

## Insights into receptor-like kinases-activated downstream events in plants

Xuming Luo<sup>1,2</sup> & Jun Liu<sup>1,2\*</sup><sup>1</sup>State Key Laboratory of Plant Genomics, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China;<sup>2</sup>College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China

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Unlike vertebrates that have the circulating immune system, plants have evolved an efficient innate immune system to defend against invading pathogens. At cellular level, the plant innate immunity primarily relies on plasma membrane-localized receptor-like kinases (RLKs)-mediated signalings. These RLKs are typically comprised of an extracellular domain, a transmembrane domain, and an intracellular kinase domain. Upon pathogen infection, the immune related RLKs utilize their extracellular domains to perceive pathogen-associated molecular patterns and transduce these signals via kinase domains to downstream components, leading to immune activation. There are about 410 RLKs in model plant *Arabidopsis thaliana* (Tang et al., 2017). Based on the functions of the extracellular domains, they are classified into leucine-rich repeat (LRR) RLK, lysine motifs (LysM) RLK, lectin RLK, and epidermal growth factor (EGF)-like RLK subfamilies (Zhou and Yang, 2016). The LRR domains and LysM domains are responsible for binding peptide and chitin, respectively, whereas the ligands of lectin RLKs are largely unknown. Ligand perception activates RLKs and, in turn results in the subsequent immune activation, including mitogen-activated protein kinase (MAPK) cascade activation, reactive oxygen species (ROS) burst, and transcriptional reprogramming.

Receptor-like cytoplasmic kinases (RLCKs) belong to the RLK superfamily but lack the ectodomain and transmem-

brane domains. They often act as substrates for RLKs and function at downstream responses. After sensing ligand, RLKs associate with and phosphorylate RLCKs in several cases, and the phosphorylated RLCKs are dissociated from RLKs complex afterwards. These phosphorylated RLCKs are able to phosphorylate and activate downstream components, such as NADPH oxidase RbohD and MAPK cascades, leading to immune activation (Li et al., 2016; Tang et al., 2017). For example, a well-studied RLCK BOTRYTIS-INDUCED KINASE1 (BIK1) directly phosphorylates RbohD for ROS burst (Tang et al., 2017), and other members in receptor-like cytoplasmic kinase VII (RLCK VII) phosphorylate MAP kinase kinase 5 (MAPKKK5) for MPK3/6 activation (Bi et al., 2018). Interestingly, some RLCKs not only localize at the plasma membrane, but also are observed in the cytoplasm or the nucleus. Lal et al. found that BIK1 can enter the nucleus and phosphorylates specific WRKY transcription factors, leading to the activation of defense hormone salicylic acid (SA) and jasmonic acid (JA) signaling (Lal et al., 2018). These discoveries uncover the substantial roles of RLCKs in plant RLKs-mediated immune signaling.

However, lectin RLKs-activated immune responses may not require RLCKs. ROS burst is one of the early responses of RLKs-mediated signaling. In *Arabidopsis* immunity, extracellular ROS is mainly produced by NADPH oxidase RbohD. We recently demonstrated that an L-type lectin RLK LecRK-IX.2 interacts with RbohD and recruits calcium-de-

\*Corresponding author (email: [Junliu@im.ac.cn](mailto:Junliu@im.ac.cn))

pendent protein kinases to phosphorylate RbohD, which eventually results in activation of SA signaling and systemic acquired resistance (Luo et al., 2017). Interestingly, Chen and the colleagues also reported that the plant eATP receptor DORN1 (Does not Respond to Nucleotides 1), an L-type lectin RLK, directly phosphorylates RbohD to control stomatal immunity (Chen et al., 2017). These evidences point out to a fact that RLCKs may not be essential in lectin RLKs-mediated immune activation.

Transcriptional reprogramming is a virtually late response of RLKs mediated signaling compared to ROS burst, which is largely dependent on plant hormones. For example, JA is an important defense hormone. The transcription factor MYC2 regulates majority of the JA-responsive genes. A recent study demonstrated that a malectin RLK FERONIA (FER) can phosphorylate and destabilize MYC2 to inactivate JA signaling pathway, thereby positively regulating the disease resistance to biotrophic bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 in *Arabidopsis* (Guo et al., 2018). This is the first line of evidence that a RLK can directly modulate the central regulator of defense hormone to reprogram defense gene expression and to control immunity. These findings reveal a new avenue for discovering the direct targets of RLKs in plant immunity.

Phytopathogens mainly live in host apoplast. The ability to acquire metabolites from host is critical for their proliferation. The metabolites that pathogens can use include sugar, lipid, and amino acids. Therefore, pathogens may inevitably compete with host plants for nutrition during infection. However, whether plant immunity is involved in the process remains unclear. It is known that metabolites influx or efflux is executed by multiple plasma membrane localized transporters. Interestingly, Yamada and the colleagues discovered that the activity of sugar transporter 13 (STP13) could be induced by flg22, a conserved 22 amino acid peptide of bacterial flagellin, in *Arabidopsis* (Yamada et al., 2016). They further found that BRI1-ASSOCIATED RECEPTOR KINASE1 (BAK1), which is known as the co-receptor of LRR receptor kinase FLAGELLIN SENSITIVE2 (FLS2), mediated phosphorylation of STP13 and facilitated the influx of sugar from plant apoplast. The sugar influx results in constrained availability of nutrition to *Pst* (Yamada et al., 2016). This study highlights the importance of RLKs in apoplastic immunity by regulating transporter activity. Because there are many transporters responsible for nutrition transportation, it is plausible to hypothesize that plant immunity may intrinsically recruit the plasma membrane-located transporters to suppress pathogen infection.

The activated RLKs need to be removed in order to avoid constitutive immune activation. During flg22-activated immunity, BAK1 phosphorylates two redundant U-box family E3 ubiquitin ligases PUB12 and PUB13 to promote degradation of FLS2 in *Arabidopsis* (Lu et al., 2011). This

study provides a unique regulatory circuit for RLK turnover by phosphorylation-dependent recruitment of specific E3 ligases. Recent report by Zhou et al. showed that PUB12 and PUB13 can also ubiquitinate the brassinosteroid (BR) LRR receptor kinase BRASSINOSTEROID INSENSITIVE1 (BRI1) for degradation. BRI1, on the other hand, phosphorylates the serine 344 residue of PUB13 to enhance this process (Zhou et al., 2018), implying that direct phosphorylation of E3 ligases by RLKs can promote the enzyme activities and modulate RLK levels. Therefore, ubiquitination of RLKs by RLKs-mediated phosphorylation of immune related E3 ligases plays essential roles in avoiding continuing immune activation in plants.

In the last decade, achievements on the RLKs researches have substantially improved us in understanding the plant innate immunity. However, several recent exciting reports have uncovered more hidden roles of plant RLKs. Wang and the colleagues' recent discovery showed that LRR-RLKs MALE DISCOVERER1 (MDIS1), MDIS1-INTERACTING RECEPTOR LIKE KINASE1 (MIK1), and MIK2, were involved in the plant reproductive process. These RLKs serve as male receptors that can recognize female attractant LURE1 which is a defensin-like peptide and control micro-pylar targeting of the pollen tubes during reproduction (Wang et al., 2016). Excitingly, reproductive isolation barrier could be partially broken between *Arabidopsis* and *Capsella rubella* if it is transformed with *Arabidopsis* AtMDIS1. The other interesting discovery by Nakaminami et al. showed that the immunity related PEP1 RECEPTOR 1 (PEPR1) and its ligand AtPep3 play an additional role in resisting salinity stress (Nakaminami et al., 2018). Treatment with AtPep3 significantly enhanced the salinity resistance in *Arabidopsis* in a PEPR1 dependent manner. These works indicate that RLKs are also crucial in other physiological events; however, the downstream components remain elusive.

These research advances further underpin the central roles of RLKs and RLCKs in plant biology. Nevertheless, there are still many fields remaining to be explored, especially the roles of RLKs and the downstream RLCKs in other biological processes, in addition to plant immunity. It is worth noting that our knowledge on RLCKs is largely based on the achievements from the researches of bacterial pathogens. What are the roles of RLCKs in the infection of fungal and oomycete pathogens as well as herbivores? Future studies on the RLKs and RLCKs-mediated downstream signaling will help us to understand how the plant immunity is initiated and transduced in plants.

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

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