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Big Roles of Small Kinases: The Complex Functions of Receptor-Like Cytoplasmic Kinases in Plant Immunity and Development

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Abstract

Plants have evolved a large number of receptor-like cytoplasmic kinases (RLCKs) that often functionally and physically associate with receptor-like kinases (RLKs) to modulate plant growth, development and immune responses. Without any apparent extracellular domain, RLCKs relay intracellular signaling often via RLK complex-mediated transphosphorylation events. Recent advances have suggested essential roles of diverse RLCKs in concert with RLKs in regulating various cellular and physiological responses. We summarize here the complex roles of RLCKs in mediating plant immune responses and growth regulation, and discuss specific and overlapping functions of RLCKs in transducing diverse signaling pathways.

Keywords

Phosphorylation; plant development; plant immunity; receptor-like cytoplasmic kinases

Introduction

Being sessile, plants have evolved complex defense mechanisms to cope with potential microbial invasions while maintaining and maximizing their growth and development. In addition to the physical and mechanical barriers of plant cells, the innate immune system is an essential part of a formidable defense plants use to confront microbial threats. In contrast to animals, plants lack a sophisticated adaptive immune system. However, plants have built an effective “danger” detecting surveillance system by expanding the receptor-like kinase (RLK) family to a staggering number of genes. These proteins can function as radar antennae to sense the presence of microbial components termed as microbe or pathogen-associated molecular patterns (MAMPs or PAMPs) (Boller and Felix 2009). Precise recognition of MAMPs by RLKs triggers conformational changes, which often leads to

rapid receptor homo- or hetero-dimerization with other RLKs. Typical plant RLKs consist of a signal sequence, an extracellular motif, a single transmembrane region and a cytoplasmic kinase domain, which is structurally similar with mammalian receptor tyrosine kinases (RTKs) (Shiu and Bleecker 2001; Antolin-Llovera et al. 2012). In contrast to the limited number of RTKs (58 known RTKs in humans) or Toll-like receptors (TLR; 10 known TLRs in humans) that perceive MAMPs in mammals, plants have evolved a large repertoire of RLKs that form the largest family of plant membrane receptors with 610 members in *Arabidopsis* and 1,100 members in rice (Shiu et al. 2004). In addition to their roles in plant immunity, RLKs regulate a wide range of biological processes including plant growth and development via perceiving diverse signals through the extracellular domain. CLAVATA1 (CLV1) binds to a small protein peptide CLV3 produced by the plant itself and functions as an important developmental regulator in balancing cell proliferation and differentiation in the meristem (Ogawa et al. 2008). BRI1 is the receptor of BRs, a class of plant hormone with essential roles in growth and development (Belkhadir et al. 2006). ERECTA family LRR-RLKs regulate organ shape, inflorescence architecture, and stomata patterning (Shpak et al. 2005).

Intriguingly, plants have also evolved a significant number of receptor-like cytoplasmic kinases (RLCKs) with 147 members in *Arabidopsis* and 379 members in rice (Shiu and Bleecker 2001; Vij et al. 2008). Compared to RLKs, the majority of RLCKs only contain a Ser/Thr-specific cytoplasmic kinase domain and some have additional domains similar to the RLK extracellular domains, such as leucine rich repeat (LRR), lectin, epidermal growth factor (EGF), domain of unknown function (DUF), U-BOX, and WD40 (Vij et al. 2008). However, similar with RLKs, some RLCKs are potentially anchored to the plasma membrane through N-terminal putative myristoylation and/or palmitoylation motifs (Veronese et al. 2006; Tang et al. 2008) (Figure 1). More importantly, RLCKs often functionally and physically associate with RLKs to relay intracellular signaling via transphosphorylation events. Although the biological functions of RLCKs are less characterized than RLKs, recent advances have suggested essential roles of diverse RLCKs in concert with RLKs in regulating plant growth, development and immunity. Accompanying other reviews in this issue about the functions of RLKs in diverse biological processes, we attempt to summarize the fundamental roles of RLCKs in mediating plant immune responses and regulating plant growth and development with an elaboration on recent progress (Figure 1).

RLCKs in Mediating Plant Immune Responses

The first line of plant inducible defense is triggered by the recognition of MAMPs or PAMPs and is termed PAMP-triggered immunity (PTI). As a part of basal defense to fend off a broad spectrum of microbial invasions, the elicitation of PTI is accompanied by profound transcriptional reprogramming, production of reactive oxygen species (ROS), deposition of callose to reinforce the cell wall and stomatal closure to block pathogen entry (Boller and Felix 2009). To achieve an effective infection, successful pathogens further evolved various virulence mechanisms to dampen off the host immune systems or interfere with host physiological and cellular responses. For instance, many Gram-negative bacteria have obtained the ability to inject a repertoire of virulence effector proteins into host cells

through type III secretion system (TTSS) to block immune responses (Shan et al. 2007; Feng and Zhou 2012). To confine or eliminate infection, certain plants have evolutionarily acquired polymorphic intracellular receptors, also termed as disease resistance proteins (R proteins), to directly or indirectly recognize effectors and elicit a second line of plant inducible defense defined as effector-triggered immunity (ETI) (Dodds and Rathjen 2010; Spoel and Dong 2012). Accumulating evidence has supported various roles of different RLCKs in multiple stages of plant-microbe arms race.

RLCKs in PTI

One of the best-characterized plant PTI signaling is mediated by LRR-RLK Flagellin sensing 2 (FLS2) that recognizes a conserved 22-amino acid peptide (flg22) of bacterial flagellin (Gomez-Gomez and Boller 2000). Upon ligand perception, FLS2 rapidly associates with BRI1-associated receptor kinase 1 (BAK1), thereby initiating downstream signaling (Chinchilla et al. 2007; Heese et al. 2007). BAK1 belongs to a subfamily of LRR-RLK, termed as Somatic embryogenesis receptor kinase (SERK), involved in plant immunity and development (Chinchilla et al. 2009; Du et al. 2012a). Mitogen-activated protein kinase (MAPK) cascades and calcium-dependent protein kinases (CDPKs) act downstream of LRR-RLK receptor complexes in transducing intracellular signaling events, thereby leading to transcriptional reprogramming (Asai et al. 2002; Boudsocq et al. 2010). To attenuate the prolonged activation of PTI signaling, two closely related E3 ubiquitin ligases PUB12 and PUB13 are recruited to the FLS2 receptor upon flg22 perception. Activation of PUB12 and PUB13 by BAK1 phosphorylation results in the polyubiquitination of FLS2 and leads to FLS2 degradation for downregulation of flg22 signaling (Lu et al. 2011). FLS2 also undergoes endocytosis upon ligand perception (Robatzek et al. 2006). Apparently, the PUB12/13-mediated FLS2 ubiquitination is uncoupled with FLS2 endocytosis (Lu et al. 2011). It is likely that FLS2 endocytosis is mediated by other E3 ubiquitin ligases or ubiquitination-independent mechanisms.

In mammals, RLCKs IL-1 receptor-associated protein kinases (IRAKs) play essential roles in TLR-mediated innate immunity. MAMP perceptions via all TLRs (except TLR3) lead to the recruitment of adaptor protein Myeloid differentiation factor 88 (MyD88), and IRAK1 and IRAK 4. Once IRAK1 associates with MyD88, it is phosphorylated by the activated IRAK4, and subsequently released from MyD88 to propagate the signal by association with an E3 ubiquitin ligase (Takeuchi and Akira 2010). Significantly, a plant RLCK, Botrytis-induced kinase 1 (BIK1), is rapidly phosphorylated upon flagellin perception in an FLS2- and BAK1-dependent manner (Lu et al. 2010a; Zhang et al. 2010) (Figure 2). The *bik1* mutants are compromised in diverse flagellin-mediated responses and immunity to the nonpathogenic bacterial infection. In addition to flagellin signaling, BIK1 is also involved in bacterial elongation factor Tu (EF-Tu)-mediated immune responses (Zhang et al. 2010). Accordingly, BIK1 interacts with both FLS2 and EF-Tu receptor EFR, another LRR-RLK, suggesting that BIK1 resembles mammalian IRAKs as a convergent component involved in signaling triggered by multiple MAMPs. Both FLS2 and EFR are non-RD kinases with little kinase activity, whereas BAK1 is a RD kinase with strong *in vitro* kinase activity (Schwessinger et al. 2011). BAK1 phosphorylates BIK1 *in vitro* and *in vivo*. Interestingly, BIK1 also directly phosphorylates BAK1 and FLS2 (Lu et al. 2010a). Several other

subfamily VII RLCKs, including PBS1 and PBL1 (PBS1-like 1), which have high homology with BIK1, are also phosphorylated upon flg22 treatment and play redundant roles with BIK1 in PTI responses (Lu et al. 2010b; Zhang et al. 2010). However, it remains unclear as to how BIK1 and its close family members positively regulate PTI signaling. The current model suggests that BIK1 functions either upstream or independently of MAPK cascades in flagellin signaling. Identification of BIK1 phosphorylation targets will fill in the missing link of BIK1 activation and downstream immune responses.

In addition to bacterial resistance, BIK1 is also an important player for plant resistance to necrotrophic fungi and insects. BIK1 was originally identified as a *Botrytis*-induced gene by microarray analysis of whole genome transcriptome changes upon necrotrophic fungal *B. cinerea* infection. Compared to wild-type plants, the *bik1* mutant displayed enhanced susceptibility to *B. cinerea* and *Alternaria brassicicola* infection with attenuated induction of jasmonate- and ethylene-regulated defense gene *PDF1.2*. However, the *bik1* mutant displayed enhanced resistance to the virulent bacterial pathogen *Pseudomonas syringae* pv. tomato (*Pst*) DC3000 (Veronese et al. 2006). The seemingly contradictory disease responses with the role of *bik1* in PTI signaling are largely due to the enhanced defense hormone salicylic acid (SA) level in the *bik1* mutant, as the *bik1*NahG plants, in which the elevated SA is removed, was more susceptible to *Pst* DC3000 infection (Veronese et al. 2006). *bik1* also functions in defense against necrotrophic ascomycete fungus *Sclerotinia*. The *Sclerotinia* oxalic acid (OA)-deficient mutant A2, which is nonpathogenic in WT plants, regains the pathogenicity in the *bik1* mutant, suggesting the interplay between *bik1* and the key pathogenicity factor OA (Williams et al. 2011). In addition, tomato *bik1* ortholog Tomato protein kinase 1b (TBK1b) plays important roles in mediating plant defense to hornworms (Abuqamar et al. 2008). It is not clear whether *bik1* functions in plant fungal or insect resistance also through association with pattern recognition receptors (PRRs) that perceive MAMPs from fungi or insects. Belonging to the same RLCK VII subfamily as *bik1*, *Capsicum annuum* receptor-like cytoplasmic protein kinase 1 (CaPIK1) from pepper and Broad-spectrum resistance 1 (BSR1) from rice have been reported to play roles in disease resistance to various pathogens (Dubouzet et al. 2011; Kim and Hwang 2011). In addition to subfamily VII, RBK1, a subfamily VI RLCK, was induced by *Phytophthora infestans* and *B. cinerea* infection. RBK1 and RBK2 could be potentially involved in plant immunity through association with cysteine rich RLK and plant-specific small GTPases ROPs (Molendijk et al. 2008). It will be interesting to determine whether RLCKs functionally interact with ROPs in mediating plant disease resistance.

RLCKs in ETI

In the presence of pathogen effectors, plant R proteins elicit a myriad of immune responses, including defense-related gene activation, programmed cell death (PCD) and ROS production. As most effector-R protein pairs could not be detected for a direct interaction, a guard hypothesis has been articulated in which R proteins function as a guard to monitor cellular and physiological changes of guardee triggered by corresponding effectors, thereby eliciting ETI responses (Dangl and Jones 2001). Guardees are often required for effector-mediated virulence functions and play roles in plant PTI responses. Recent studies have

shown that some RLCKs could function as guardees or interact with guardees in ETI signaling.

PBS1, a subfamily VII RLCK, is required for RPS5-mediated ETI responses to bacterial effector AvrPphB. As one of the typical intracellular R proteins, RPS5 carries a nucleotide-binding domain and a leucine-rich repeat (NB-LRR), a structural feature shared by mammalian NOD-like receptors (NLRs) that perceive intracellular MAMPs and danger signals to initiate inflammation and immunity (DeYoung and Innes 2006; Maekawa et al. 2011). RPS5 forms a complex with PBS1 via the coiled-coil (CC) domain at its N-terminus (Ade et al. 2007). Instead of direct interaction with RPS5, bacterial effector AvrPphB interacts with and proteolytically cleaves PBS1 and multiple members of PBLs including *bik1* (Shao et al. 2003; Zhang et al. 2010) (Figure 2). Therefore, RPS5 functions as a guard to monitor the changes of PBS1 triggered by AvrPphB and elicits ETI responses. In the absence of RPS5, the AvrPphB-mediated protein cleavage could potentially suppress PBL/*bik1*-mediated PTI responses including ROS production, callose deposition and immune gene activation, thereby promoting pathogen virulence (Shao et al. 2003; Zhang et al. 2010). As pathogens often deliver minute amounts of effectors into host cells, it will be intriguing to see if AvrPphB could cleave multiple members of PBL proteins *in vivo*.

Tomato Pto is among the very first characterized R proteins in plants and confers race-specific disease resistance to *P. syringae* carrying effector AvrPto or AvrPtoB (Martin et al. 2003). Unlike most of the R proteins carrying NB-LRR structure, Pto encodes a cytoplasmic kinase and directly interacts with AvrPto and AvrPtoB. This interaction is crucial for the activation of race-specific ETI responses (Martin et al. 2003). Thus, Pto functions as a guardee in AvrPto- and AvrPtoB-mediated resistance although the evidence of Pto as a AvrPto or AvrPtoB virulence target is lacking. Interestingly, Fen, a close homolog of Pto interacts with the N-terminal region of AvrPtoB (Rosebrock et al. 2007). An NB-LRR protein Prf forms a complex with both Pto and Fen, and is required for AvrPto- and AvrPtoB-mediated resistance (Balmuth and Rathjen 2007; Rosebrock et al. 2007). Functional and structural studies postulate a model in which Prf is negatively regulated by Pto through AvrPto/AvrPtoB-interacting loops, and binding of AvrPto or AvrPtoB to Pto releases Pto inhibition and activates Prf-mediated defense signaling (Xing et al. 2007; Dong et al. 2009; Du et al. 2012b).

RPM1-induced protein kinase (RIPK), a subfamily VII RLCK, has been shown to be involved in NB-LRR protein RPM1-mediated ETI responses triggered by *P. syringae* effector AvrB or AvrRpm1. RIN4, a plasma membrane localized protein, directly interacts with both plant RPM1 protein and bacterial effectors AvrB and AvrRpm1. Recognition of AvrRpm1 and AvrB leads to RIN4 phosphorylation, which could be monitored by RPM1 protein to elicit ETI signaling. RIN4 T166 is phosphorylated *in vivo* in the presence of AvrB or AvrRpm1 and T166 phosphomimic mutations cause effector-independent RPM1-dependent ETI signaling activation (Chung et al. 2011). Since AvrB possesses certain limited structural homology to protein kinases (Desveaux et al. 2007), it is hypothesized that AvrB may directly phosphorylate RIN4. However, the recombinant AvrB protein does not exhibit any kinase activity (Liu et al. 2011). Alternatively, RIN4 could be phosphorylated by plant kinases. A proteomic approach identified RIPK as an RIN4 interacting kinase. RIPK

phosphorylates RIN4 *in vitro* and *in vivo* at several residues including T166 (Liu et al. 2011). The *ripk* mutant displayed reduced RIN4 phosphorylation and RPM1-mediated defense responses to AvrB. Interestingly, RIPK also interacts with and phosphorylates AvrB *in vitro* (Liu et al. 2011). Although the exact mechanism of how RIPK is activated remains elusive, it is likely that interaction with AvrB stimulates RIPK phosphorylation on RIN4. It is also possible that AvrB possesses *in vivo* kinase activity and directly phosphorylates RIN4 or RIPK to initiate RPM1-mediated defense. Recent studies revealed that a group of specific CDPKs could sense sustained increase of cytoplasmic Ca^{2+} concentration in RPM1-mediated defense. Distinct and overlapping CDPKs regulate the bifurcate defense responses via phosphorylation of different substrates and subcellular dynamics (Gao et al. 2013). Apparently, CDPKs are phosphorylated in the presence of AvrRpm1 or AvrB. It will be interesting to test whether certain RLCKs could phosphorylate CDPKs *in vitro* and *in vivo*.

RLCKs in Orchestrating Plant Growth and Development

Plant growth and development are controlled by the integration of many environmental factors, endogenous signaling substances and intrinsic genetic programs. The signaling substances are also known as plant hormones including cytokinin (CK), auxin, gibberellins (GAs), abscisic acid (ABA), ethylene (ET), and brassinosteroids (BRs) (Vanstraelen and Benkova 2012). SA and jasmonic acid (JA) are considered as plant defense hormones as their production is often induced during pathogen infections. Plant hormones usually have pleiotropic effects with dynamic changes in different tissues and developmental stages and intricately intertwine with each other. In addition to the roles in plant growth and development, the homeostasis of individual hormones and their crosstalk affect the outcomes of plant-pathogen interactions (Robert-Seilanianz et al. 2011). A recent genome-wide transcriptional profiling survey of rice *RLCK* genes revealed that about 100 out of 379 *OsRLCKs* are differentially expressed during distinct growth stages, suggesting their involvement in diverse developmental processes in plants (Vij et al. 2008). Indeed, multiple RLCKs have been reported to function in BR, ET and SA-mediated signaling pathways and modulate various processes of plant growth and development.

RLCKs in BR signaling

Brassinosteroids, a group of polyhydroxylated steroidal hormones, are perceived by *Arabidopsis* LRR-RLK Brassinosteroid insensitive 1 (BRI1). BR perception induces BRI1–BAK1 interaction and subsequent transphosphorylation, which leads to the release of inhibition from BRI1 C-terminal domain and BRI 1 kinase inhibitor protein (BKI1) to fully activate BRI1 (Kim and Wang 2010; Ye et al. 2011). The activated BRI1 phosphorylates downstream RLCKs BR signaling kinases (BSKs) and Constitutive differential growth 1 (CDG1) (Muto et al. 2004; Tang et al. 2008; Kim et al. 2011). BSK1 and CDG1 belong to RLCK subfamily VIIc and XII, respectively (Shiu et al. 2004), which function in parallel to positively regulate BR signaling. Both BSKs and CDG1 interact with and are activated by BRI1 trans-phosphorylation. Interestingly, BRI1 phosphorylates a conserved site in the activation loop of CDG1 and BSK1 (S234 in CDG1 and S230 in BSK1) (Tang et al. 2008; Kim et al. 2011), indicating a shared activation mechanism of individual RLCKs by BRI1. The activated CDG1 and BSK1 dissociate from the BRI1 receptor and relay the signaling

downstream by targeting the same component, the phosphatase *bri1* suppressor 1 (BSU1). BSK1 together with BSK2 and BSK3 were originally identified from BR-induced phosphoproteomics assay (Tang et al. 2008). Although BSKs lack apparent kinase activity, phosphorylation of BSK1 by BRI1 promotes BSK1 binding to BSU1. In contrast, CDG1 is able to directly phosphorylate BSU1 in the absence of BRI1, and the phosphorylation is further enhanced upon BRI1 phosphorylation. Interestingly, BSK1 and CDG1 possess distinct tissue expression patterns, suggesting that BSK1 and CDG1 may modulate BR signaling in a tissue-specific manner (Tang et al. 2008; Kim et al. 2011).

Recently, BSK1 was found to physically associate with FLS2 and regulate plant innate immunity. BSK1 was identified from a suppressor screen of powdery mildew resistance caused by a mutation in enhanced disease resistance 2 (EDS2) (Shi et al. 2013). The *bsk1* mutant displayed enhanced susceptibility to different bacterial and fungal pathogens, and reduced ROS production to flg22 treatment. Similar with *bik1*, the BSK1 is released from FLS2 upon flg22 perception (Shi et al. 2013). Despite unclear mechanisms, BSK1 is a shared component in flagellin and BR signaling. BAK1 is another shared component in flagellin and BR signaling via heterodimerization with corresponding receptors FLS2 and BRI1 (Li et al. 2002; Nam and Li 2002; Chinchilla et al. 2007; Heese et al. 2007). Recent studies have shown that BR homeostasis and signaling unidirectionally modulate FLS2-mediated immune responses (Albrecht et al. 2012; Belkhadir et al. 2012). The essential role of BAK1 in both BR and flagellin signaling pathways suggests that it may function as a rate-limiting factor to make a trade-off between growth and immunity. Interestingly, BR antagonizes FLS2 signaling in both BAK1-dependent and -independent manners (Albrecht et al. 2012; Belkhadir et al. 2012). Certain RLCKs downstream of receptors, such as BSK1 and *bik1*, could potentially function as cross-regulators to balance FLS2 and BRI1 pathways. Further in-depth studies on protein association dynamics, activation mechanisms and differential phosphorylation events of BSK1 and *bik1* in FLS2 and BRI1 receptor complexes will provide insight into the mechanisms of shared components that modulate plant immunity and hormone signaling.

RLCKs in ET signaling

Ethylene is perceived by an endoplasmic reticulum (ER) membrane-localized protein complex consisting of Ethylene Responses (ETRs) and Ethylene Response Sensors (ERSs), which function redundantly to negatively regulate ET responses via Constitutive Triple Response 1 (CTR1), a Raf-like protein kinase (Bleecker and Kende 2000). Downstream of CTR1 is ER-tethered Ethylene Insensitive2 (EIN2), which is cleaved and translocates to the nucleus, thereby activating ET responses (Qiao et al. 2012). Intriguingly, the *bik1* mutant exhibits the altered triple response, a hall mark of ET signaling, and is partially insensitive to exogenous application of ET. ET also induces phosphorylation of *bik1* and inhibition of ET perception blocks both ET and flg22-induced *bik1* phosphorylation (Laluk et al. 2011). It will be intriguing to mechanistically understand how the plasma membrane-localized *bik1* interacts with largely ER-tethered ET perception and signaling complex. One possibility is that *bik1* is released from the plasma membrane and translocates into endosomes to relay ET signaling. Recent research indicates that *bik1* regulates ET signaling through interaction with PEPR1, a LRR-RLK perceiving Arabidopsis endogenous peptide Pep1, a damage-

associated molecular pattern (Liu et al. 2013). Interestingly, the Pep1 and Pep2 receptor *pepr1/pepr2* mutants showed reduced sensitivity to ET-induced seedling growth inhibition and defense gene expression. It has been hypothesized that the Peps/PEPRs/*bik1* ligand-receptor complex acts downstream of the canonical ET signaling cascade to regulate ET responses (Liu et al. 2013). It remains unknown how ER-localized ET signaling activates the plasma membrane-resident PEPRs/*bik1* receptor complex although *PROPEP* genes are known to be induced by ET. But it is not known whether EIN3 could bind to the promoter of *PROPEP* genes. Instead, it has been reported that EIN3 directly binds to the promoter region of *BIK1* to regulate its transcription (Laluk et al. 2011).

RLCKs in embryonic patterning and organ abscission

Plants start life as a single cell, called a zygote. The Arabidopsis zygote elongates about three-fold before dividing into a small apical and a large basal cell. The apical cell divides and forms the spherical pre-embryo while the basal cell produces the filamentous suspensor. The subfamily II RLCK, Short suspensor (SSP), has been shown to play important roles in the embryonic patterning process (Bayer et al. 2009). The *ssp* mutant suppresses zygote elongation and suspensor formation. Interestingly, *ssp*-mediated embryonic phenotypes possess a unique parent-of-origin effect. It has been proposed that SSP mRNA produced in sperm cells remains untranslated and is delivered to the zygote, where SSP proteins accumulate transiently upon fertilization. Genetic analyses indicate that a MAPK cascade constituting of YDA (a MAPK kinase)-MPK3/6 (MAPKs) and putative transcription factor Grounded (GRD) act downstream of SSP. Transient accumulation of SSP protein triggers activation of YDA signaling (Lukowitz et al. 2004; Bayer et al. 2009). The study provides an example that RLCKs function genetically upstream of the MAPK cascade in transducing RLK signaling (Figure 2). However, it remains unknown whether SSP directly activates YDA by phosphorylation or via other mechanisms.

Plant organ abscission mediates the natural separation of flowers, fruits, or leaves from plants (Roberts et al. 2002; Liljegren 2012). The signal transduction pathway for organ abscission resembles the framework of PTI signaling, which consists of peptide ligand, receptors, MAPK cascade and transcription factors. Yet the distinct features exist at each step. A secreted peptide Inflorescence deficient in abscission (IDA) activates a pair of redundant LRR-RLKs, HAESA (HAE), and HAESA-like 2 (HSL2), which subsequently activate a MAPK signaling cascade leading to cell separation (Jinn et al. 2000; Cho et al. 2008). Two additional RLKs Evershed (EVR) and SERK1 negatively regulate organ separation likely through promoting receptor internalization. A subfamily VII RLCK, Cast away (CST), acts as a spatial and temporal inhibitor of organ separation (Leslie et al. 2010; Lewis et al. 2010; Burr et al. 2011). CST primarily localizes at the plasma membrane through putative myristoylation and palmitoylation motifs. Intriguingly, CST was also observed at unknown internal punctuate structures. Moreover, CST interacts with EVR and HAE at the restricted sub-domain of the plasma membrane. Yet, it remains unknown whether the spatial localization is required for CST functions. Phenotypic analyses of the kinase-dead mutant *cst-1* indicate that the kinase activity is required for CST1 functions (Burr et al. 2011). It will be interesting to determine whether transphosphorylation events occur in the CST1, EVR and HAE complexes. As CST, SERK1 and EVR promote the

internalization of the receptor HAE through NVE-dependent endocytosis (Burr et al. 2011), it is tantalizing to hypothesize that the phosphorylation events in the receptor complex constitute an essential step for HAE internalization.

RLCKs in self-incompatibility signaling

Plant self-incompatibility (SI) is to prevent inbreeding and to encourage outcrossing by rejecting self-related pollens. A single locus, termed S locus, controls the discrimination of self and nonself between pollen and pistil, and only allows non-self pollen to germinate and fertilize the ovules (Takayama and Isogai 2005). The S-locus encodes two proteins – one expressed in the pistil and the other in the pollen. The protein-protein interactions lead to the arrest of pollen germination and/or pollen tube elongation. In Brassica, SI recognition is controlled by SP11/SCR, the male determinant S-locus protein 11 and SRK, the female determinant S-receptor kinase. The interaction between SRK and its cognate ligand SP11 from the same haplotype results in the activation of SRK, which in turn triggers a specific signaling cascade leading to SI responses (Takayama and Isogai 2005). The subfamily VII RLCK, M locus protein kinase (MPLK), which is anchored to the plasma membrane through N-terminal myristoylation or hydrophobic regions, is essential for SI responses (Murase et al. 2004). Notably, two MPLK transcripts, MPLKf1 and MPLKf2, the products of alternative splicing were detected in Brassica. Both isoforms directly interact with SRK and positively regulate SI responses (Kakita et al. 2007). SRK is able to phosphorylate MPLK *in vitro*. The SRK/MPLK complex also binds and phosphorylates ARC1, an E3 ubiquitin ligase sharing similar structure motifs with PUB12/13 in PTI signaling (Gu et al. 1998). The phosphorylated MPLK and ARC1 are released from the complex and translocate to the proteasome for degradation (Stone et al. 2003), whereas phosphorylated SRK undergoes endocytosis for degradation (Ivanov and Gaude 2009). MPLK appears to be analogous to *bik1* in terms of association with the receptors and phosphorylation dynamics in SI signaling.

Conclusions

As exemplified by the studies discussed above, despite the obviously distinct physiological responses from immunity, hormone-mediated growth, organ abscission, embryonic patterning to self-incompatibility, the framework of signal transduction cascades shares remarkable similarity. The perception of ligands by corresponding receptors often elicits dimerization dynamics and transphosphorylation between receptor complexes and RLCKs, thereby activating intracellular signaling events (Figure 2). Lacking obvious extracellular motifs, RLCKs are mainly involved in the signal relays via direct association and phosphorylation with receptors rather than signal perception. For instance, *bik1* and its homolog PBL1 transduce PTI signaling from the FLS2/BAK1 complex whereas CDG1 and BSKs transduce BR signaling from the BRI1/BAK1 complex (Tang et al. 2008; Lu et al. 2010a; Zhang et al. 2010; Kim et al. 2011). In contrast, association and phosphorylation of CST promote HAE internalization, resulting in the attenuation of signaling in organ abscission (Burr et al. 2011).

Apparently, some RLCKs are involved in multiple signaling pathways with distinct and complex functions. For instance, *bik1* has been shown to be involved in PTI and ET signaling pathways, and BSK1 is involved in PTI and BR signaling pathways (Figure 1). How the shared signaling components maintain the specificity of signaling outputs remains an interesting question in the field. It is possible that the differential and dynamic phosphorylation events of RLCKs by RLKs elicit specific RLCK modification, subcellular localization and/or substrate interaction and activation to regulate distinct signaling outputs. Another major unsolved question is how RLCKs connect with downstream signaling events. Genetic studies positioned SSP upstream of the MAPK cascade in embryonic patterning. However, the biochemical evidence is still lacking for this seemingly logical pathway. It is also possible that RLCKs function independently of the MAPK cascade or directly activate transcription factors or other effector proteins to regulate gene expression and other physiological responses (Figure 2). Future studies on the identification of specific phosphorylation sites and substrates, other posttranslational modifications and ligand-induced subcellular dynamics of RLCKs will provide insights into how this group of small kinases play big roles in diverse cellular and physiological responses.

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Figure 1. Examples of receptor-like cytoplasmic kinases (RLCKs) functions in plant immunity and development

These RLCKs are potentially anchored to the plasma membrane through N-terminal putative myristoylation and/or palmitoylation motifs. Pto, RPM1-induced protein kinase (RIPK) and PBS1 are involved in effector-triggered immunity (ETI); *Capsicum annuum* receptor-like cytoplasmic protein kinase 1 (CaPIK1), tomato BIK1 ortholog Tomato protein kinase 1b (TBK1b) and PBLs are involved in PAMP-triggered immunity (PTI); BIK1 is involved in PTI and ET signaling; brassinosteroid signaling kinases (BSKs) are involved in PTI and BR signaling; Constitutive differential growth 1 (CDG1) is involved in BR signaling; Short suspensor (SSP) is involved in embryonic patterning; Cast away (CST) is involved in abscission and M locus protein kinase (MPLK) is involved in self-incompatibility (SI).

Figure 2. A model of receptor-like cytoplasmic kinase (RLCK) functions in different biological processes through association and transphosphorylation with RLK receptor complexes
 RLCK/RLK complexes could activate mitogen-activated protein kinase (MAPK) cascade (MEKK-MKK-MPK) and/or MAPK-independent signaling to regulate gene expression via transcription factors (TFs) or other physiological responses.