GPI-ANCHORED PROTEINs (LLGs), other RLKs, and RLPs [91]. LLG1 has been shown to positively regulate FLS2-mediated immunity and *mekk1*-induced cell death [92,93]. However, whether and how GPI plays a role in these pathways remains unknown. Rice CHITIN ELICITOR BINDING PROTEIN (OsCEBiP) is a GPI-anchored PRR for its PM targeting [94]. It remains unknown whether the GPI of PRRs plays other roles in addition to PM targeting.

## Proteolysis of PRR complexes

Proteolytic cleavage of the small secreted peptide ligands of RKs is essential for peptide secretion and PRR signaling activation [95]. Proteolytic cleavage is also emerging as a necessary regulatory process of PRR complex functions. Rice OsXA21 was reported to be cleaved, and the cleaved intracellular domain might translocate to the nucleus [96]. *Arabidopsis* CERK1 is also likely cleaved, and the cleaved and uncleaved CERK1 may have uncoupled functions in plant immunity and cell death control [97]. The legume RK SYMBIOSIS RECEPTOR-LIKE KINASE (SYMRK) undergoes proteolytic cleavage, and the cleaved ectodomain promotes complex assembly with another RK NOD FACTOR RECEPTOR5 (NFR5) in root symbiosis [98]. An ATP-binding proteomics analysis predicted that two uncharacterized LRR-RKs (AT3G02880 and AT5G16590) exist only as cytoplasmic domains, suggesting that the extracellular domains are cleaved [99]. The coreceptor BAK1 and other SERKs undergo a $Ca^{2+}$-dependent proteolytic cleavage, which is required for BAK1-regulated plant growth, development, and immunity [100]. The BAK1 mutant with a defect in cleavage impairs its phosphorylation and PM localization. The PM-tethered BIK1 was also observed in the nucleus [48**]. It will be interesting to determine whether BIK1 is cleaved and translocated into the nucleus. Nevertheless, there are still many unsolved questions for PRR complexes' proteolysis. For example, what are the proteases that regulate this process? Where does each fragment go after cleavage? Is the proteolysis induced upon ligand perception? What is the relationship of proteolysis and other PTMs in regulating PRR complexes?

## Perspectives: what is next?

Over the past decades, different PTMs have been reported to regulate PRR complex assembly, homeostasis, activation, and subcellular dynamics. Different PTMs can simultaneously or sequentially act on the same target and synergistically or antagonistically regulate target proteins. There are some examples of the cross talk of phosphorylation and ubiquitylation in the regulation of PRR complexes. However, detailed regulatory mechanisms among different PTMs await to be discovered. PTMs are usually reversible processes. Much more knowledge about the modifying enzymes is known than that of demodifying enzymes. Although phosphorylation and ubiquitylation are extensively studied in plant immunity, protein dephosphorylation and deubiquitylation are much less understood. Some other types of PTMs, such as ADP-

ribosylation, have been implicated in plant immunity [101]. It is not surprising that they may also regulate PRR complexes.

Our understanding of PTMs on PRR regulation will benefit from the emerging technical advances, especially the drastically increased sensitivity, resolution, and mass accuracy of proteomics instrumentation. MS-based proteomics provides a quantitative atlas of near-complete *Arabidopsis* proteomes and phosphoproteomes in 30 different tissues [102**]. Large-scale quantitative proteomics following the enrichment of specific PTMs in plants treated with different MAMPs will continuously reveal the PRR-associated PTM dynamics in a native state at the whole organismal level. A recent study using a large-scale MS analysis uncovered the quantitative effect of phosphorylation on protein stability regulation in a site-specific manner and in the context of surrounding amino acids, local structure, and phosphoprotein functions [103]. Single-cell proteomics, combined with cell type−specific knockouts, will help us understand the difference of expressed proteome from cell to cell and the specific function of PTMs at single-cell resolution [104]. Gene targeting or base editing tools make it possible to evaluate the function of specific PTM sites with loss-of-function (i.e. phosphoinactive) and gain-of-function (i.e. phosphomimetic) mutations in the endogenous proteins. Together, these advances and others will help us better understand PRR complex regulation by PTMs and provide insight into strategic development for improved plant disease resistance.

## Declaration of competing interest

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

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