Trends in Plant Science

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Review

Receptor-like cytoplasmic kinases: orchestrating plant cellular communication

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The receptor-like kinase (RLK) family of receptors and the associated receptor-like cytoplasmic kinases (RLCKs) have expanded in plants because of selective pressure from environmental stress and evolving pathogens. RLCKs link pathogen perception to activation of coping mechanisms. RLK–RLCK modules regulate hormone synthesis and responses, reactive oxygen species (ROS) production, Ca^{2+} signaling, activation of mitogen-activated protein kinase (MAPK), and immune gene expression, all of which contribute to immunity. Some RLCKs integrate responses from multiple receptors recognizing distinct ligands. RLKs/RLCKs and nucleotide-binding domain, leucine-rich repeats (NLRs) were found to synergize, demonstrating the intertwined genetic network in plant immunity. Studies in arabidopsis (*Arabidopsis thaliana*) have provided paradigms about RLCK functions, but a lack of understanding of crop RLCKs undermines their application. In this review, we summarize current understanding of the diverse functions of RLCKs, based on model systems and observations in crop species, and the emerging role of RLCKs in pathogen and abiotic stress response signaling.

Highlights

Receptor-like cytoplasmic kinases (RLCKs) have become major players in plant immunity regardless of the pathways involved.

RLCKs form regulatory nodes that link receptors to downstream regulators that modulate plant hormones, Ca^{2+} signaling, reactive oxygen species (ROS) accumulation as well as activation of mitogen-activated protein kinases (MAPKs), transcription regulators, and immune gene expression.

Phosphorylation, ubiquitination, and other post-translational modifications by effectors regulate RLCKs, enabling functional versatility and homeostasis.

As major integrators of signals from receptors, RLCKs are potential targets for biotechnological applications.

RLCKs play pivotal roles in plant immunity by contributing to both PTI and ETI. Due to their critical functions, these kinases are targets for manipulation by pathogen effectors to attenuate PTI. RLCKs also directly or indirectly recognize effectors and activate ETI.

The function of RLCKs in crop plant immunity is emerging.

RLCKs function in plant immune signaling, responses to other environmental cues, and plant growth and development

Plant responses to pathogens, environmental stress, and growth signals are regulated by intricate genetic, molecular, and biochemical mechanisms that sense and orchestrate adaptive and developmental responses. Plant RLK proteins are receptors for extracellular and endogenous signals that shape the specificity of responses. They are characterized by extracellular ligand-binding, transmembrane, and intracellular kinase domains. Receptor-like proteins (RLPs) are also transmembrane proteins that lack the cytoplasmic kinase domain but retain the extracellular leucine-rich repeat (LRR) domains required for ligand binding. RLCKs are often functionally and physically associated with RLKs and link receptors to downstream response regulators underlying different biological functions. Structurally, RLCKs lack the extracellular and transmembrane domains that characterize RLKs, but share similar kinase domains, suggesting that RLKs and RLCKs have a monophyletic origin [1]. However, recent evolutionary analyses of cell surface receptors suggest that RLK and RLCK are from ancient plant lineages that may have evolved in parallel [2]. Regardless, in plants, RLKs and RLCKs expanded into hundreds of genes as a result of selective pressure from environmental assault and pathogen challenge, indicative of their critical role in plant adaptation [3]. The animal RLK/Pelle family of proteins are far fewer, but also function in immune response regulation, suggesting that this protein family evolved before the divergence of plants and animals [1]. Broadly, RLCKs function in pathogen response, hormone signaling, insect resistance, symbiosis, embryonic patterning, plant reproduction, and nutrient deficiency, as reviewed recently elsewhere [4]. RLKs are cell surface-localized receptors for pathogen-associated molecular patterns (PAMPs; also called general elicitors), pathogen or host-derived elicitors, such as the bacterial flg22, fungal chitin, plant cell wall fragments, and phytocytokines (peptides) the recognition of which activates PAMP-triggered immunity (PTI) [5]. RLKs that recognize PAMPs are also referred to as pattern

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recognition receptors (PRRs). By contrast, NLRs are intracellular receptors of pathogen effectors that activate effector-triggered immunity (ETI), which is a particularly strong form of resistance to certain strains of pathogens. RLKs, RLCKs, and NLRs form an intertwined network of immune response regulators that function synergistically in PTI and ETI [6,7].

Despite accumulating knowledge of the functions of arabidopsis RLCKs, their crop counterparts remain understudied, limiting the identification of functionally conserved plant RLCKs and undermining their application. Although a few complete ligand–RLK–RLCK signaling modules have been established [8–12], the functions of a substantial number of RLKs and RLCKs remain unknown even in model systems. Additionally, most observations emerged from arabidopsis interactions with the bacterial pathogen *Pseudomonas syringae* (*Pst*) and some fungal pathogens, but there is a lack of data on the role of RLCKs and RLKs in plant responses to nematodes, viruses, fungal, and oomycete pathogens. In addition, climate change brings new pathogen and pest dynamics and expands the ecological reach of pathogens, which can contribute to an increased frequency of disease epidemics. The role of these critical regulators in modulating disease under changing climate variables is unknown. A detailed understanding of the functions of RLK–RLCK modules is important for the rationale design of disease-resistant plants under changing environmental and pathogen dynamics. RLCKs integrate responses from multiple cues, which makes them targets of choice for biotechnological applications. Gene-editing tools are expected to fuel new discoveries in RLCK functions in crop plants.

A simplified model of immune response signaling, starting from signal recognition to activation of resistance mechanisms involving RLKs and RLCKs, is presented in Figure 1. Multiple recent reviews have focused on the fundamental aspects of immune signaling through RLCKs [4,13–17]; thus, this review emphasizes recent observations from crop plants, including tomato (*Solanum lycopersicum*), rice (*Oryza sativa*), wheat *(Triticum aestivum)*, and other crops. Tomato is not only an important vegetable with major contributions to human nutrition, but also amenable to genetic and molecular studies. It is host to bacterial, fungal, oomycete, viral, and nematode pathogens that impact its productivity, making it an economically important experimental system. The relative ease of transformation for gene editing also makes it a model crop of choice to dissect RLCK functions. Available information on RLCK immune function in rice, maize, and wheat, which are all global mainline food crops, is summarized in Table 1. RLCK data are lacking for many agronomic and horticultural crops. In this review, we focus on RLCKs as linkers of surface-localized receptors to downstream immune response regulators, paradigms deciphered from model systems, observations in crop species, and the emerging role of RLCKs as regulators of abiotic stress responses as well as fungal and insect resistance.

Structural and functional paradigms of the BIK1-related family of RLCK subfamily VII

RLCKs are divided into 17 subfamilies based on their sequence similarities [18]. Structurally, RLCKs contain a large central kinase domain with 11 conserved subdomains, including the highly conserved activation segment [1]. There are 379 RLCKs in rice [19], 149 in arabidopsis [18], and 128 in tomato [20]; the functions of most of which are unknown. In arabidopsis, some of the 46 members of RLCK-VII have been widely studied in pathogen response signaling [21]. This group is further divided into nine subgroups involved in plant immunity, growth, and development (Figure 2). Among these, RLCK VII subgroup 4 is required for chitin-triggered ROS production and activation of MAPKs in arabidopsis and tomato [21,22]. Higher order mutants in members of subgroups 5, 7, and 8 are susceptible to the nonpathogenic strain of *Pst* (hrcC-) and show reduced flg22-, elf18-, and chitin-triggered ROS production [21]. In addition, RLCK VII-5 has been implicated in immune responses to the fatty acid (FA) PAMP mc-3-OH-FAs from Gram-

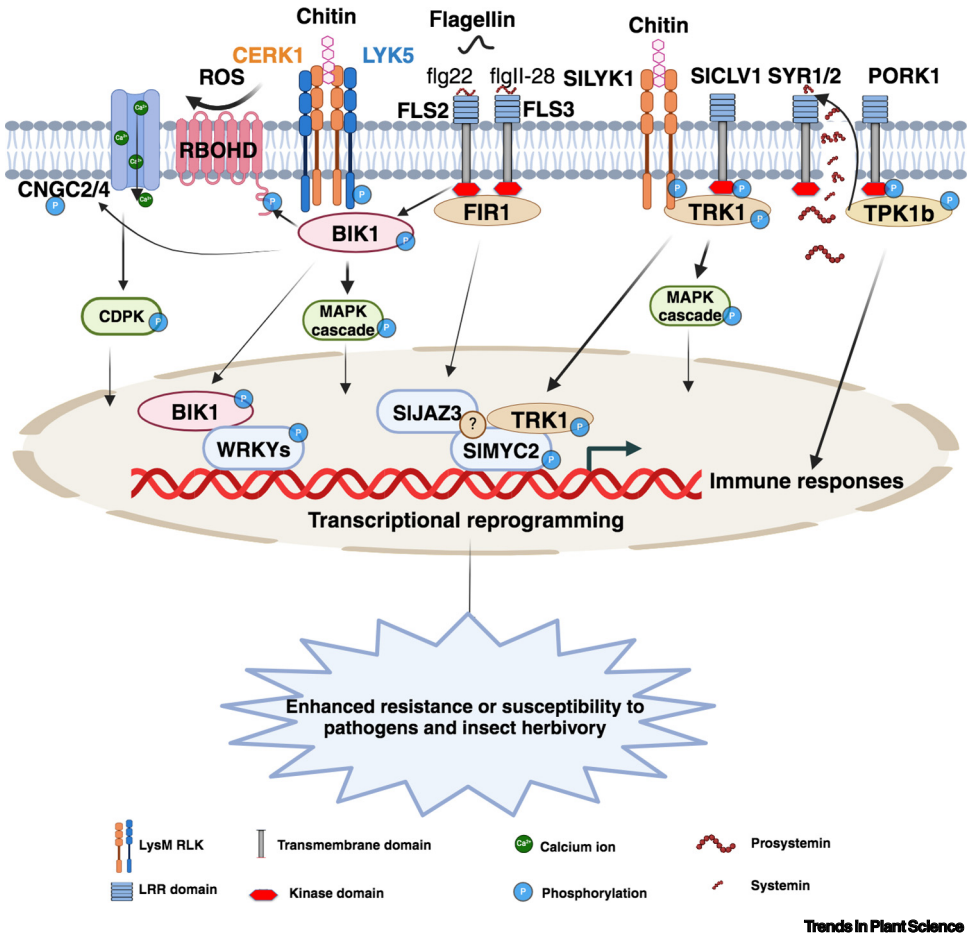

Figure 1. A simplified model showing receptor-like kinases (RLKs), receptor-like cytoplasmic kinases (RLCKs), and other genetic components of quantitative resistance mediated by recognition of elicitors. Representative and major regulators of tomato (Solanum lycopersicum) and arabidopsis (*Arabidopsis thaliana*) RLCKs, RLKs, and downstream components are shown. Receptor burst oxidase homolog protein D (RBOHD) is phosphorylated by many different RLCKs, but only BOTRYTIS-INDUCED KINASE 1 (BIK1) is shown here for simplicity. Most RLCKs are positive regulators of disease resistance, but some susceptibility factors have been isolated. Immune responses in quantitative resistance represent the accumulation of plant diverse plant hormones, the expression of a battery of defense genes, hormone-regulated genes, such as those encoding protease inhibitors in tomato, and array of secondary metabolites. Abbreviations: CDPK, calcium-dependent protein kinase; CERK1, CHITIN ELICITOR RECEPTOR KINASE1; CNGC2/4, cyclic nucleotide-gated channels 2/4; FIR1, FLS/FLS3-interacting receptor-like cytoplasmic kinase 1; flg22/flgII-28, flagellin 22/28; FLS2/3, flagellin-sensing 2/3; LYK1/5, LysM receptor kinase 1/5; MAPKs, mitogen-activated protein kinases; PORK1, PEPR1 Orthologue Receptor-like Kinase1; ROS, reactive oxygen species; SlCLV1, tomato CLAVATA1; SlJAZ3, Jasmonate ZIM domain3; SYR1/2, Systemin Receptor1/2;TPK1b, Tomato Protein Kinase1b; TRK1, TPK1b-related kinase; WRKYs, DNA-binding proteins.

negative bacteria [23], whereas RLCK VII-8 contributes to Pep2-triggered MAPK activation [21]. Overall, some RLCK subgroups appear to be specific, while others integrate signals from multiple PAMP recognitions, suggesting a level of functional specialization of RLCK subgroups in plant immunity.

BOTRYTIS-INDUCED KINASE 1 (BIK1) is a RLCK widely studied in plant immunity. Consistently, BIK1 interacts with an increasing number of proteins, including the RLKs/RLPs FLAGELLIN SENSITIVE2 (FLS2) [24], elongation factor thermo unstable (EF-Tu) receptor (EFR) [25], PEP

Table 1. Characterized RLCKs in arabidopsis and crop plants, their interacting partners, and functions[a]

Gene	Function	Interactor proteins	RLCK family in arabidopsis	Refs
Arabidopsis				
BIK1	PTI and ETI, positive or negative regulator of plant immunity depending on the nature of pathogens	Diverse interacting proteins, including RLKs, RBOHD, TFs, protein phosphatase, protein ubiquitinase, MAPKs, CPKs, DGK5, etc.	VII-8	[24–31,34,70,99,100,104–112]
PBL1	Cleaved by AvrPphB, which activates RPS5 and, subsequently, ETI	PBS1; RPS5	VII-8	[12]
PBL34/35/36	PTI through interaction with the RLK LORE, which is the receptor for the mc-3-OH-FAs; root quiescent center stem cell maintenance	LORE	VII-5	[23]
PBL27	Complexes with CERK1 and LYK5 for chitin-mediated PTI, and with ZED1-ZAR1 complex, impacting ETI	LysM RLK, CERK1, ZRK3, MAPKKK5, SLAH3	VII-1	[38,39,75]
PBL27, 17, 8, 15, 13	HopZ1a acetylates PBLs to promote ZED1-PBL-ZAR1 interaction in ZAR1-dependent plant immunity	HopZ1a; ZED1	VII-1, VII-6	[64]
ZED1	ETI; associates with different ZRKs to recognize effectors	HopZ1a; PBLs; ZRKs	XII-2	[61,65]
PBL2	Uridylated by AvrAC, ETI	AvrAC; ZRK1; ZAR1	VII-9	[67]
SZE1/2	Components of plant resistance to *Pseudomonas syringae* (*Pst*) carrying HopZ1a	ZAR1; HopZ1a	XII-2	[66]
ZRK1/RKS1	Required for ZAR1-mediated ETI	ZAR1; PBL2	XII-2	[67]
ZRK3	Recognition of *Pst* effector HopF2a	ZAR1	XII-2	[65]
PBL8 & PBL17	*Phytophthora infestans* effector RXLR25 interacts with PBL8 and PBL17 to suppress resistance	RXLR25	VII-6	[72]
CRPK1	Negative regulator of cold stress; phosphorylates 14-3-3λ, destabilizing cold-responsive C-repeat-binding factor proteins	14-3-3λ	NG	[113]
PCRK1/2	Enhances plant immunity against *Pst pv. maculicola* ES4326 through interacting with FLS2 and regulation at SA-biosynthetic gene *ICS1* and TFs, *SARD1* and *CBP60g*	FLS2	VII-4	[114]
BSK1–8	BSK1 modulates plant immunity against *Pst* through upstream receptors, such as RLK902 and FLS2. BSK1 function in plant resistance to fungi and oomycetes is extended through interaction of RD19. In addition, BSKs are involved in brassinosteroid (BR) signaling pathway	BSK1 interacts with RLK902, FLS2, MAPKKK5, PDL2, and RD19; BSK1–8 interact with BRI1	XII	[115–117]
CRCK3	NLR protein SUMM2 senses disruption of MPK4 phosphorylation to its substrate CRCK3 to activate ETI	SUMM2	IV	[118]
RLCK-VI_A3	AtRLCK VI_A3 interacts with small monomeric G protein AtROP to regulate basal resistance to *Erysiphe cruciferarum* and cellular differentiation during trichome morphogenesis	AtROPs	VI	[119]
CDG1	Negatively regulates flg22 and chitin-induced ROS by promoting degradation of FLS2 and CERK1; also phosphorylates RIN4 to enhance AvrRpm1-induced ETI and is involved in BR signaling pathway	BRI1, FLS2, CERK1, RIN4	VII-NG	[120]
SSP (BSK12)	SSP transcripts are produced in mature pollen without translation to protein. SSP protein is transiently expressed after delivery from sperm	ND	XII	[121]

Table 1. (continued)

Gene	Function	Interactor proteins	RLCK family in arabidopsis	Refs
	cells to zygote and then activates MAPK YODA-mediated signaling activity, providing essential temporal cue for regulation of asymmetric first division			
MRI	Novel component of CrRLK1L-mediated signaling pathway that coordinates CW integrity and tip growth; interacts with OXI1 to regulate pollen tube growth and root hair elongation	OXI1	VIII	[122,123]
CST	Acts as spatial inhibitor of cell separation by interacting with the LRR-RLKs, HAESA and EVERSHED, in floral organ abscission	HAE, EVR	VII-7	[124]
ARCK1	Cysteine-rich repeat RLCK; negatively controls ABA- and osmotic-mediated signal transduction through interacting with RLK CRK36	CRK36	NG	[125]
PTI1-1/PTI1-2/PTI1-3/ PTI1-4/PTI1-5	All interact with, and are phosphorylated by, protein kinase OXI1 in response to phosphatidic acid, H2O2, flg22, and xylanase. PTI1-1, PTI1-2, and PTI1-5 may be involved in biotic and abiotic stress, and male gametophyte sterility, respectively	OXI1	VIII	[122,126,127]
PBL13	Negative regulator of arabidopsis immune responses to *Pst* through association with RBOHD before pathogen perception	PIRE; RBOHD	VII-6	[47,128]
RIPK (PBL14)	Important for XopAC, AvrRpm1, or AvrB-specific ETI, and arabidopsis resistance to Xcc or *Pst*, respectively	XopAC, RIN4, FERONIA, NADP-malic enzyme 2, RBOHD	VII-6	[129]
SGN1 (PBL15)	Establishment of Casparian strip formation in root endodermis by locally inducing ROS-dependent lignification	CIF2- SGN3-SGN1-NADPH oxidase complexes	VII-6	[130,131]
LIP1/2	Pollen tube guidance into micropyle	Unknown	VII-3	[132]
Brassica rapa				
MLPK	Two MLPK isoforms localize to papilla cell membrane and interact directly with ligand-activated S-locus receptor kinase SRK to transduce self-incompatibility signaling.	SRK	VII-8	[133,134]
Solanum lycopersicum (tomato)				
TPK1b	Fungal and insect defense through systemin and ethylene responses	PORK1; FLS2	VII-8	[35,76]
TRK1	Fungal defense through interaction with SlLYK1, SlMYC2-mediated responses to chitin; meristem homeostasis through interaction with CLV1 and WUS	SlLYK1, SlCLV1, SlMYC2, SlWUS2	VII-4	[22]
Fir1 (AtBSK7)	Flagellin response signaling and pre-invasion immunity to *Pst*; negative regulator of JA signaling	JAZ3; FLS2; FLS3	XII-1	[55]
Pto	Confers resistance to bacterial speck disease caused by *Pst* (AvrPto/AvrPtoB)	Prf; TFT3; AvrPto/AvrPtoB; Pti1	NG	[69]
Pti1a/b	Involved in immune responses to *Pst* pv tomato	Pto; Prf	VIII	[135]
SlZRK1	Negative regulator in wound-induced jasmonate accumulation and insect resistance	ND	XII-2	[78]
SlRIPK	Enhances plant resistance to various pathogens but does not sacrifice plant growth	ND	VII-6	[136]

(continued on next page)

Table 1. (continued)

Gene	Function	Interactor proteins	RLCK family in arabidopsis	Refs
Fen	Involved in effector perception and activates NLR Prf in a Fen kinase-dependent manner	Prf	NG	[73,74]
ACIK1	Has essential role in LRR Cf-4 and Cf-9, which confers recognition of secreted *Cladosporium fulvum* effectors Avr4 and Avr9	Cf-4, Cf-9	VII-6	[137]
Oryza sativa (rice)				
BSR1 (PBL19, PBL20)	Broad-spectrum resistance to multiple pathogens in different plant species through signaling of chitin, OsPeps, peptidoglycan, and lipopolysaccharides	ND	VII-4	[56,57,103,138]
OsRLCK185 (PBL27)	Chitin-mediated plant resistance through direct phosphorylation by OsCERK1	CERK1-CEBiP; OsCNGC9; OsDRE2a	VII-1	[41,53]
STRK1	Enhances salt and oxidative stress tolerance through activation of CatC by phosphorylation, thereby regulating H_2O_2 homeostasis	CatC	NG	[94]
GUDK (RIPK; PBL13)	Signaling of drought stress and salinity responses	OsAP37	VII-6	[96]
OsRLCK 57, 107, 118, 176 (BIK1)	Function in chitin- and PGN-mediated rice immunity	OsRLCK176 and 107 interact with OsCERK1	VII-8	[42,139]
CSK1; OsRLCK269 (PBL28)	Negative regulator of secondary cell wall (SCW) formation, ABA-mediated cell growth and SCW deposition	Phosphates TF VND6	VII-NG	[140]
RLCK102	Positively regulates receptor kinase XA21-mediated immunity and negatively regulates rice development through BR signaling in rice	XA21, OsBRI1	VII-9	[141]
OsBSK1-2; OsBSK3	OsBSK1-2, an ortholog of BSK1, negatively regulates plant resistance to *Magnaporthe oryzae*, but positively responds to chitin- or flg22-triggered immunity. OsBSK3 interacts upstream (OsBRI1) or downstream (OsPPKL1 and OsGSK3) to regulate grain length and weight through BR-signaling pathway	OsBRI1, OsPPKL1 (AtBSU1) and OsGSK3 (AtBIN2)	XII	[142,143]
OsRLCK253	OsRLCK253 may interact with OsSAP1/11 to improve water-deficit and salt stress tolerance	OsSAP1/11	NG	[144]
PSTOL1	Identified in *Oryza rufipogon* accessions; enhances rice root weight and phosphorus content	ND	NG	[145]
Triticum aestivum (wheat)				
TaPsIPK1	Binds to fungal effector PsSpg1 to trigger wheat susceptibility to *Puccinia striiformis*	PsSpg1; TaCBF1d	VII-NG	[71]
TaRLCK1B	Rapidly and markedly elevated by *Rhizoctonia cerealis* infection in resistant wheat cultivars	ND	VII-8	[146]
Stpk-V	Leads to cell death; confers durable and broad-spectrum resistance to wheat powdery mildew	ND	NG	[147]
Marchantia polymorpha				
MpPlSLa (PBL8)	Mediates ROS accumulation in response to chitin	MpRBOH1	VII-6	[49]
Hordeum vulgare (barley)				
RBK1	Identified in interaction of small monomeric G-proteins of plant RHO (HvROP); might function in basal resistance to powdery mildew by influencing microtubule organization	HvROPs, HvRACB, HvRAC1	VI	[148]
Lophopyrum elongatum (wheatgrass)				
Esi47 (AtPCRK1)	Regulates salt stress and ABA signaling	ND	VII-4	[149]

Table 1. (continued)

Gene	Function	Interactor proteins	RLCK family in arabidopsis	Refs
Glycine soja (soybean)				
GsRLCK	Positively regulates plant tolerance to drought and salt stress in arabidopsis	ND	NG	[150]
GsCBRLK	Positively regulates plant tolerance to salt and ABA by interacting with late embryogenesis abundant protein; also interacts with GsMSRB5a to activate ROS signaling to regulate carbonate alkaline stress	GsPM30, GsMSRB5a	IV	[151,152]
Zea mays (corn)				
ZmBLK1	Increases resistance to *Clavibacter michiganensis* subsp. *Nebraskensis*, a bacterial pathogen causing Gross's wilt	ZmWIK/ZmWAKL; ZmRBOH4	VII-8	[36,153]
ZmSTK1, ZmSTK2	Mediate pollen development and glycolytic pathway through enolases	Enolases	IX	[154,155]
Capsicum annuum (pepper)				
CaPIK1 (AtPBL5)	Elevates ROS bursts and enhances basal resistance to *Pst* pv. tomato and *Hyaloperonospora arabidopsidis*; also associated with salicylic acid-dependent defense response	CaGRP1	VII-1	[156,157]

[a]Abbreviations: ACIK1, AVR9/CF-9-INDUCED KINASE 1; ARCK1, ABA- AND OSMOTIC-STRESS-INDUCIBLE RECEPTOR-LIKE CYTOSOLIC KINASE1; BIK1, BOTRYTIS-INDUCED KINASE 1; BSK1, BRASSINOSTEROID-SIGNALING KINASE1; BSR1, BROAD-SPECTRUM RESISTANCE 1; CST, CAST AWAY; CDG1, CONSTITUTIVE DIFFERENTIAL GROWTH1; CRCK3, CALMODULIN-BINDING RECEPTOR-LIKE CYTOPLASMIC KINASE 3; CRK36, CYSTEINE-RICH RLK (RECEPTOR-LIKE PROTEIN KINASE) 36; CRLK1/2, CALCIUM/ CALMODULIN-REGULATED RECEPTOR-LIKE CYTOPLASMIC KINASE1/2; CRPK1, COLD-RESPONSIVE PROTEIN KINASE 1; CSK1, CELLULOSE SYNTHASE CO-EXPRESSED KINASE; EVR, EVERSHED; FER, FERONIA; GsCBRLK, CALCIUM-DEPENDENT CALMODULIN-BINDING RECEPTOR-LIKE KINASE; GsMSRB5a, methionine sulfoxide reductase (MSR) B5a protein; GsPM30, group 3 late embryogenesis abundant protein 30; GUDK, GROWTH UNDER DROUGHT KINASE; HAE, HAESA; MAPKKK5, MEK KINASE5; MLPK, M-locus protein kinase; MRI, MARIS; ND, not determined; NG, not grouped; OsPPKL1, protein phosphatase with Kelch-like repeat domain1; OXI1, OXIDATIVE SIGNAL INDUCIBLE1; PBS1, AVRPPHB SUSCEPTIBILE 1; PCPR1/2, PATTERN-TRIGGERED IMMUNITY COMPROMISED RECEPTOR-LIKE CYTOPLASMIC KINASE 1/2; PCRK1, PATTERN-TRIGGERED IMMUNITY COMPROMISED RECEPTOR-LIKE CYTOPLASMIC KINASE1; PCRK1/2, PTI COMPROMISED RECEPTOR-LIKE CYTOPLASMIC KINASE 1/2; PORK1, PEPR1 ORTHOLOGUE RECEPTOR-LIKE KINASES1; *Pst*, *Pseudomonas syringae*; PSTOL1, phosphorus-starvation tolerance 1; Pti1, Pto interaction protein 1; RBK1, ROP binding protein kinase1; RBOHD, RESPIRATORY BURST OXIDASE PROTEIN D; RD19, RESPONSE TO DEHYDRATION 19; RDL2, RD19-LIKE 2; RLK902, RECEPTOR-LIKE KINASE 902; ROP, Rho of plants; ROS, reactive oxygen species; RPS5, RESISTANT TO P. SYRINGAE 5; PBL, PBS1-LIKE KINASE; SAP, stress-associated protein; SCW, secondary cell wall; SRK, S-receptor kinase; SSP, SHORT SUSPENSOR; STRK1, SALT TOLERANCE RECEPTOR-LIKE CYTOPLASMIC KINASE 1; SUMM2, SUPPRESSOR OF MKK1 MKK2; SYR1/2, SYSTEMIN RECRPTOR 1/2; TaPsIPK1, *Puccinia striiformis*-induced Protein Kinase 1; TF, transcription factors; TRK1, TPK1B RELATED KINASE1; VND6, VASCULAR-RELATED NAC-DOMAIN 6; WUS, WUSCHEL; Xcc, *Xanthomonas campestris* pv campestris; ZAE1/2, SUPPRESSOR OF ZED1-D1; ZAR1, HOPZ-ACTIVATED RESISTANCE 1; ZED1, HOPZ-ETI-DEFICIENT 1; ZmBLK1, *Zea mays* BIK1-LIKE KINASE 1; ZRK1, ZED1-RELATED KINASE 1; LIP1/2, LIGHT INSENSITIVE PERIOD1; CaGRP1, GLYCINE-RICH RNA-BINDING PROTEIN1; DGK5, DIACYLGLYCEROL KINASE 5.

RECEPTOR1/2 (PEPR1/2) [26], CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1) [27], BRASSINOSTEROID INSENSITIVE 1 (BRI1)-ASSOCIATED KINASE 1 (BAK1) [28], BRI1 [29], and CYSTEINE-RICH RECEPTOR-LIKE KINASE 36 (CRK36) [30]. In addition, BIK1 interacts with many proteins with diverse biochemical and molecular functions (Table 2), and more interacting proteins are likely to be identified. BIK1 has the ability to interact with many proteins, which may explain its multifunctionality. The structural and sequence variations in RLCKs that underlie their specific or broad molecular interactions and biological functions need further investigation. Interestingly, BIK1 phosphorylation status and its stability determine its interacting partners [8]. The crystal structure of BIK1 protein has been resolved through X-ray diffraction at 2.35-Å resolution [9]. Comparison of BIK1 crystal structure with other related kinases demonstrates that the β2–β3 loop in BIK1 is ten residues longer and forms a structure that was suggested to be a platform for interaction with other proteins [9]; however, the structure of the BIK1 complex has not been studied to elucidate the interacting ability of BIK1. Whether this structural feature underpins the broad interaction of BIK1 with other proteins and its diverse functions needs further studies.

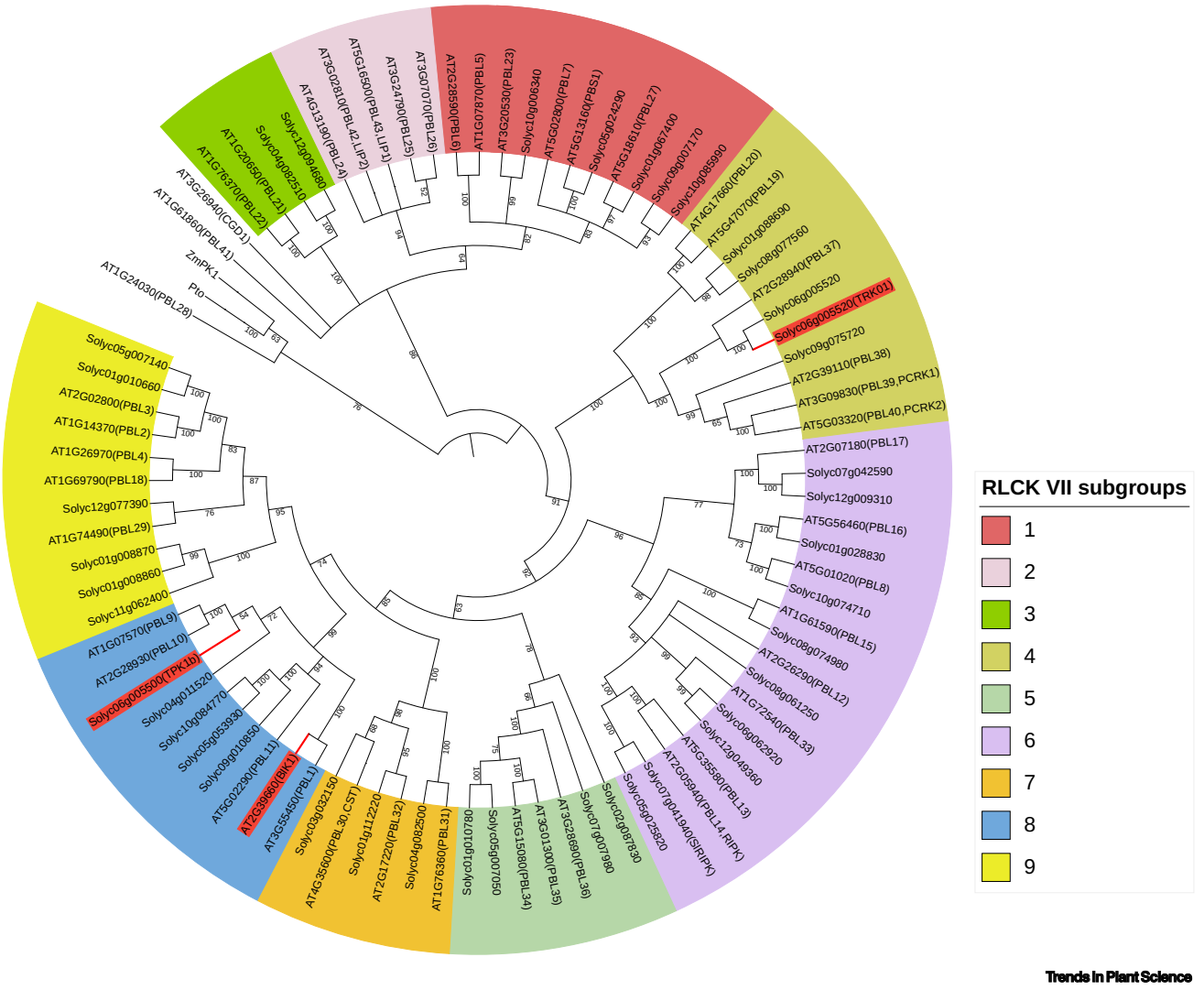

Figure 2. Phylogenetic analysis of arabidopsis (*Arabidopsis thaliana)* and tomato (*Solanum lycopersicum*) receptor-like cytoplasmic kinases (RLCK) VII proteins. This phylogenetic tree represents a small proportion of the large RLCK protein family, which remains largely uncharacterized. Only three RLCK genes have been characterized from the numerous members of RLCK VII in tomato.

Arabidopsis BIK1 was identified because of its increased expression in response to *Botrytis cinerea*, hence its name. The increased susceptibility of the arabidopsis *bik1* mutant to the necrotrophic fungi *B. cinerea* and *Alternaria brassiciola* and enhanced resistance to virulent strains of *Pst,* established its genetic significance and laid the foundation for subsequent studies [10]. The mechanism of resistance of the *bik1* mutant to *Pst* and other pathogens has been documented, but was not followed with subsequent studies. In addition, BIK1 is required for multiple growth traits, including flowering time, seed set, root hair growth, and seedling growth responses to ethylene [11,26,29]. Only a single loss-of-function allele of BIK1 has been studied, and its growth defects can be severe depending on growth conditions. Subsequent studies demonstrated that BIK1 is required for PTI [11,12,30], ETI [12,31], responses to insect pests [32], fungal, and bacterial pathogens [10,12] as well as double-strand (ds)RNA-induced immunity to viral infection [33] and control of stomatal opening [34]. Studies of BIK1 have advanced

Table 2. Arabidopsis BIK1-interacting proteins implicated in plant immune response, hormone, and growth response pathways[a]

BIK1-interacting protein	Response to elicitor or roles	Refs
FLS2	Receptor for flg22 epitope of bacterial flagellin. When flg22 is sensed by FLS2, the latter forms a complex with BAK1 and BIK1, and BIK1 is phosphorylated to activate plant immunity	[24]
EFR	Similar to FLS2, EFR perceives elf18 from EF-Tu. When elf18 is sensed by EFR, the latter forms a complex with BAK1 and BIK1, and BIK1 is phosphorylated to activate plant immunity	[25]
PEPR1 and PEPR2	PEPR1 and PEPR2 recognize pep1 and pep2, respectively, considered damage-associated molecular patterns (DAMPs, phytocytokines). PEPR1 interacts and phosphorylates BIK1 to mediate Pep1-induced plant immunity	[26]
CERK1	LysM-containing receptor that perceives chitin to activate chitin-mediated plant immunity; phosphorylates BIK1 to regulate chitin-induced ROS production and callose deposition	[12,38]
BRI1	Associates with BRI1 receptor; is released from it upon brassinosteroid (BR) treatment; can be phosphorylated directly by BRI1 in BAK1-independent manner	[29]
ERECTA	An RLK. BIK1 interacts with ERECTA family proteins and phosphorylates ERECTA to modulate leaf morphogenesis and inflorescence architecture	[158]
CRK36	Interacts with BIK1 and enhances flg22-triggered BIK1 phosphorylation through NADPH oxidases to promote stomatal immunity	[30]
BAK1	Acts as coreceptor for FLS2 and has critical role in FLS2-mediated plant immunity; phosphorylates, and is phosphorylated by, BIK1	[28]
CALCIUM-DEPENDENT PROTEIN KINASE 28 (CPK28)	Acts as negative regulator in plant immunity; interacts and phosphorylates BIK1 to maintain BIK1 stability	[109,159]
SERINE/THREONINE KINASE1 (SIK1)	Positively regulates plant immunity through direct phosphorylation of BIK1 and RBOHD	[111]
MITOGEN-ACTIVATED PROTEIN4 (MAP4KKKK; MAP4K4)	Phosphorylates BIK1 on multiple sites to regulate BIK1 stability and BIK1-mediated immunity	[110]
DIACYLGLYCEROL KINASE 5 (DGK5)	BIK1 phosphorylates DGK5 at S506, which leads to induction of cellular messengers, phosphatidic acid (PA), ROS, and immunity. However, PRR-activated intracellular MPK4 phosphorylates DGK5 at T446, which subsequently suppresses DGK5 activity and PA production, resulting in attenuated plant immunity	[112]
EXTRA LARGE G-PROTEIN 2 (XLG2)	Flg22 causes BIK1 phosphorylation of XLG2 at its N terminus, dissociating XLG2 from BIK1-FLS2 complex; XLG2 positively regulates RBOHD in ROS production and resistance to *Pst*	[104]
CYCLIC NUCLEOTIDE-GATED CHANNEL (CNGC) 20, CNGC2, and CNGC4	CNGCs are required for calcium ion channels. BIK1 interacts and stabilizes CNGC20 or phosphorylates CNGC2/CNGC4 to elevate Ca^{2+} influx for plant immunity	[105]
REDUCED HYPEROSMOLALITY-INDUCED CA^{2+} INCREASE1.3 (OSCA1.3)	Ca^{2+}-permeable channel; BIK1 associates and phosphorylates OSCA1.3 at S54 site for Ca^{2+} permeability to regulate stomatal immunity	[34]
Plant U-BOX (PUB25/26)	Nonactivated BIK1 is targeted and degraded by PUB25/26; CPK28 phosphorylates PUB25/26 to enhance degradation. Heterotrimeric G proteins AGB1 and XLG2 inhibit PUB25/26 activity and stabilize BIK1	[100]
PUB4	In absence of PAMP elicitation, BIK1 is degraded by	[31]

(continued on next page)

Table 2. (continued)

BIK1-interacting protein	Response to elicitor or roles	Refs
	ubiquitin-protein ligase PUB4. After elicitation, PUB4 promotes activated BIK1 accumulation. However, *Ralstonia solanacearum* effector, RipAC, suppresses PUB4 accumulation and phosphorylation	
RING-H2 FINGER A3A (RHA3A)	BIK1 is monoubiquitinated by RHA3A. The monoubiquitinated BIK1 dissociates from FLS2 and BAK1, and is endocytosed	[106]
RING DOMAIN LIGASE 1/2 (RGLG1/2)	BIK1 homeostasis is controlled by balance between ubiquitin ligases RGLG1/2 and PUB25. RGLG1/2 prefers to interact with hypophosphorylated BIK1 and ubiquitinate it. When flg22 is perceived, RGLG1/2 suppresses hyperphosphorylated BIK1 degradation through PUB25. PUB25 also mediates RGLG2 degradation	[99]
PROTEIN PHOSPHATASE 2C38 (PP2C38)	Suppresses BIK1 phosphorylation and its phosphorylation of RBOHD. BIK1–PP2C38 interaction is dissociated after flg22 or elf18 treatment	[107]
BRI1 SUPPRESSOR 1 (BSU1)	BIK1 interacts and phosphorylates BSU1 at S251 residue for MAPK activation in FLS2 immune signaling but not BR signaling	[108]
RESPIRATORY BURST OXIDASE HOMOLOG PROTEIN D (RBOHD)	BIK1 regulates RBOHD activation, ROS production, and BIK1-dependent stomatal closure through phosphorylation of RBOHD at S39, S343, and S347 residues	[160]
Necrosis-inducing secreted protein 1 (NIS1)	Fungal effector NIS1 blocks BIK1-RBOHD interaction and inhibits PAMP-triggered ROS production	[70]
WRKY33/WRKY50/ WRKY57	BIK1 continuously phosphorylates WRKYs to suppress SA and JA-mediated immunity. Suppression is released when BIK1 is phosphorylated at S89 and T90 and loses phosphorylation of WRKYs after *Pseudomonas syringae* infection	[25]

[a]Most effector proteins that interact with, and modify, BIK1 were not included. More BIK1 interactors are likely to emerge, given that BIK1 and other RLCK proteins appear to have a wide range of interactors.

knowledge of the functions of RLCKs, leading to significant paradigms [10,12], and have opened avenues to study RLCKs in model and crop plants [35,36]. As receptor-associated proteins, many RLCKs are required to transmit pathogen perception and activate immune responses through PTI.

RLCKs are key players in PAMP-triggered immunity to diverse pathogens

Quantitative resistance to pathogens is a genetically complex mechanism that provides partial resistance to biotrophic and necrotrophic pathogens. Extensive studies of RLCKs have helped dissect molecular mechanisms of PTI, which is a recognition-dependent quantitative resistance regardless of the nature of the pathogen. This contrasts with simply inherited qualitative resistance, which provides complete or near-complete but race-specific resistance to biotrophic, hemibiotrophic, and some necrotrophic pathogens [37]. Response signaling of PAMPs by RLCKs downstream of many PRRs is key to quantitative resistance to bacterial and fungal pathogens, and has been extensively studied (Tables 1 and 2). One of these, the G-type lectin receptor kinase LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION (LORE), is the receptor for medium-chain mc-3-OH-FAs. LORE is phosphorylated in response to mc-3-OH-FAs, in turn phosphorylating BIK1-related proteins PBS1-like kinase (PBL)34, PBL35, and PBL36 (all RLCK VII-5 members) mediating immunity to *Pst* [23]. PBL27 (VII-1) complexes with chitin receptor LYSIN MOTIF RECEPTOR KINASE 5 (LYK5) or CERK1 [38], which is

required for responses to chitin [39]. In arabidopsis, tomato, and rice, LysM RLKs form complexes with RLCKs and induce chitin-mediated resistance, showing the conserved nature of this mechanism [22,38,40–42].

PTI signaling and activation of immune responses rely on the regulation of ROS production, Ca^{2+} signaling, and MAPK phosphorylation by RLCKs. Phosphorylation of MAPKs, calcium-dependent protein kinases (CDPKs), and receptor burst oxidase homolog protein D (RBOHD) by RLCKs mediate the activation of immune responses that correlate with increased PTI to different pathogens. Lately, a limited number of selected immune responses have been used as markers of immune activation regardless of the pathways involved. The actual contributions of these vary between different pathosystems, and the impact of immune response markers, such as ROS, on the resistance phenotype is debatable, especially when different pathogen lifestyles are considered. Among the initial responses following receptor activation by elicitors is the rapid and transient increase in cytosolic calcium levels and the generation of ROS. In turn, this leads to initiation of the protein kinase cascades CDPK and MAPK, which subsequently channel the signal to the nucleus, resulting in expression of a battery of defense-related genes [43]. The roles of CDPKs in the regulation of plant immunity have been discussed in recent reviews [44,45]. ROS burst has been a classical marker for effector-NLR interactions, with a clear distinction in the levels and kinetics of ROS production between compatible and incompatible interactions [46]. It is now a widely used marker for PTI, an immune response in a compatible interaction [21,47]. Besides regulation of calcium in flux, RLCKs phosphorylate RBOHD to regulate ROS production [47,48]. Interestingly, RLCK-RBOHD-mediated ROS production through phosphorylation is conserved in land plants, including liverworts (*Marchantia polymorpha),* which is a non-flowering plant [49]. The exception is that PBL13 negatively regulates ROS production through RBOHD phosphorylation and ubiquitination [47].

Finally, ROS burst and Ca^{2+}, both signaling molecules in plant immunity, are functionally interlinked. During PTI, RLCKs mediate the activation of Ca^{2+} channels, which leads to increased cytoplasmic Ca^{2+} signals [50]. Ca^{2+} ions then not only activate RBOHD through binding to its N-terminal EF-hands, but also induce the activity of $Ca2^{+}$-regulated kinases, which phosphorylate RBOHD [51].

BIK1 phosphorylation of one component of the calcium channel, cyclic nucleotide-gated channel4 (CNGC4), induces Ca^{2+} influx [52]. Similar mechanisms have been demonstrated in rice, where OsRLCK185 was activated when chitin was perceived by CERK1-CEBiP, triggering Ca^{2+} influx through OsCNGC9 (VII-1) phosphorylation [53]. The additional roles of Ca^{2+} signals in plant immunity are detailed elsewhere [54]. MAPK activation is a rapid response in PTI and ETI pathways, and RLCKs are involved in activation of the MAPK cascade. Arabidopsis PBL27 (VII-1) interacts with LYK5 [38] and phosphorylates MAPKKK5 to induce chitin-induced MAPK activation [39]. This function is conserved in rice, where the arabidopsis ortholog of PBL27, OsRLCK185 (VII-1), activates MAPK upon chitin perception [41]. Overall, RLCK phosphorylation of ROS regulators and protein kinase cascades are major factors in plant immunity.

The functions of RLCKs in quantitative resistance in crop plants have been less studied, but are slowly emerging (Table 1). TOMATO PROTEIN KINASE1B (TPK1b) and TPK1B-RELATED KINASE1 (TRK1) function in resistance to fungal pathogens (Table 1 and Figure 1). TPK1b functions through ethylene-dependent fungal resistance [35], which was subsequently demonstrated in BIK1 [26], whereas TRK1 is required for resistance to *B. cinerea* through chitin signaling [22]. TRK1 links chitin perception to plant hormone signaling that modulates resistance to *B. cinerea*. Another tomato RLCK, FLS/FLS3-interacting receptor-like cytoplasmic kinase 1 (Fir1), is essential for flagellin-induced defense response and resistance to *Pst* [55]. Interestingly, Fir1 also interacts with JASMONATE-ZIM DOMAIN3 (JAZ3), a negative regulator of jasmonic acid (JA) signaling, to

activate resistance (Figure 1). A rice RLCK, BROAD-SPECTRUM RESISTANCE 1 (BSR1), confers broad-spectrum resistance to multiple pathogens in different crops, including sugarcane and tomato [56,57]. Furthermore, the simultaneous inactivation of six members of RLCK in rice impaired ROS production and callose deposition in response to flg22 and chitin and resulted in susceptibility to bacterial and fungal pathogens [58]. Thus, understanding the functions of key RLCKs in quantitative resistance to damaging crop pathogens can lead to improved diseases resistance and other traits.

RLCKs in ETI

In most cases, PTI and ETI are superimposed in genotypes despite the seemingly separate pathways that have been historically attributed to them. NLRs are deployed in the presence of other quantitative resistance regulators, which then provide the increased resistance. For example, loss of NLRs abrogates systemic acquired resistance (SAR), a resistance in systemic tissue of plants that occurs in response to prior infection by pathogens [59]. Recent work demonstrated that plant immune pathways controlled by RLKs, RLCKs, and NLRs synergize defenses against *Pst* [7]. NLRs enhance the abundance of kinases and NADPH oxidases, which are components of PTI [7]. Consistently, BIK1 is required for the expression of RBOHD and bacterial resistance during ETI [6]. Similarly, the hypersensitive response is strongly enhanced by the activation of PRRs. In the case of tomato RLP Cf-4, targeted by effector Avr4 from *Cladosporium fulvum*, the intensity and kinetics of the biphasic ROS burst associated with ETI differed in various *rlck* mutant plants, revealing the role of RLCKs in ETI [60]. In addition, NLRs recognize different bacterial effectors through association with different combinations of RLCKs, suggesting the centrality of RLCK in ETI [61]. Furthermore, studies of the NLR HOPZ-ACTIVATED RESISTANCE 1 (ZAR1) suggested an evolutionarily conserved partnership with RLCKs [62]. Thus, an increasing number of studies are demonstrating the intertwined nature of PTI and ETI.

Several RLCKs interact with pathogen-effector proteins. For instance, the *Xanthomonas campestris* effector XopAC uridylates arabidopsis RLCKs, PBL2 (VII-9), RPM1-induced protein kinase (RIPK; VII-6), and BIK1 (VII-8) [63]. The *Pst* effector HopZ1a acetylates the RLCK, HOPZ-ETI-DEFICIENT1 (ZED1; RLCK XII-2) to activate ZAR1 [64]. HOPZ1a also promotes the interaction of ZED1 with multiple PBLs to complex with ZAR1 [64]. ZED1 or ZAR1 are associated with combinations of RLCKs (called ZRKs; RLCK XII-2) to sense different effectors and broaden the spectrum of effector recognition [61,65]. The arabidopsis RLCKs, SUPPRESSOR OF ZED1-D1/2 (SZE1 and SZE2, RLCK XII-2), form a functional complex with ZAR1 and mediate ETI [66]. Similarly, PBL2 uridylation by effector AvrAC forms a complex with ZRK1 (aka RKS1) and ZAR1 [67]. The ZRK3-ZAR1 complex is required for HopF2a recognition [65]. The AvrPphB effector cleaves arabidopsis AVRPPHB SUSCEPTIBILE 1 (PBS1), which subsequently activates the NLR RPS5, leading to ETI [68]. The tomato RLCK, Pto, interacts with *Pst* effectors, AvrPto and AvrPtoB, triggering ETI [69]. BIK1 is targeted by the conserved fungal effector necrosis-inducing secreted protein 1 (NIS1), which promotes fungal virulence on crops, further enhancing the role of BIK1 in broad-spectrum resistance [70]. The rust fungal effector, PsSpg1, binds to the wheat RLCK *Puccinia striiformis*-induced protein kinase 1 (TaPsIPK1) to trigger wheat susceptibility to yellow rust [71]. The oomycete effector RXLR25 from *Phytophthora infestans* interacts with the RLCKs BIK1 (VII-8), PBL8 (VII-6), and PBL17 (VII-6), inhibiting phosphorylation and resulting in PTI suppression [72]. In rice, the effector Xoo1488 from *Xanthomonas oryzae* interacts with OsRLCK185 (VII-1) to block chitin-triggered immunity [40]. Overall, RLCKs are subject to manipulation by diverse pathogen effectors that can dampen PTI but also directly or indirectly promote ETI.

Overall, RLCKs are targeted by effectors for suppression of PTI and also serve as a bridge that connects effectors and resistant proteins. Recently, a small molecule (zaractin), which mimics bacterial effectors, was described to enhance the ZRK3/PBL27 interaction and activate ZAR1-

dependent immunity. Similar to zaractin, the insecticide fenthion also activates NLR-mediated plant immunity through the RLCK Fen and the NLR Prf [73,74]. Zaractin and fenthion serve as chemical activators of the NLR-mediated immune response, providing an approach to design chemical immune activators based on the understanding of effector, RLCK, and NLR interactions [75].

RLCKs mediate defense against insect herbivory

Tomato RLKs and RLCKs are implicated in pathogen and insect resistance through systemin and ethylene signaling [76,77]. Systemin is a Solanaceae-specific peptide involved in resistance to insect herbivory and fungal infection. Interestingly, TPK1b interacts with the RLK PEPR1/2 ORTHOLOG RECEPTOR-LIKE KINASE1 (PORK1) and mediates resistance to tobacco hornworm (*Manduca sexta*) and seedling growth responses to systemin [76]. The tomato RLKs, SYSTEMIN RECEPTOR1/2 (SYR1/2) are systemin receptors important for defense against insect herbivory [77]. The relationships between the PORK1-TPK1b module and SYR1 and SYR2 are yet to be defined. Tomato PORK1, SYR1, and SYR2 are all required for systemin responses and, thus, may form complexes for systemin recognition and signaling, which warrants further studies. By contrast, the tomato RLCK ZED1-RELATED KINASE 1 (SlZRK1; RLCK XII-2) acts as a negative regulator in wound-induced JA accumulation and insect resistance [78]. In arabidopsis, BIK1 mediates resistance to green peach aphids via PHYTOALEXIN DEFICIENT4, whereas PBL27 (VII-1) complexes with CALCIUM-DEPENDENT PROTEIN KINASES (CPK)-RELATED PROTEIN KINASES (CRK2) to induce defense responses to the general herbivore *Spodoptera litura* (tobacco cutworm) [79]. Overall, RLCKs regulate responses required for resistance to insect herbivory.

Plant RLCKs are emerging regulators in plant responses to abiotic stress

In nature, plants are challenged by numerous biotic and abiotic stressors, often simultaneously, or sequentially. The spectrum and specificity of RLCKs to abiotic stressors is unexplored because most studies are conducted by specialists who focus on specific biotic or abiotic stress conditions. In general, downstream plant cellular responses overlap regardless of the stressor. RLCKs form a regulatory hub channeling signals to MAPKs, transcription factors (TFs), Ca^{2+}, ROS, and regulators of hormone response and biosynthesis pathways that have roles in plant responses to biotic and abiotic stress. MAPK functions in stress tolerance responses are addressed in several recent reviews [80–83]. The regulation of ROS homeostasis through scavenging and detoxification of excess ROS, and protection from cellular damage have critical functions in biotic and abiotic stress tolerance [84–88]. RLCKs regulate ROS accumulation, which changes in response to many abiotic stress factors [47], and the accumulation of cytosolic Ca^{2+}, which is also important for both biotic and abiotic stress responses [89,90]. Consistently, several RLCKs have been implicated in abiotic stress responses [91–93] (Table 1). Rice RLCK SALT TOLERANCE RECEPTOR-LIKE CYTOPLASMIC KINASE 1 (STRK1) phosphorylates the ROS-scavenging enzyme, catalase C (CatC) to regulate the plant response to salt and H_2O_2 homeostasis [94]. In rice, GROWTH UNDER DROUGHT KINASE (GUDK, VII-6) mediates drought stress signaling through phosphorylation of the ethylene response TF OsAP37 [95]. In some instances, distinct functions of RLCKs in biotic versus abiotic stress and even distinct responses to different pathogens have been observed [96,97]. Understanding mechanisms that fine-tune plant responses to simultaneous or sequential challenges by biotic and abiotic stressors and the role of RLCKs will be important in the context of climate change.

Transcriptional and post-translational regulation of RLCKs and impact on gene expression

The complex regulation of RLCKs helps fine-tune and balance their versatile function in signaling of responses to pathogens, environmental cues, and plant growth. This likely involves diverse

regulatory mechanisms, such as phosphorylation and ubiquitination, which govern protein translocation, association, and dissociation with other proteins and their impact on gene expression. In addition, RLCKs show significant variation at the gene expression level, although mechanisms governing their gene expression have not been a significant focus of research. For example, the expression of ~23% of rice RLCKs changes in response to cold, salt, and dehydration [19], which signifies that RLCKs are recruited to coordinate different processes as direct and indirect regulators of gene expression in networks connecting receptors to downstream responses. Altered pathogen-induced expression and subsequent validation through reverse genetic approaches of tomato and arabidopsis RLCKs have been used to identify functionally important RLCKs [10,22,35], suggesting that transcriptional changes of RLCK genes are key functional indicators.

Post-translational modifications (PTMs) are fundamental cellular processes in cell signaling regulating protein localization, stability, and protein–protein interactions. RLCKs are regulated through various PTMs, including phosphorylation and ubiquitination, which modulate their signaling functions. RLCKs impact the expression of genes through localization to the nucleus and interaction with TFs, connecting pathogen sensing to immune gene expression. The phosphorylation of BIK1 by the PRR EFR triggers localization to the nucleus and interaction with TFs WRKY33, WRKY50, and WRKY57 and modulates JA and salicylic acid (SA) levels (Table 2) [25]. Phosphorylation also triggers other regulatory processes, such as ubiquitination [71]. CPK28 phosphorylates and activates the ubiquitin ligases PUB25 and PUB26, which target proteosome degradation of BIK1 (Table 2) [98]. Thus, BIK1 turnover is tightly controlled through ubiquitination by ubiquitin ligases [31,99,100]. In addition, RLCKs are acetylated and uridylated by bacterial effectors, which modulate their functions [101,102].

RLCK signaling involves auto- and *trans*-phosphorylation of serine/threonine (Ser/Thr) and tyrosine (Tyr) residues [11,103]. Receptor activation by ligand recognition results in RLCK phosphorylation, which initiates downstream biochemical and physiological responses that correlate with resistance. In tomato, TRK1 interacts with the tomato ortholog of the chitin receptor SlLYK1 and the TF MYC2 [22], linking chitin perception, JA signaling, and fungal resistance. Chitin- and *Botrytis*-induced expression of tomato *PROTEASE INHIBITOR1* and MYC target genes are dependent on TRK1 [22], and MYC2 phosphorylation by TRK1 [22]. Interestingly, TRK1 shows contrasting chitin-induced gene expression in meristem and leaf tissues; it suppresses chitin-induced gene expression in shoot apices, but promotes expression in leaves [22]. Accordingly, TRK1 complexes with SlLYK1 to regulate plant response to chitin and SlCLV1 for meristem growth homeostasis, respectively [22]. These observations provide avenues to untangle tissue-specific TRK1-containing complexes to understand distinct RLCK functions. Similarly, wheat RLCK TaPsIPK1 translocates from the plasma membrane to the nucleus, phosphorylates TaCBF1 to dampen the expression of resistance-related genes, and promotes stripe rust diseases caused by *P. striiformis* [71]. In sum, the complex regulation of RLCKs from gene expression to protein accumulation and stability by endogenous and pathogen-derived proteins involving different biochemical and molecular processes confers functional specificity and versatility to RLCKs.

Concluding remarks and future perspectives

RLCKs are early components of pathogen response signaling. Perception of environmental and endogenous signals and generation of specific responses are under cellular and environmental control. Plant pathologists have long described the impact of the environment on diseases through the classical disease triangle. However, the role of RLCKs in modulating plant–pathogen interactions and stability of plant resistance under changing temperature, and other climate variables, such as increased CO_2 concentrations, is unknown. A better insight into regulators,

Outstanding questions

Many RLCKs interact with different RLKs and RBOHD, which raises questions about how RLCKs assume specific or broad molecular and biological functions.

What mechanisms underlie RLCK functions that promote resistance or susceptibility?

What is the mechanism for some RLCKs showing broad molecular interactions and pleiotropic effects whereas closely related RLCKs show no major immunity and/or growth phenotypes?

What are the cellular responses mediated by characterized and more recently discovered RLCKs? What are the immune response genes, immune response markers, and metabolites that best associate with quantitative resistance function of RLCKs?

Some RLCKs regulate distinct gene expression in different plant tissues. What are the upstream and downstream partners of RLCKs, and tissue-specific RLCK-containing complexes?

RLKs and RLCKs share similar kinase domains. Do the kinase domains of RLKs interact/phosphorylate RBOHD? Why are RLCKs needed as intermediaries?

What are the nuclear targets of RLCKs beyond the few TFs identified? This knowledge will aid dissection of the mechanisms of RLCK regulation of gene expression.

What are the mechanisms of specificity and PTMs of RLCKs that regulate their diverse functions? For example, are there patterns of RLCK phosphorylation profiles that regulate specific functions? Comprehensive structure–function studies on selected RLCKs will be important.

What drives the evolution of RLCKs? Are there evolutionarily conserved core RLCKs with significant genetic impacts on plant phenotypes?

How are RLCKs regulated in infected and non-infected cells and what is their role in systemic resistance?

and molecular and biochemical mechanisms of plant responses to pathogens and abiotic stress, as well as crosstalk with plant growth traits, will be critical to develop plants that thrive under our changing climate. Significant progress has been made in understanding the functions of RLCKs in model plant–bacterial interactions, but progress has been limited in crop plants and economically important foliar and soil-borne diseases caused by fungi, nematodes, and viruses.

RLCKs are highly related proteins and functional information is difficult to decipher from sequence analyses. Thus, analyses of signaling mechanisms, specificity of biological functions, protein–protein interactions, and structure–function studies are important. Some RLCKs show broad molecular interactions and pleiotropic effects on diverse traits. Why there is such an outsized genetic contribution by some RLCKs, such as BIK1, whereas loss of function alleles in closely related RLCKs show no apparent phenotypes is unknown. RLCKs interact with many RLKs and RBOHD, but how specific outputs are generated is often unclear.

Complex, interacting mechanisms regulate RLCKs, which, in turn, have biological impacts. Deeper insights into model experimental systems should be accompanied by validation and studies on evolutionary conservation in crop plants as well as isolation of new RLCKs in understudied crop plants, which may help discover novel RLCK and their alleles with new functions. Isolation of nuclear RLCK targets and understanding their impact on gene expression, and how signals reach the nucleus to activate responses require additional research. Understanding the role of relocalization of RLCKs in generating specific responses, the upstream and downstream partners of RLCKs, and the characterization of tissue-specific complexes will also be important. In addition, dissecting the functions of RLCKs in soil-borne bacterial, nematode, and fungal diseases, and isolation of RLCKs that serve as susceptibility factors that can be edited and used in crop protection require a new focus. There is also a need to leverage some of the regulatory processes established in model systems for crop improvement. Gene editing is predicted to expedite functional studies on crop RLCKs and also to generate edited genotypes in otherwise desirable and well-adapted crop cultivars. Key biological questions that need future studies are summarized in Outstanding questions.

What regulates RLCK subcellular movement?

Is there crosstalk between RLCKs?

Considering data showing the impact of RLCKs on the accumulation of ROS, Ca^{2+}, and MAPK signaling, which are implicated in both biotic and abiotic stress, what is the extent of RLCK involvement in abiotic stress tolerance in plants?

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Declaration of interests

The authors declare no competing interests.

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