Insight

ABA perception is modulated by membrane receptor-like kinases

Dorota Konopka-Postupolska* and Grazyna Dobrowolska

Institute of Biochemistry and Biophysics, Polish Academy of Science, Warsaw, Poland

*Correspondence: konopka@ibb.waw.pl

In this issue of the Journal of Experimental Botany, Shang et al. (2020) characterize the possible role of membrane receptor-like protein kinase 1 (RPK1) in abscisic acid (ABA) signaling. The authors show that upon ABA accumulation, RPK1 can form a complex and possibly regulate the ABA-mediated activation of the OST1 kinase—the major regulator of ABA-induced responsive pathways. Arabidopsis thaliana mutants that lack functional RPK1 are ABA resistant and show increased water loss, whereas overexpressing plants have an increased tolerance to drought stress (Osakabe et al., 2010). The existence of a positive feedback regulation loop among RPK1, calmodulin 4, and NADPH oxidase F (respiratory burst oxidase homolog F, RBOHF) at the transcriptional level was reported (Koo et al., 2017). Thus, RPK1 may constitute a link to the plasma membrane perception of ABA.

Physiological effects of ABA—puzzling diversity of biosynthesis and the basic pathway of intracellular perception

The vast majority of studies conducted so far focused on the physiological effects of ABA on plants. Endogenous ABA regulates almost all aspects of plant life, beginning from sprouting up to senescence. During growth, ABA determines organ and body size and root growth, modulates plant metabolism, and controls fertility (Harris, 2015). During stresses, such as drought, salt, and, to a lesser extent, low temperature, it protects plants acting as a key regulator of adaptation to biotic and abiotic stresses. Functional diversity, which is inherent to ABA signaling, requires a very sophisticated and spatiotemporally specific detection and reaction system. In the last few years, significant progress has been made towards understanding the mechanisms contributing to ABA biosynthesis, transport, perception, and identification of intracellular targets. However, apart from its existence, relatively little is known about ABA sensing at the plasma membrane, and understanding of the interplay between independent sensing units remains a matter for future research.

One of the most thoroughly studied ABA signaling pathways is found in guard cells, leading to stomatal closure during soil dehydration or lowering of air humidity. For many years it has been known that phosphorylation and dephosphorylation of proteins are essential for this process (Yang et al., 2017). In protoplasts of Arabidopsis thaliana (Arabidopsis) guard cells more than half of the kinase genes identified in the Arabidopsis genome (689 out of 1019) and all genes encoding phosphatase catalytic subunits (Wang et al., 2007) are expressed. Plants with defects in certain phosphatases or kinases display ABA-hypersensitive or ABA-insensitive phenotypes. Mutations in a specific group of phosphatases, specifically the A clade of PP2C (ABI1, ABI2, and
Box 1. Plant membrane perception

Plant genomes contain the greatly expanded monophyletic gene family that code for receptor-like protein kinases (RPKs/RLKs), structurally and functionally related to animal receptor kinases from the Pelle family. In individual species, RLKs account for ~2% of the total number of coding genes (i.e. ~610 in Arabidopsis thaliana and 1100 in Oryza sativa). They establish a surveillance system for the detection of environmental factors as well as perceiving diverse internal signals to orchestrate growth and development, and to control self-incompatibility. The typical RLKs consist of an N-terminal extracellular ‘receptor’ domain, a single transmembrane domain, and a C-terminal intracellular domain with protein kinase activity. According to the structure of their extracellular domains, RLKs are classified into several subfamilies. The best recognized is a subfamily containing extracellular leucine-rich repeat motifs (LRR-RLK) whose members are BRASSINOSTEROID INSENSITIVE 1 (BRI1), BRI1-associated kinase 1 (BAK1), CLAVATA1, PEP RECEPTOR 1 (PEPR1) and PEPR2 pattern recognition receptors (PRRs), FLAGELLIN SENSING2 (FLS2), and EF-Tu receptor (EFR). Broadly, they are involved in the perception and initiation of signaling. Another group are the cysteine-rich receptor-like kinases (CRKs) (44 in Arabidopsis) with the extracellular cysteine-rich motif of unknown function (C-X8-C-X2-C, DUF26) available for extracellular signal perception. The precise role of the DUF26 domain is unknown, but it was suggested that it is involved in redox regulation and protein–protein interaction (Bourdais et al., 2015). Some CRKs were proposed to assist in ROS perception during the response to biotic and abiotic environmental factors (Bourdais et al., 2015). Approximately 25% of Arabidopsis RLKs do not have an ectodomain (and, in some cases also lack a transmembrane domain) and thus are called the receptor-like cytoplasmic protein kinases (RLCKs) (Liang and Zhou, 2018). RLCKs are often functionally and/or physically associated with RLKs and can transmit their activation downstream via transphosphorylation (Liang and Zhou, 2018).

The only ABA receptor proteins identified so far are localized intracellularly but the hormone is also perceived at the plasma membrane, although no bona fide membrane ABA receptor has been identified. Arabidopsis thaliana plants with a functional knockout of RPK1 are more resistant than the wild type to exogenous ABA. It was shown that RPK1 together with calmodulin 4 and NADPH oxidase F (respiratory burst oxidase homolog F, RBOHF) mediate the transient accumulation of H2O2 and trigger age-dependent cell death (Koo et al., 2017). ABA is able to activate the ROS burst by the BAK1/OST1 complex containing RPK1 can associate with OST1 and modify its activity by phosphorylation. Therefore, it is tempting to speculate that RPK1 could form a complex with BAK1 and CRKs during ABA-mediated responses. Activation of RPK1 could happen by transphosphorylation in a complex with CRK or with mobile signaling molecules that have not yet been identified. For example, RPK1 mediates signaling by a small peptide, CLE, in the pathway that controls root growth (Racolta et al., 2018).
HAB1), result in ABA hypersensitivity manifested, among others, by enhanced tolerance to drought. In turn, a kinase from the sucrose non-fermenting-1 (SNF1)-related protein kinases 2 family (SNRK2)—SNRK2.6, also called open stomata 1 (OST1)—was identified as a critical positive regulator of ABA signal transduction in guard cells and its inactivation resulted in complete inhibition of ABA-induced stomatal closure (Acharya et al., 2013). In the absence of ABA, OST1 activity is inhibited by dephosphorylation and direct physical interaction between kinase and PP2C phosphatases (Ng et al., 2014). When ABA is present, this complex dissociates and OST1 is released from phosphatase inhibition (Box 1). This results in activation of the kinase via autophosphorylation of Ser175 in the kinase activation loop. Dissociation of the kinase–phosphatase complex in the presence of ABA results from binding of phosphatase to the holo form of the soluble ABA receptor (PYR/PYL/RCAR). Once it has bound to a ligand, the receptor undergoes a conformational change that results in the creation of a platform for tight binding and inactivation of PP2Cs (Ng et al., 2014). Therefore, the shortest basic ABA-sensing pathway identified in guard cells comprises PYR/PYL/RCAR receptor (ABA receptor), ABI1/HAB1 (a phosphatase), OST1 (a protein kinase), and a target protein. Among downstream targets of free OST1 in guard cells, there is the slow anion channel–associated 1 (SLAC1), quickly activating anion channel (QUAC1), a major anion channel in guard cells, potassium inward rectifying channel (KAT1s), and RBOHF (Arnaud and Hwang, 2015). However, experimental results suggest that ABA signaling in guard cells is also regulated by several additional pathways initiated at the cellular membrane by other hormones or environmental factors (Cutler et al., 2010).

**Perception of ABA at the plasma membrane**

Plants perceive extracellular signals at the plasma membrane by receptor-like kinases (RLKs), whose structural organization and mode of activation are comparable with those of animal receptor tyrosine kinases (RTKs) (Box 1). For activation, plant RLKs require ligand-induced oligomerization followed by the recruitment of receptor-like cytoplasmic kinases (RLCKs). In the receptor complex, RLCKs are phosphorylated by kinase domains of receptors, after which they dissociate and modulate the activity of their respective downstream targets (Liang and Zhou, 2018). For example, it was shown that RLCK *Botrytis*-induced kinase 1 (BIK1) interacts directly with several RLKs to regulate plant immune response, phytohormone signaling, or stress tolerance (Box 2A). Experimental data indicate that...
downstream targets of RLCKs comprise common signaling nodes, such as reactive oxygen species (ROS) production or mitogen-activated protein kinase (MAPK) cascades (Liang and Zhou, 2018).

Some of the plant RLKs were already reported to be important for ABA signaling (Box 2B). Among them, besides receptor-like protein kinase 1 (RPK1), there are RLKs from the cysteine-rich family, CRKs (Tanaka et al., 2012; Zhang et al., 2013; Bourdais et al., 2015; Lu et al., 2016), proline-rich extensin-like receptor-like kinase RLK4 (PERK4) (Bai et al., 2009), lectin receptor-like kinases (LecRK) (Deng et al., 2009), GUARD CELL HYDROGEN PEROXIDE-RESISTANT 1 receptor-like kinase (GHR1) (Hua et al., 2012), receptor DEAD-kinase 1 (RDK1) (Kumar et al., 2017), and FERONIA receptor kinase (Yu et al., 2012). Still, our knowledge about the cross-talk of membrane perception pathways with the basic ABA signaling pathway managed by OST1 remains limited. RLKs have either a positive or a negative effect on ABA signaling, and some of them were shown to interfere differently with the basic ABA-mediated pathway. For example, upon co-expression in Xenopus laevis oocytes, GHR1 interacts with SLAC1 (Hua et al., 2012). It can be assumed that it activates channel conductance by phosphorylation. The activation was inhibited by phosphatase ABI2 but not by ABI1. In turn, RDK1 was shown to mediate recruitment of ABI1 to the plasma membrane (Kumar et al., 2017).

Interaction between membrane RPK1 and the basic intracellular ABA pathway

In this issue, Shang et al. (2020) analysed the molecular mechanism of RPK1 activity in ABA-induced stomatal closure and its possible interaction with the basic ABA-mediated pathway. An initial experiment showed that similarly to BAK1, RPK1 functions in the guard cells upstream of OST1. BAK1 itself does not bind a ligand but functions as a co-receptor, and its activity is critical for the formation of multimeric complexes, with many (if not all) RLKs involved in a wide range of physiological processes, such as immunity, growth, or development. Thus, it can be assumed that upon ABA-induced stomatal closure, membrane receptor RPK1 recruits BAK1 and OST1 to the common complex near the plasma membrane, in which BAK1 functions as a master regulator while OST1 functions...
in a similar manner to RLCK. The authors show that upon ABA stimulation RPK1 forms complexes with both BAK1 and OST1 but with different kinetics. Based on the gene expression, and two techniques of monitoring of in vivo complex formation (fluorescence resonance energy transfer and multicolour bimolecular fluorescence complementation), the authors conclude that BAK1–OST1 is created first from pre-existing proteins (with a peak at 30 min after ABA treatment), whereas the RPK1–OST1 complex is formed from the de novo synthesized protein later on (a gradual increase between 30 min and 2 h after ABA). Finally, the authors find that phosphatase ABI1 that regulates OST1 activity in the shortest basic ABA signaling pathway can also dephosphorylate RPK1, in a time-dependent manner, but not BAK1. RPK1 directly interacts with OST1, regulating phosphorylation and affecting the activity of the latter.

Overall, the results of this study indicate that during stomatal closure, RPK1-mediated response converges with the shortest basic ABA signaling pathway on the level of OST1 activation. However, in such a scenario, an important question remains open: the molecular mechanisms of RPK1 activation upon ABA treatment. To activate kinase activity of RLKs they have to oligomerize upon ligand binding. Therefore, it is tempting to speculate that, with the action of ABA, the RPK1 receptor at a plasma membrane becomes organized in multiprotein complex/complexes with BAK1 and redox-activated ABA-responsive CRK, similarly to FLAGELLIN SENSING 2 (FLS2), EFRs, and BAK1 during the pathogen-induced response. Such oligomerization may be induced by H$_2$O$_2$ accumulation in the apoplast resulting from OST1-mediated activation of RBOHF (Box 2). This concept sheds new light on our understanding of cross-talk among membrane-perceived and intracellular plant signaling pathways.

**Keywords:** ABA-induced stomatal closure, ABI1, abscisic acid, BAK1, OST1, RPK1, sequential complex formation.

**References**

Acharya BR, Jeon BW, Zhang W, Assmann SM. 2013. Open Stomata 1 (OST1) is limiting in abscisic acid responses of Arabidopsis guard cells. New Phytologist 200, 1049–1063.

Arnaud D, Hwang I. 2015. A sophisticated network of signaling pathways regulates stomatal defenses to bacterial pathogens. Molecular Plant 8, 566–581.

Bai L, Zhang G, Zhou Y, Zhang Z, Wang W, Du Y, Wu Z, Song C-P. 2009. Plasma membrane-associated proline-rich extensin-like receptor kinase 4, a novel regulator of Ca signalling, is required for abscisic acid responses in Arabidopsis thaliana. The Plant Journal 60, 314–327.

Bourdais G, Burdiak P, Gauthier A, et al. 2015. Large-scale phenomics identifies primary and fine-tuning roles for CRKs in responses related to oxidative stress. PLoS Genetics 11, e1005373.

Cutler SR, Rodríguez PL, Finkelstein RR, Abrams SR. 2010. Abscisic acid: emergence of a core signaling network. Annual Review of Plant Biology 61, 651–679.

Deng K, Wang Q, Zeng J, Guo X, Zhao X, Tang D, Liu X. 2009. A lectin receptor kinase positively regulates ABA response during seed germination and is involved in salt and osmotic stress response. Journal of Plant Biology 52, 493.

Harris JM. 2015. Abscisic acid: hidden architect of root system structure. Plants 4, 548–572.

Hua D, Wang C, He J, Liao H, Duan Y, Zhu Z, Guo Y, Chen Z, Gong Z. 2012. A plasma membrane receptor kinase, GHR1, mediates abscisic acid- and hydrogen peroxide-regulated stomatal movement in Arabidopsis. The Plant Cell 24, 2546–2561.

Kimura S, Waszczak C, Hunter K, Wrazcezk M. 2017. Bound by fate: the role of reactive oxygen species in receptor-like kinase signaling. The Plant Cell 29, 638–654.

Koo JC, Lee IC, Dai C, et al. 2017. The protein trio RPK1–CaM4–RbohF mediates transient superoxide production to trigger age-dependent cell death in Arabidopsis. Cell Reports 21, 3373–3380.

Kumar D, Kumar R, Baek D, Hyun T-K, Chung WS, Yun D-J, Kim J-Y. 2017. Arabidopsis thaliana RECEPTOR DEATH KINASE1 functions as a positive regulator in plant responses to ABA. Molecular Plant 10, 223–243.

Lee DS, Kim YG, Kwon SJ, Ryu CY-M, Park OK. 2017. The Arabidopsis cysteine-rich receptor-like kinase CRK36 regulates immunity through interaction with the cytoplasmic kinase BIK1. Frontiers in Plant Science 8, 1856.

Liang X, Zhou J-M. 2018. Receptor-like cytoplasmic kinases: central players in plant receptor kinase-mediated signaling. Annual Review of Plant Biology 69, 267–299.

Lu K, Liang S, Wu Z, Bi C, Yu Y-T, Wang X-F, Zhang D-P. 2016. Overexpression of an Arabidopsis cysteine-rich receptor-like protein kinase, CRK5, enhances abscisic acid sensitivity and confers drought tolerance. Journal of Experimental Botany 67, 5009–5027.

Nambara E, Marion-Poll A. 2005. Abscisic acid biosynthesis and catabolism. Annual Review of Plant Biology 56, 165–185.

Ng LM, Melcher K, Teh BT, Xu HE. 2014. Abscisic acid perception and signaling: structural mechanisms and applications. Acta Pharmacologica Sinica 35, 567–584.

Osakabe Y, Mizuno S, Tanaka H, et al. 2010. Overproduction of the membrane-bound receptor-like protein kinase 1, RPK1, enhances abiotic stress tolerance in Arabidopsis. Journal of Biological Chemistry 285, 9190–9201.

Racolta A, Nadine MD, Davies K, Lee C, Rowe S, Velazco Y, Wellington R, Tax FE. 2018. A common pathway of root growth control and response to CLE peptides through two receptor kinases in Arabidopsis. Genetics 208, 667–704.

Shang Y, Yang D, Ha Y, Shin H-Y, Nam KH. 2020. RPK1 and BAK1 sequentially form complexes with OST1 to regulate ABA-induced stomatal closure. Journal of Experimental Botany 71, 1491–1502.

Shi C-C, Feng C-C, Yang M-M, Li J-L, Xiao-Xu Li, Zhao B-C, Huang Z-J, Ge R-C. 2014. Overexpression of the receptor-like protein kinase genes AIRPK1 and OsRPK1 reduces the salt tolerance of Arabidopsis thaliana. Plant Science 217–218, 63–70.

Shinozawa A, Otake R, Takezawa D, et al. 2019. SnRK2 protein kinases represent an ancient system in plants for adaptation to a terrestrial environment. Communications Biology 2, 30.

Tanaka H, Osakabe Y, Katsura S, Mizuno S, Maruyama K, Kusakabe M, Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K. 2012. Abiotic stress-inducible receptor-like kinases negatively control ABA signaling in Arabidopsis. The Plant Journal 70, 599–613.

Wang H, Chevalier D, Larue C, Ki Cho S, Walker JC. 2007. The protein phosphatases and protein kinases of Arabidopsis thaliana. The Arabidopsis Book 5, e0106.

Wasilewska A, Vlad F, Sirichandra C, Redko Y, Jammes F, Valon C, Frei dit Frey N, Leung J. 2009. An update on abscisic acid signaling in plants and more... Molecular Plant 1, 198–217.

Yang W, Zhang W, Wang X. 2017. Post-translational control of ABA signalling: the roles of protein phosphorylation and ubiquitination. Plant Biotechnology Journal 15, 4–14.

Yu F, Qian L, Nibau C, et al. 2012. FERONIA receptor kinase pathway suppresses abscisic acid signaling in Arabidopsis by activating ABI2 phosphatase. Proceedings of the National Academy of Sciences, USA 109, 14693–14698.

Zhang X, Yang G, Shi R, Han X, Qi L, Wang R, Xiong L, Li G. 2013. Arabidopsis cysteine-rich receptor-like kinase 45 functions in the responses to abscisic acid and abiotic stresses. Plant Physiology and Biochemistry 67, 189–198.