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SPECIAL TOPIC: Plant receptor kinases: one size fits all
• REVIEW •

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Diverse roles of SERK family genes in plant growth, development and defense response

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Plant receptor-like protein kinases (RLKs) are transmembrane proteins with an extracellular domain and an intracellular kinase domain, which enable plant perceiving diverse extracellular stimuli to trigger the intracellular signal transduction. The somatic embryogenesis receptor kinases (SERKs) code the leucine-rich-repeat receptor-like kinase (LRR-RLK), and have been demonstrated to associate with multiple ligand-binding receptors to regulate plant growth, root development, male fertility, stomatal development and movement, and immune responses. Here, we focus on the progress made in recent years in understanding the versatile functions of *Arabidopsis* SERK proteins, and review SERK proteins as co-receptor to perceive different endogenous and environmental cues in different signaling pathway, and discuss how the kinase activity of SERKs is regulated by various modification.

RLK, SERK, signal transduction

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INTRODUCTION

Plants are non-motile organisms and therefore through evolution they have developed multiple strategies to sense surrounding cues in order to adapt to the changing environment. Due to their unique structural properties, receptor-like protein kinases (RLKs) play central roles in perception of extracellular stimuli and initiation of intracellular signaling cascades to make plant cells response differently under various environmental challenges (Morris and Walker, 2003). A typical plant RLK contains an extracellular ligand-binding domain, a single-pass transmembrane domain and an intracellular kinase domain. *Arabidopsis* genome encodes more than 610 RLKs, including 135 receptor-like cytoplasmic kinase (RLCKs), which lack extracellular domains. Based on their N-terminal domain, RLKs were classified into more than 10 subfamilies, among which the leucine-rich repeat RLKs (LRR-RLKs) belong to the largest subfamily containing at least 223 members, featured by 1–32 copies of LRRs in their extracellular domain (Gou et al., 2010). LRR-RLKs play crucial roles in regulating many aspects of plant growth, development and stress responses such as embryo pattern formation, inflorescence architecture, stomata patterning and differentiation, brassinosteroid signaling, innate immunity and so on (Morris and Walker, 2003).

The somatic embryogenesis receptor kinases (SERKs), members of subfamily II LRR-RLKs, have been found to have a broader range of functions in plant growth, development and defense response (Li, 2010). SERK gene was first isolated from *Daucuscarota* cell cultures, in which it was marked single somatic cells competent to form embryos (Schmidt et al., 1997). In *Arabidopsis*, there are five SERK proteins, named SERK1, SERK2, SERK3/BRI1-associated

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kinase 1 (BAK1), SERK4/BAK1-like kinase 1 (BKK1), and SERK5, which redundantly participate in various plant growth and defense processes (Li, 2010). SERK1 and SERK2 are crucial in regulating male fertility (Albrecht et al., 2005; Colcombet et al., 2005), BAK1, SERK1/2/5, BKK1 interact with BRI1 to positively regulate BR response (Gou et al., 2012; Li et al., 2002; Nam and Li, 2002; Wu et al., 2015), and BAK1 associates with multiple pattern recognition receptors (PRRs) that recognize specific pathogen-associated molecular patterns (PAMPs) commonly present in microbes to control plant immunity (Chinchilla et al., 2007; Halter et al., 2014; Heese et al., 2007; Roux et al., 2011; Wang et al., 2011). In this review, we summarize current knowledge of the functional repertoire of SERKs in diverse signaling pathway, and discuss the recent advance in our understanding of the regulatory mechanisms of SERK proteins.

Bioinformatic AnalysEs of SERKs

SERK proteins contain a small extracellular domain consisting of 5 LRRs, a serine-proline rich domain (SPP), a single-pass transmembrane domain, and an intracellular kinase domain (Aan den Toorn et al., 2015). The SPP domain has been considered to be one of the hallmarks of the SERKs, and hypothesized to act as a hinge providing the flexibility for the SERK extracellular domain (Hecht et al., 2001). Phylogenetic analysis of the SERKs revealed that genes encoding SERK have been identified in all known plant genomes including monocots, dicots, and lower plant moss *Physcomitrella patens*. The SERK family evolutionally branched to four major clusters: non-vascular SERK proteins, monocot SERK proteins, and two distinct clusters of dicot SERK proteins (Aan den Toorn et al., 2015). In *Arabidopsis*, SERK proteins are clustered to two distinct groups, one group containing SERK1 and SERK2 and the other containing BAK1, BKK1, and SERK5 (Aan den Toorn et al., 2015). Protein sequence analysis showed both extracellular domain and cytoplasmic domain of two groups of *Arabidopsis* SERKs contain divergent sequences that could provide SERK proteins with different functional specificity. For instance, the extracellular domain of BAK1 cannot replace that of SERK1 and SERK2 in male fertility. Besides, neither the SERK2 extracellular nor the kinase domain can replace BAK1 domains in brassinosteroids (BRs) signaling and immune response (Aan den Toorn et al., 2015).

VERSATILE FUNCTIONS OF SERK PROTEINS IN ARABIDOPSIS

SERKs interact with BRI1 to regulate brassinosteroid signaling pathway

SERK3 also named BRI1-associated receptor kinase 1 (BAK1), was independently identified by a genetic screen for suppressors of a weak BR receptor mutant *bri1-5* (Li et al., 2002), and by a yeast two-hybrid screen for the interacting proteins with BRI1 (Nam and Li, 2002). Recent genetic results demonstrated that BAK1 and its homologs are essential for BR signal transduction via a direct association with BRI1. *serk1 bak1 bkk1* triple mutant exhibits an extreme dwarf phenotype and insensitivity to BR, which is similar to the null *bri1* mutant. While overexpression of all *SERK* genes suppressed the phenotype of *bri1-5* (Gou et al., 2012; Wu et al., 2015). Crystal structure analyses showed that the extracellular LRR domains of SERK and BRI1 extremely twisted and formed an integral BR-binding pocket (Hothorn et al., 2011; Santiago et al., 2013; She et al., 2011). BR acts as a molecular glue to promote the association of SERKs and BRI1, which triggers the conformational change of their cytoplasmic domain, and subsequently activates the BR signaling pathway, ultimately reprograms the transcriptome to regulate various aspects of plant growth and development, including seed germination, photomorphogenesis, cell elongation and division, and vascular differentiation (Clouse and Sasse, 1998; Kim and Wang, 2010; Wang et al., 2012).

Recently two group reported that an immunophilin-like FK506-binding protein twisted dwarf1 (TWD1) physically interacts with BRI1 and BAK1 to modulate BR signal transduction (Chaiwanon et al., 2016; Zhao et al., 2016). The *TWD1* loss-of-function mutants display reduced cell elongation, twisted organ and dark green leaf phenotype, which are similar to BR-signaling or biosynthesis mutants. Root growth and hypocotyl elongation assays indicated that *twd1* mutants are less sensitive to BR, but hypersensitive to the BR biosynthesis inhibitor, brassinazole (BRZ). Bimolecular fluorescence complementation (BiFC) assay indicated TWD1 interacts with BRI1 and BAK1 in plasma membrane, and gel blot overlay assay further confirmed that TWD1 directly binds to the kinase domain of BRI1 and BAK1, indicating TWD1 affects BR signal transduction by changing BRI1 and BAK1 kinase activity. Consistent with this, *TWD1* mutation reduced the BR-induced auto-phosphorylation levels of BRI1 and BAK1, but did not affect BRI1 stability and BRI1 localization to the plasma membrane. TWD1 appears critical for BR-induced phosphorylation of BRI1 and BAK1. However, further studies are required for understanding the mechanism by which TWD1 regulates the BRI1 and BAK1 function.

SERKs regulate *Arabidopsis* root development mainly via BR-independent actions

Promoter-Gus analyses showed that all *SERK* genes are expressed in root, but their expression patterns are quite different. *BAK1* is expressed ubiquitously in root, and more abundantly in root meristem. *SERK1* is highly expressed in root vascular, and *BKK1* is detected only in the elongation zone (Du et al., 2012). Consistent with the expression data,

serk1 bak1 bkk1 triple mutant shows an extremely shortened root, and the meristem size of the triple mutant is dramatically reduced. BR signaling is completely blocked in the *serk1 bak1 bkk1* triple mutant, but the root of the triple mutant is much shorter than those of BR deficiency or signaling null mutants, such as *cpd* and *bri1-101* (Du et al., 2012). Further analyses showed that the expression of numbers of genes that are critical for root growth and development were dramatically reduced in the *serk1 bak1 bkk1* triple mutant, but not in *cpd* and *bri1-701*, indicating SERKs control root growth and development mainly through BR-independent actions (Du et al., 2012).

Phytosulfokine (PSK) is a secreted sulfated pentapeptide with the amino acid backbone YIYTQ, and has been described as a peptide hormone that promotes plant growth and development (Sauter, 2015). Perception and transduction of the PSK peptide requires the membrane-localized receptor protein PSKR1 and PSKR2 in Arabidopsis, but largely relies on PSKR1(Sauter, 2015). The null *pskr1* mutant displays a shortened root phenotype, which is similar to the phenotype of *serk1 bak1 bkk1* triple mutant (Matsubayashi et al., 2006a; Matsubayashi et al., 2006b; Wang et al., 2015a). Interestingly, PSK promotes root growth in wild-type plants, but not in *pskr1* and *serk1 bak1 bkk1* (Wang et al., 2015a), suggesting SERK members may function as co-receptors with PSKR1. Biochemical and structural results demonstrated that PSK promotes PSKR1-SERK heterodimerization, and initiates the signal transduction (Ladwig et al., 2015; Wang et al., 2015a). Whether PSK triggers the transphosphorylation of PSKR1 and SERKs to transduce the peptide signals from cell surface to nucleus remains to further study.

SERK-mediated stomatal patterning and movement

Stomata are epidermal pores to be used for water and gas exchange between plants and atmosphere. Stomata are positioned on the leaf surface which is regulated by the epidermal paterning factors (EPF) peptide signaling pathway in *Arabidopsis*. EPF and EPF-like STOMAGEN differently bind to the cell surface-located receptor complex, including at least ERECTA (ER), ERECTA-like1 and 2 (ERL1, ERL2), and TOO MANY MOUNTHS (TMM), to activate the MAP kinase cascade and several transcription factors to define the stomatal patterning (Pillitteri and Torii, 2012). Recent study from Libo Shan's laboratory demonstrated that SERKs associated with ER and TMM to regulate stomatal development (Meng et al., 2015). Systematical genetic analyses exhibited that the *serk1 serk2 bak1 serk4* quadruple mutant disrupted stomatal patterning and showed an elevated stomatal index, which was close to the phenotype of *er erl1 erl2* triple mutant. Biochemical results showed SERKs interact with ER and ERL1 in an EPF-dependent manner, whereas their associations with TMM were independent of EPF ligand. Further studies showed that the cytoplasmic kinase domains of BAK1 and ER interact and transphosphorylate each other. All these results revealed that SERKs act as co-receptors for ER family RLK in EPF signaling pathway to regulate stomatal patterning (Cheung and Wu, 2015; Meng et al., 2015).

SERKs are not only required for stomatal patterning, but also play important roles for stoamtal movement (Shang et al., 2015). ABA treatment promotes the stomatal closure in wild-type plants, but has no obvious effect in BAK1 loss-of-function mutant *bak1-3* (Shang et al., 2015). ABA-INSENSITIVE 1 (ABI1) and OPEN STOMATA1 (OST1), two key components of ABA signaling pathway, were reported to interact with BAK1 near the plasma membrane. BAK1 binds and phosphorylates OST1 to enhance OST1 activity, but ABI1 associates with BAK1 to block BAK1 binding to OST1. Given the important role of BAK1 in BR signaling and the positive effects of BR on stomatal closure, BAK1 are proposed to regulate stomatal movement downstream of ABA and BR signaling pathways. In contrast to this idea, ABA and BR have opposite effects on the interaction between BAK1 and OST1. ABA stimulates, but BR represses the formation of BAK1-OST1 complex (Shang et al., 2015). Future study of BR effect on the stomatal closure of SERKs and OST1 mutants will provide insights into the dissection the crosstalk of BR and ABA on stomatal movement.

SERK-mediated anther development

In flowering plants, male gametophytes are generated in anther from microsporocyte. *SERK1* and *SERK2* genes are highly expressed in anther primordia up to the second parietal division (Albrecht et al., 2005; Colcombet et al., 2005). Single knockout mutants of *SERK1* and *SERK2* show no obvious phenotype, but double mutant *serk1 serk2* is completely male sterile due to lack of mature pollen grains. Detailed analyses showed that the double mutant only developed three cell layers surrounding the sporogenous cell mass, and lack development of tapetal cell layer, which is essential for anther development, demonstrating SERK1 and SERK2 redundantly control male sporogenesis (Albrecht et al., 2005; Colcombet et al., 2005).

The phenotypes of *serk1 serk2* are similar to that of *ems1/exs* and *tpd* mutants. *TAPETUM DETER-MINENT1* (*TPD*) encodes a secreted small protein, which can directly binds to the extracellular domain of LRR-RLK protein, EXCESS MICROSPOROCYTES1/EXTRA SPOROGENOUS CELLS (EMS1/EXS) (Jia et al., 2008). The phenotypic similarity of these mutants, EMS1/EXS and BRI1 belong to the same LRR-RLK subfamily, and BRI1 association with SERK to transduce BR signal, raised an intriguing hypothesis that TPD promotes the dimerization of EMS1/EXS and SERKs, and subsequently phosphorylation to initiate TPD signal transduction and control anther development. This is indeed an exciting hypothesis that need to be tested in the future.

SERK-mediated immune responses

As sessile organisms, plants are continuously exposed to numerous assaults by different plant pathogens. To counteract the actions of invading pathogens, plants have evolved a sophisticated immune system to fight against pathogenic microbes (Antolin-Llovera et al., 2012). A critical step in immune system is ligand-induced homo- or hetero-oligomerization of LRR-RLKs (Antolin-Llovera et al., 2012). The best characterized RLK in immune system is flagellin sensing 2 (FLS2), which recognizes the conserved 22-amino acid of bacterial flagellin, named flg22 (Gomez-Gomez and Boller, 2000). In the presence of flg22, FLS2 associates with BAK1 in a few seconds, and then transphosphorylates each other to initiate plant immune responses (Chinchilla et al., 2007; Heese et al., 2007; Lu et al., 2010, 2011; Roux et al., 2011). Crystallography study showed that flg22 peptide directly binds to the LRR domains of FLS2 and BAK1 to form a signaling-active complex. BAK1 associates with FLS2 through some conserved residues that are also involved in the interaction between BAK1 and BRI1 (Sun et al., 2013). However, FLS2 and BRI1 use different domains to contact with BAK1. In FLS2, the residues of LRR18-20 were used to interact with BAK1, but in BRI1, the residues interacting with co-receptors are located at the island domain, the last LRR and the juxtamembrane domain (Hothorn et al., 2011; She et al., 2011; Sun et al., 2013).

Botrytis-induced kinase 1 (BIK1), a receptor-like cytoplasmic kinase, plays an additive role in defense by associating with FLS2 in unstimulated plants. Upon flg22 treatment, BIK1 is phosphorylated by BAK1 in an FLS2-dependent manner (Lu et al., 2010; Zhang et al., 2010). BIK1 also phosphorylates BAK1 and FLS2 *in vitro* to enhance the flg22 signaling by further phosphorylating BIK1 and other possible substrates (Lin et al., 2014; Lu et al., 2010). The importance of BIK1 in flg22 signal transduction is further indicated by the findings that BIK1-specific phosphorylation of RbohD and calcium increase that control the flg22-induced ROS production and stomatal defense (Kadota et al., 2014; Li et al., 2014).

BAK1 is functionally required for responses triggered by multiple pathogen associated molecular patterns (PAMPs) in *Arabidopsis*. In addition to FLS2, BAK1 heterodimerizes with several RLKs including elongation factor-Tu receptor (EFR), DAMP peptide receptor 1 (AtPEPR1), BAK1-interacting RLK1-3 (BIR1-3) (Halter et al., 2014; Roux et al., 2011; Tang et al., 2015; Wang et al., 2011). *BAK1* knockout mutant showed dramatically reduced defense responses such as seedling growth inhibition, oxidative burst, and MAPK activation upon treatments with multiple PAMPs, indicating that BAK1 regulates plant immunity by interacting with numerous ligand-binding LRR-RLKs (Chinchilla et al., 2007; Halter et al., 2014; Heese et al., 2007; Roux et al., 2011).

REGULATORY MECHANISM OF SERK PROTEINS

Phosphorylation and dephosphorylation

The activation of plant LRR-RLK is initiated by ligand-induced dimerization and phosphorylation (Antolin-Llovera et al., 2012). As co-receptors, SERKs positively regulate the phosphorylation level of the receptor LRR-RLKs on specific kinase-domain residues that are critical for these receptors function. SERKs also can be phosphorylated by the interacting main receptor LRR-RLKs, quantitatively increasing the kinase activity of SERKs toward specific substrates (Chinchilla et al., 2007; Heese et al., 2007; Li et al., 2002; Meng et al., 2015; Nam and Li, 2002; Wang et al., 2008). BAK1 is phosphorylated on multiple kinase domain residues, and differential phosphorylation patterns of RLK partners result from altered BAK1 phosphorylation status. Mutating specific phosphorylation sites of BAK1 leads the BAK1 transgenic plants to exhibit different BR response (Wang et al., 2008, 2014). Using a new BAK1 mutant allele *bak1-5*, Zipfel's lab recently demonstrated that BAK1 controls plant growth, innate immunity, and cell death by phosphorylation-dependent differential regulation of ligand-binding RD and non-RD LRR-RLKs (Schwessinger et al., 2011).

BAK1, a dual-specificity kinase, not only has Ser/Thr kinase activity, but also exhibits Tyr kinase specificity (Oh et al., 2010). Mutagenic analyses identified Tyr-463 as an essential residue for kinase activity, as the BAK1Y463F was almost completely inactive. In addition to Tyr-463, BAK1 is also auto-phosphorylated on Tyr-610 (Oh et al., 2010). Plants expressing *BAK1Y610F* showed dwarf phenotype and dramatically attenuated response to exogenous BR. The mutagenesis of BAK1 on Tyr-610 leads the transphosphorylation activity of BRI1 to be severely impaired, indicating the phosphorylation of BAK1 at Tyr-610 is essential for enhanced BR signaling (Oh et al., 2010, 2011). However, flg22-mediated growth inhibition exhibits similar pattern in plants expressing wild-type *BAK1* and *BAK1Y610F*, suggesting the phosphrylation of Tyr-610 is not required for, at least, flg22-mediated defense response (Oh et al., 2010, 2011).

Phosphorylation and dephosphorylation can function as an on-off switch for a protein's activity. Biochemical analysis showed that cantharidin, a well-established specific PP2A inhibitor, induced the PAMP-triggered immunity and restricted the pathogen growth (Segonzac et al., 2014). Cantharidin-triggered ROS production was strongly reduced in the null *bak1-4* and *bik1 pbl1* mutants, while it was unaffected in *fls2* and completely absent in *rbohD*, suggesting PP2A acts upstream or at the level of BAK1 and/or BIK1. Coimmunoprecipitation experiments showed that the PP2A subunits A1, B'η, B'ζ, and C4 are parts of a constitutive BAK1 complex. The kinase activity of BAK1 is also regu-

lated by PP2A. BAK1 kinase activity was increased by ~40% in the *pp2a-c4* mutant line and conversely reduced by ~60% in the *PP2A-C4* overexpressing line (Segonzac et al., 2014). In the presence of ligands, BAK1 is induced to dimerize with LRR-RLK BRI1 and FLS2, and active the kinase to auto- and trans-phosphorylation to transduce the signal from plasma membrane to cytoplasm. In the absence of ligands, PP2A associates with BAK1, and dephosphorylates BAK1 to fine-tune receptor complex activation. Recent study report that The dephosphorylation of BRI1 by PP2A to regulate brassinosteroid signaling provides a new evidence for this model (Wang et al., 2015b). But how distinct PP2A holoenzymes regulate multiple cellular processes needs to be analyzed in the future.

BAK1 targeting by effectors secreted by pathogens

Successful pathogens have evolved strategies to interfere the general defense components to weaken the PAMP-mediated plant immunity (Macho and Zipfel, 2015). Many Gram-negative bacteria deliver a battery of effectors into plant cells through the type III secretion system to target important host components and sabotage plant immunity (Xin and He, 2013). AvrPto and AvrPtoB are type III effectors of *Pseudomonas syringae pv. tomato strain DC3000*, which is a pathogen of both tomato and *Arabidopsis* (Xin and He, 2013). AvrPto and AvrPtoB have been demonstrated to interact with BAK1, and then interfere with ligand-dependent association of FLS2 with BAK1 to inhibit PAMP signaling (Cheng et al., 2011; Shan et al., 2008). AvrPto and AvrPtoB also can directly associate with FLS2 and EFR to inhibit their kinase activity (Gohre et al., 2008; Xiang et al., 2008). AvrPtoB contains an E3 ubiquitin ligase domain at its C terminus and can degrade FLS2 (Gohre et al., 2008; Xiang et al., 2008). In addition to AvrPto and AvrPtoB, another type III effector HopF2 was reported to suppress plant immunity by targeting BAK1 (Zhou et al., 2014). HopF2 directly interacts with BAK1 via transmembrane and kinase domain in an FLS2-independent manner. HopF2 virulence function is associated with its suppression of BIK1 phosphorylation, and BIK1 is rapidly phosphorylated upon flg22 perception by BAK1 (Zhou et al., 2014), indicating HopF2 binding might inhibit the kinase acitvity of BAK1. These effectors impede BAK1-dependent host immune responses to diverse other PAMPs, brassinosteroid signaling, and EPF-mediated stomatal patterning (Macho and Zipfel, 2015).

Glutathionylation

Glutathionylation, a redox-dependent modification of proteins, is recognized as an important protective mechanism against irreversible protein oxidation in redox-mediated signaling pathway (Zaffagnini et al., 2012). The glutaredoxin protein GRXC2 interacts with BAK1, and catalyses glutathionylation of BAK1 *in vitro* (Bender et al., 2015). Oxidizing agents such as hydrogen peroxide, diamide, dramatically inhibit the kinase activity of BAK1. The BAK1 cytoplasmic domain contains four cysteine residues at positions 353, 374, 408 and 545. Matrix-assisted laser desorp-

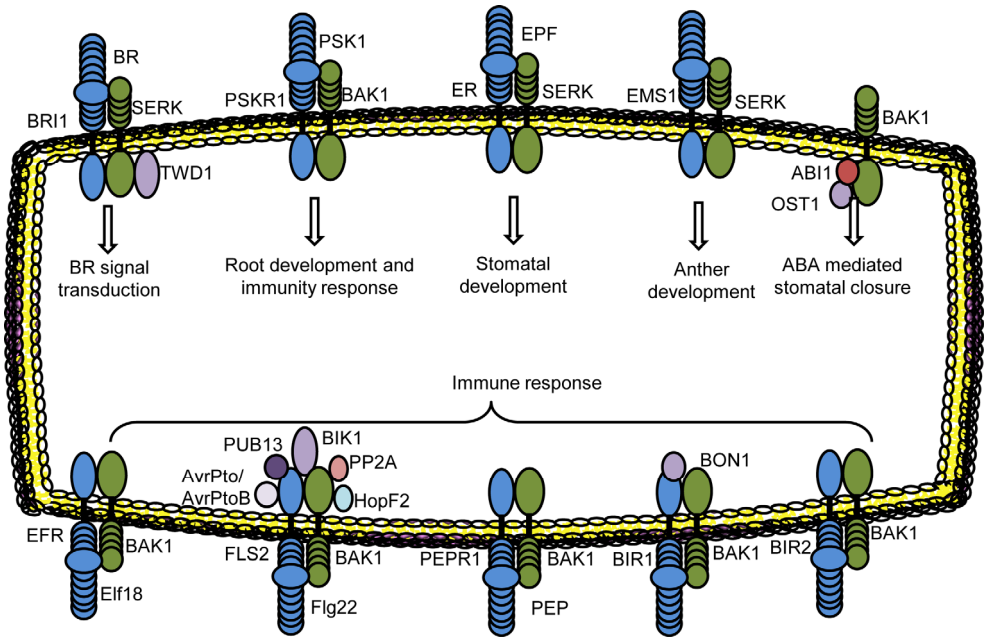

Figure 1 Signaling network mediated by SERKs in *Arabidopsis*. The SERK proteins function as co-receptors by physically interacting with numerous cell-surface receptors including BRI1, PSKR1, ER, EMS1, EFR, FLS2, PEPR1, BIR1 and BIR2 to regulate BR signal transduction, root development, stomatal patterning, anther development, and immune response. BAK1 associates with OST1 and ABI1 to control ABA-mediated stomatal closure. Type III effectors AvrPto, AvrPtoB and HopF2 target BAK1 to inhibit plant immunity. In the presence of flg22, BAK1 phosphorylates BIK1 in an FLS2-dependent manner, and BIK1 in turn phosphorylates BAK1 to enhance the flg22 signaling. In the absence of ligands, PP2A associates with BAK1, and dephosphorylates BAK1 to fine-tune receptor complex activation.

tion/ionization time of flight mass spectrometry (MALDI-TOF MS) identified that Cys353 and Cys408 are the major sites of glutathionylation catalyzing by AtGRXC2, whereas Cys374 might only be glutathionylated at high concentration of GSSG (Bender et al., 2015). Mutating Cys408 to tyrosine in *bak1-5* allele background increases the BAK1 kinase activity. Interestingly, *bak1-5* mutant shows compromised EFR- and FLS2-dependent immune responses, but not BR response or cell death (Schwessinger et al., 2011), indicating that redox might participate in regulation of specificity among BAK1-mediated pathways.

CONCLUSIONS

Owing to the extraordinary progresses made in understanding the association with numerous cell-surface receptors in a ligand-dependent manner, SERKs are emerging as co-receptors to perceive diverse internal and external signals, and regulate plant growth, root development, male fertility, stomatal development and movement, and stress responses (Table 1 and Figure 1). SERKs interact with a diversity of LRR-RLK receptors to involve in different signaling pathway, whereas it is unclear how SERKs successfully determine the specificity. One possibility is that different SERK-RLK complexes have different substrates or different regulation for the same substrate. For example BIK1 is the substrate of both BRI1 and FLS2, and it negatively regulates BR response but positively regulates plant immunity. BRI1 directly phosphorylates BIK1 in a BAK-independent manner, whereas FLS2 signal transduction depends on BAK1 to phosphorylate BIK1. BIK1 plays inverse functions in BR signaling pathway and immunity via different phosphorylation events. Another interesting issue is that SERK proteins recognize the ligands and their main receptors through the conserved residues, but the different main receptors use different residues to contact with SERKs and ligands. The residue basis of main receptors coupling mathematical deduction opens up new avenues to screen the corresponding ligands of different SERK interacting LRR-RLKs. To identify the new partners and characterize the ligand-receptor-SERK complexes in the future will help us to understand better that how endogenous and environmental signals were integrated and branched by SERKs in plant cells.

Table 1 Established SERK-interacting proteins in *Arabidopsis*

Gene locus	Protein name	Validation	Physiological function	Reference
At4g39400	BRI1	Co-IP	BR signaling and plant growth	(Li et al., 2002; Nam and Li, 2002)
At1g55610	BRL1	Co-IP	BR signaling and root growth	(Fabregas et al., 2013)
At3g13380	BRL3	Co-IP	BR signaling and root growth	(Fabregas et al., 2013)
At3g21640	TWD1	Co-IP	BR signaling	(Chaiwanon et al., 2016; Zhao et al., 2016)
At2g02220	PSKR1	Co-IP	PSK signaling and root growth	(Wang et al., 2015a)
At2g26330	ERECTA	Co-IP	EPF signaling and stomatal patterning	(Meng et al., 2015)
At5g62230	ERL1	Co-IP	EPF signaling and stomatal patterning	(Meng et al., 2015)
At4g33950	OST1	BiFC	ABA signaling and stomatal closure	(Shang et al., 2015)
At4g26080	ABI1	BiFC	ABA signaling and stomatal closure	(Shang et al., 2015)
At1g80080	TMM	Co-IP	EPF signaling and stomatal patterning	(Meng et al., 2015)
At5g46330	FLS2	Co-IP	Flg22 signaling and plant defense response	(Chinchilla et al., 2007; Heese et al., 2007)
At5g20480	EFR	Co-IP	ELF18 signaling and plant defense response	(Roux et al., 2011)
At1g73080	PEPR1	Co-IP	PEPP signaling and plant defense response	(Tang et al., 2015)
At5g48380	BIR1	Co-IP	Cell death	(Wang et al., 2011)
At3g28450	BIR2	Co-IP	Plant defense response	(Halter et al., 2014)
At2g39660	BIK1	Co-IP	Plant defense response	(Lu et al., 2010)
At3g46510	PUB13	Co-IP	Plant defense response	(Zhou et al., 2015)
At5g40370	AtGRXC2	Y2H	Glutathionylation	(Bender et al., 2015)
At1g25490	PP2A-A1	Co-IP	Plant defense response	(Segonzac et al., 2014)
At2g26300	GPA1	BiFC	Plant defense response	(Aranda-Sicilia et al., 2015)
At4g34460	AGB1	BiFC	Plant defense response	(Aranda-Sicilia et al., 2015)
At3g63420	AGG1	BiFC	Plant defense response	(Aranda-Sicilia et al., 2015)
At3g22942	AGG2	BiFC	Plant defense response	(Aranda-Sicilia et al., 2015)
At3g23750	BARK1	BiFC	Root development	(Kim et al., 2013)
At5g61900	BON1	Co-IP	cell death	(Wang et al., 2011)
At5g52240	MSBP1	Co-IP	BR signaling and BAK1 endocytosis	(Song et al., 2009)
	AvrPto	Co-IP	Suppression of plant immunity	(Shan et al., 2008)
	AvrPtoB	Co-IP	Suppression of plant immunity	(Shan et al., 2008)
	HopF2	Co-IP	Suppression of plant immunity	(Zhou et al., 2014)

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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Aan den Toorn, M., Albrecht, C., and de Vries, S. (2015). On the origin of SERKs: Bioinformatics analysis of the somatic embryogenesis receptor kinases. Mol Plant 8, 762–782.

Albrecht, C., Russinova, E., Hecht, V., Baaijens, E., and de Vries, S. (2005). The *Arabidopsis thaliana* SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASES1 and 2 control male sporogenesis. Plant Cell 17, 3337–3349.

Antolin-Llovera, M., Ried, M.K., Binder, A., and Parniske, M. (2012). Receptor kinase signaling pathways in plant-microbe interactions. Annu Rev Phytopathol 50, 451–473.

Aranda-Sicilia, M.N., Trusov, Y., Maruta, N., Chakravorty, D., Zhang, Y., and Botella, J.R. (2015). Heterotrimeric G proteins interact with defense-related receptor-like kinases in *Arabidopsis*. J Plant Physiol 188, 44–48.

Bender, K.W., Wang, X., Cheng, G.B., Kim, H.S., Zielinski, R.E., and Huber, S.C. (2015). Glutaredoxin AtGRXC2 catalyses inhibitory glutathionylation of *Arabidopsis* BRI1-associated receptor-like kinase 1 (BAK1) *in vitro*. Biochem J 467, 399–413.

Chaiwanon, J., Garcia, V.J., Cartwright, H., Sun, Y., and Wang, Z.Y. (2016). Immunophilin-like FKBP42/TWISTED DWARF1 interacts with the receptor kinase BRI1 to regulate brassinosteroid signaling in *Arabidopsis*. Mol Plant 9, 593–600.

Cheng, W., Munkvold, K.R., Gao, H., Mathieu, J., Schwizer, S., Wang, S., Yan, Y.B., Wang, J., Martin, G.B., and Chai, J. (2011). Structural analysis of *Pseudomonas syringae* AvrPtoB bound to host BAK1 reveals two similar kinase-interacting domains in a type III Effector. Cell Host Microbe 10, 616–626.

Cheung, A.Y., and Wu, H.-M. (2015). Stomatal patterning: SERKs put the mouths in their right place. Curr Biol 25, R827–R844.

Chinchilla, D., Zipfel, C., Robatzek, S., Kemmerling, B., Nurnberger, T., Jones, J.D., Felix, G., and Boller, T. (2007). A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. Nature 448, 497–500.

Clouse, S.D., and Sasse, J.M. (1998). BRASSINOSTEROIDS: essential regulators of plant growth and development. Annu Rev Plant Physiol Plant Mol Biol 49, 427–451.

Colcombet, J., Boisson-Dernier, A., Ros-Palau, R., Vera, C.E., and Schroeder, J.I. (2005). *Arabidopsis* SOMATIC EMBRYOGENESIS RECEPTOR KINASES1 and 2 are essential for tapetum development and microspore maturation. Plant cell 17, 3350–3361.

Du, J., Yin, H., Zhang, S., Wei, Z., Zhao, B., Zhang, J., Gou, X., Lin, H., and Li, J. (2012). Somatic embryogenesis receptor kinases control root development mainly via brassinosteroid-independent actions in *Arabidopsis thaliana*. J Int Plant Biol 54, 388–399.

Fabregas, N., Li, N., Boeren, S., Nash, T.E., Goshe, M.B., Clouse, S.D., de Vries, S., and Cano-Delgado, A.I. (2013). The brassinosteroid insensitive1-like3 signalosome complex regulates *Arabidopsis* root development. Plant Cell 25, 3377–3388.

Gohre, V., Spallek, T., Haweker, H., Mersmann, S., Mentzel, T., Boller, T., de Torres, M., Mansfield, J.W., and Robatzek, S. (2008). Plant pattern-recognition receptor FLS2 is directed for degradation by the bacterial ubiquitin ligase AvrPtoB. Curr Biol 18, 1824–1832.

Gomez-Gomez, L., and Boller, T. (2000). FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in *Arabidopsis*. Mol Cell 5, 1003–1011.

Gou, X., He, K., Yang, H., Yuan, T., Lin, H., Clouse, S.D., and Li, J. (2010). Genome-wide cloning and sequence analysis of leucine-rich repeat receptor-like protein kinase genes in *Arabidopsis thaliana*. BMC Genomics 11, 19.

Gou, X., Yin, H., He, K., Du, J., Yi, J., Xu, S., Lin, H., Clouse, S.D., and Li, J. (2012). Genetic evidence for an indispensable role of somatic embryogenesis receptor kinases in brassinosteroid signaling. PLoS Genet 8, e1002452.

Halter, T., Imkampe, J., Mazzotta, S., Wierzba, M., Postel, S., Bucherl, C., Kiefer, C., Stahl, M., Chinchilla, D., Wang, X., Numberger,T., Zipfel,C., Clouse, S., Borst,J., Boeren,S., De Vries,S.C., Tax,F., Kemmerling,B. (2014). The leucine-rich repeat receptor kinase BIR2 is a negative regulator of BAK1 in plant immunity. Curr Biol 24, 134–143.

Hecht, V., Vielle-Calzada, J.P., Hartog, M.V., Schmidt, E.D., Boutilier, K., Grossniklaus, U., and de Vries, S.C. (2001). The *Arabidopsis* SOMATIC EMBRYOGENESIS RECEPTOR KINASE 1 gene is expressed in developing ovules and embryos and enhances embryogenic competence in culture. Plant Physiol 127, 803–816.

Heese, A., Hann, D.R., Gimenez-Ibanez, S., Jones, A.M., He, K., Li, J., Schroeder, J.I., Peck, S.C., and Rathjen, J.P. (2007). The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. Proc Natl Acad Sci USA 104, 12217–12222.

Hothorn, M., Belkhadir, Y., Dreux, M., Dabi, T., Noel, J.P., Wilson, I.A., and Chory, J. (2011). Structural basis of steroid hormone perception by the receptor kinase BRI1. Nature 474, 467–471.

Jia, G., Liu, X., Owen, H.A., and Zhao, D. (2008). Signaling of cell fate determination by the TPD1 small protein and EMS1 receptor kinase. Proc Natl Acad Sci USA 105, 2220–2225.

Kadota, Y., Sklenar, J., Derbyshire, P., Stransfeld, L., Asai, S., Ntoukakis, V., Jones, J.D., Shirasu, K., Menke, F., Jones, A., Zipfil,C. (2014). Direct regulation of the NADPH oxidase RBOHD by the PRR-associated kinase BIK1 during plant immunity. Mol Cell 54, 43–55.

Kim, M.H., Kim, Y., Kim, J.W., Lee, H.S., Lee, W.S., Kim, S.K., Wang, Z.Y., and Kim, S.H. (2013). Identification of *Arabidopsis* BAK1-associating receptor-like kinase 1 (BARK1) and characterization of its gene expression and brassinosteroid-regulated root phenotypes. Plant Cell Physiol 54, 1620–1634.

Kim, T.W., and Wang, Z.Y. (2010). Brassinosteroid signal transduction from receptor kinases to transcription factors. Annu Rev Plant Biol 61, 681–704.

Ladwig, F., Dahlke, R.I., Stuhrwohldt, N., Hartmann, J., Harter, K., and Sauter, M. (2015). Phytosulfokine regulates growth in *Arabidopsis* through a response module at the plasma membrane that includes CYCLIC NUCLEOTIDE-GATED CHANNEL17, H^+-ATPase, and BAK1. Plant Cell 27, 1718–1729.

Li, J. (2010). Multi-tasking of somatic embryogenesis receptor-like protein kinases. Curr Opin Plant Biol 13, 509–514.

Li, J., Wen, J., Lease, K.A., Doke, J.T., Tax, F.E., and Walker, J.C. (2002). BAK1, an *Arabidopsis* LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. Cell 110, 213–222.

Li, L., Li, M., Yu, L., Zhou, Z., Liang, X., Liu, Z., Cai, G., Gao, L., Zhang, X., Wang, Y., Chen, S., Zhou, J.M. (2014). The FLS2-associated kinase BIK1 directly phosphorylates the NADPH oxidase RbohD to control plant immunity. Cell Host Microbe 15, 329–338.

Lin, W., Li, B., Lu, D., Chen, S., Zhu, N., He, P., and Shan, L. (2014). Tyrosine phosphorylation of protein kinase complex BAK1/BIK1 mediates *Arabidopsis* innate immunity. Proc Natl Acad Sci USA 111, 3632–3637.

Lu, D., Lin, W., Gao, X., Wu, S., Cheng, C., Avila, J., Heese, A., Devarenne, T.P., He, P., and Shan, L. (2011). Direct ubiquitination of pattern recognition receptor FLS2 attenuates plant innate immunity. Science 332, 1439–1442.

Lu, D., Wu, S., Gao, X., Zhang, Y., Shan, L., and He, P. (2010). A receptor-like cytoplasmic kinase, BIK1, associates with a flagellin receptor complex to initiate plant innate immunity. Proc Natl Acad Sci USA 107, 496–501.

Macho, A.P., and Zipfel, C. (2015). Targeting of plant pattern recognition receptor-triggered immunity by bacterial type-III secretion system effectors. Curr Opin Microbiol 23, 14–22.

Matsubayashi, Y., Ogawa, M., Kihara, H., Niwa, M., and Sakagami, Y.

(2006a). Disruption and overexpression of *Arabidopsis* phytosulfokine receptor gene affects cellular longevity and potential for growth. Plant Physiol 142, 45–53.
Matsubayashi, Y., Shinohara, H., and Ogawa, M. (2006b). Identification and functional characterization of phytosulfokine receptor using a ligand-based approach. Chem Rec 6, 356–364.
Meng, X., Chen, X., Mang, H., Liu, C., Yu, X., Gao, X., Torii, K.U., He, P., and Shan, L. (2015). Differential function of *Arabidopsis* SERK family receptor-like kinases in stomatal patterning. Curr Biol 25, 2361–2372.
Morris, E.R., and Walker, J.C. (2003). Receptor-like protein kinases: the keys to response. Curr Opin Plant Biol 6, 339–342.
Nam, K.H., and Li, J. (2002). BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. Cell 110, 203–212.
Oh, M.H., Wang, X., Wu, X., Zhao, Y., Clouse, S.D., and Huber, S.C. (2010). Autophosphorylation of Tyr-610 in the receptor kinase BAK1 plays a role in brassinosteroid signaling and basal defense gene expression. Proc Natl Acad Sci USA 107, 17827–17832.
Oh, M.H., Wu, X., Clouse, S.D., and Huber, S.C. (2011). Functional importance of BAK1 tyrosine phosphorylation *in vivo*. Plant Signal Behav 6, 400–405.
Pillitteri, L.J., and Torii, K.U. (2012). Mechanisms of stomatal development. Annu Rev Plant Biol 63, 591–614.
Roux, M., Schwessinger, B., Albrecht, C., Chinchilla, D., Jones, A., Holton, N., Malinovsky, F.G., Tor, M., de Vries, S., and Zipfel, C. (2011). The *Arabidopsis* leucine-rich repeat receptor-like kinases BAK1/SERK3 and BKK1/SERK4 are required for innate immunity to hemibiotrophic and biotrophic pathogens. Plant Cell 23, 2440–2455.
Santiago, J., Henzler, C., and Hothorn, M. (2013). Molecular mechanism for plant steroid receptor activation by somatic embryogenesis co-receptor kinases. Science 341, 889–892.
Sauter, M. (2015). Phytosulfokine peptide signalling. J Exp Bot 66, 5161–5169.
Schmidt, E.D., Guzzo, F., Toonen, M.A., and de Vries, S.C. (1997). A leucine-rich repeat containing receptor-like kinase marks somatic plant cells competent to form embryos. Development 124, 2049–2062.
Schwessinger, B., Roux, M., Kadota, Y., Ntoukakis, V., Sklenar, J., Jones, A., and Zipfel, C. (2011). Phosphorylation-dependent differential regulation of plant growth, cell death, and innate immunity by the regulatory receptor-like kinase BAK1. PLoS Genet 7, e1002046.
Segonzac, C., Macho, A.P., Sanmartin, M., Ntoukakis, V., Sanchez-Serrano, J.J., and Zipfel, C. (2014). Negative control of BAK1 by protein phosphatase 2A during plant innate immunity. EMBO J 33, 2069–2079.
Shan, L., He, P., Li, J., Heese, A., Peck, S.C., Nurnberger, T., Martin, G.B., and Sheen, J. (2008). Bacterial effectors target the common signaling partner BAK1 to disrupt multiple MAMP receptor-signaling complexes and impede plant immunity. Cell Host Microbe 4, 17–27.
Shang, Y., Dai, C., Lee, M.M., Kwak, J.M., and Nam, K.H. (2015). BRI1-associated receptor kinase 1 regulates guard cell ABA signaling mediated by open stomata 1 in *Arabidopsis*. Mol Plant 9, 447–460.
She, J., Han, Z., Kim, T.W., Wang, J., Cheng, W., Chang, J., Shi, S., Wang, J., Yang, M., Wang, Z.Y., Chai, J. (2011). Structural insight into brassinosteroid perception by BRI1. Nature 474, 472–476.
Song, L., Shi, Q.M., Yang, X.H., Xu, Z.H., and Xue, H.W. (2009). Membrane steroid-binding protein 1 (MSBP1) negatively regulates brassinosteroid signaling by enhancing the endocytosis of BAK1. Cell Res 19, 864–876.
Sun, Y., Li, L., Macho, A.P., Han, Z., Hu, Z., Zipfel, C., Zhou, J.M., and Chai, J. (2013). Structural basis for flg22-induced activation of the *Arabidopsis* FLS2-BAK1 immune complex. Science 342, 624–628.
Tang, J., Han, Z., Sun, Y., Zhang, H., Gong, X., and Chai, J. (2015). Structural basis for recognition of an endogenous peptide by the plant receptor kinase PEPR1. Cell Res 25, 110–120.
Wang, J., Li, H., Han, Z., Zhang, H., Wang, T., Lin, G., Chang, J., Yang, W., and Chai, J. (2015a). Allosteric receptor activation by the plant peptide hormone phytosulfokine. Nature 525, 265–268.
Wang, R., Liu, M., Yuan, M., Oses-Prieto, J.A., Cai, X., Sun, Y., Burlingame, A.L., Wang, Z.Y., and Tang, W. (2015b). The brassinosteroid-activated BRI1 receptor kinase is switched off by dephosphorylation mediated by cytoplasm-localized PP2A B' subunits. Mol Plant 9, 148–157.
Wang, X., Kota, U., He, K., Blackburn, K., Li, J., Goshe, M.B., Huber, S.C., and Clouse, S.D. (2008). Sequential transphosphorylation of the BRI1/BAK1 receptor kinase complex impacts early events in brassinosteroid signaling. Dev Cell 15, 220–235.
Wang, Y., Li, Z., Liu, D., Xu, J., Wei, X., Yan, L., Yang, C., Lou, Z., and Shui, W. (2014). Assessment of BAK1 activity in different plant receptor-like kinase complexes by quantitative profiling of phosphorylation patterns. J Proteomics 108, 484–493.
Wang, Z., Meng, P., Zhang, X., Ren, D., and Yang, S. (2011). BON1 interacts with the protein kinases BIR1 and BAK1 in modulation of temperature-dependent plant growth and cell death in *Arabidopsis*. Plant J 67, 1081–1093.
Wang, Z.Y., Bai, M.Y., Oh, E., and Zhu, J.Y. (2012). Brassinosteroid signaling network and regulation of photomorphogenesis. Annu Rev Genet 46, 701–724.
Wu, W., Wu, Y., Gao, Y., Li, M., Yin, H., Lv, M., Zhao, J., Li, J., and He, K. (2015). Somatic embryogenesis receptor-like kinase 5 in the ecotype Landsberg erecta of *Arabidopsis* is a functional RD LRR-RLK in regulating brassinosteroid signaling and cell death control. Front Plant Sci 6, 852.
Xiang, T., Zong, N., Zou, Y., Wu, Y., Zhang, J., Xing, W., Li, Y., Tang, X., Zhu, L., Chai, J., *et al.* (2008). *Pseudomonas syringae* effector AvrPto blocks innate immunity by targeting receptor kinases. Curr Biol 18, 74–80.
Xin, X.F., and He, S.Y. (2013). *Pseudomonas syringae pv. tomato* DC3000: a model pathogen for probing disease susceptibility and hormone signaling in plants. Annu Rev Phytopathol 51, 473–498.
Zaffagnini, M., Bedhomme, M., Marchand, C.H., Morisse, S., Trost, P., and Lemaire, S.D. (2012). Redox regulation in photosynthetic organisms: focus on glutathionylation. Antioxid Redox Signal 16, 567–586.
Zhang, J., Li, W., Xiang, T., Liu, Z., Laluk, K., Ding, X., Zou, Y., Gao, M., Zhang, X., Chen, S., Mengiste, T., Zhang, Y., Zhou,J.M. (2010). Receptor-like cytoplasmic kinases integrate signaling from multiple plant immune receptors and are targeted by a *Pseudomonas syringae* effector. Cell Host Microbe 7, 290–301.
Zhao, B., Lv, M., Feng, Z., Campbell, T., Liscum, E., and Li, J. (2016). TWISTED DWARF 1 associates with BRASSINOSTEROID INSENSITIVE 1 to regulate early events of the brassinosteroid signaling pathway. Mol Plant 9, 582–592.
Zhou, J., Lu, D., Xu, G., Finlayson, S.A., He, P., and Shan, L. (2015). The dominant negative ARM domain uncovers multiple functions of PUB13 in *Arabidopsis* immunity, flowering, and senescence. J Exp Bot 66, 3353–3366.
Zhou, J., Wu, S., Chen, X., Liu, C., Sheen, J., Shan, L., and He, P. (2014). The *Pseudomonas syringae* effector HopF2 suppresses *Arabidopsis* immunity by targeting BAK1. Plant J 77, 235–245.