Novel ABP1-TMK auxin sensing system controls ROP GTPase-mediated interdigitated cell expansion in *Arabidopsis*

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ROP GTPases (Rho-like GTPase from plants), plant counterparts of animal and fungal Rho-family GTPases, have recently been shown to be key components of a novel signaling pathway activated by the plant hormone auxin. Auxin (indole acetic acid) is a key regulator of virtually every aspect of plant growth and development, yet the molecular mechanisms of auxin responses remain largely unknown. AUXIN BINDING PROTEIN1 (ABP1) is an ancient protein that binds auxin and has been implied as a receptor for a number of auxin responses, but its precise mechanism remains unresolved. A paradox for ABP1's action is that it is predominantly found in the endoplasmic reticulum (ER) lumen, while it has been implicated as a cell surface auxin receptor, functionally distinct from the nuclear TIR1/AFB auxin receptor family that regulates transcriptional responses. Since our group reported that ABP1 is required for activating two antagonizing ROP signaling pathways involved in cytoskeletal reorganization and cell shape formation in *Arabidopsis* leaf pavement cells, we recently further showed that the plasma membrane-localized TMK receptor-like kinases functionally interact in a complex with ABP1 and are required for ABP1-dependent activation of ROP GTPases by auxin. The formation of this cell surface complex is induced by auxin and requires functional ABP1. These exciting findings provide convincing evidence for this novel auxin sensing system on the cell surface and suggest intriguing mechanisms for TMKs being functional partners of ABP1 to transmit

extracellular auxin signal to intracellular ROP signaling module during polar cell expansion.

Auxins are a pivotal group of phytohormones found in all land plants and regulate a large number of plant growth and developmental processes, such as embryogenesis, morphogenesis, organogenesis, and reproduction.¹⁻³ How such a small molecule could orchestrate so versatile processes is elusive and intriguing. In the past decade, one milestone of auxin perception and signaling transduction research is the discovery of TIR1/AFB-based gene transcriptional signaling required for auxin distribution and gradients in different plant tissues.⁴⁻⁶ Auxin directly binds to TIR1/AFBs, and acts like a glue to stabilize the binding of substrate AUX/IAA proteins. Different combinations of TIR1 and Aux/IAA proteins form co-receptor complexes with a wide range of auxin-binding affinities and may contribute to the complexity of auxin responses. Although the TIR1/AFB pathway is required for the regulation of a diversity of auxin responses, some rapid cellular auxin actions appear unlikely to be transcriptionally regulated.⁷⁻⁹ Therefore, TIR1/AFB-independent auxin signaling pathways that contribute to the rapid auxin responses exist in plants as well.

AUXIN BINDING PROTEIN1 (ABP1), which is present in all green plants, is shown to be essential for numerous auxin-related processes such as cell cycle and expansion and auxin-regulated gene expression.⁹⁻¹¹ Independently of TIR1-mediated auxin perception and/or signaling pathway, ABP1-mediated auxin

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Figure 1. A working model for ABP1/TMK auxin receptor coordination of interdigitated growth in pavement cells. ABP1 and TMK forms a receptor complex on plasma membrane for auxin perception. When auxin is perceived, it will promote the self-phosphorylation of TMK to activate kinase activity. Active TMK will further phosphorylate RhoGEF and release its GEF activity. This active GEF will finally activate mutually exclusive ROP2 and ROP6 pathways, which are localized to the lobing and indenting side and promote lobe protrusion and neck indentation, respectively.

perception from the extracellular space is required for the plasma membrane (PM) or cytoplasmic responses at non-transcriptional levels. Although the majority of ABP1 was observed to reside in endoplasmic reticulum lumen, a small portion of ABP1 was found to be secreted and to associate with the outer face of the PM, which was proposed to be the major location of auxin perception by ABP1.^{12,13} Given it functions as auxin receptor, the low amount of PM-delimited ABP1 is supposed to sense extracellular auxin required for downstream cellular signaling. In the *Arabidopsis* leaf epidermal pavement cell (PC), previous findings from our group showed that ABP1 mediates auxin signaling to coordinately activate two mutually exclusive ROP signaling pathways.¹⁴ ROPs are the sole plant RHO clade GTPases and it is therefore expected that they participate in the regulation of a large number of cellular events including cell polarization, polar growth, vesicle trafficking, and cell morphogenesis.^{15–17} On the cell surface of the PCs, ABP1-based auxin signal rapidly activates both ROP2 and ROP6 pathways for local intra- and inter-cellular coordination of interdigitating lobes and indentations.¹⁴ As ABP1 lacks a transmembrane domain, it is not possible to transmit extracellular auxin signals to ROPs by ABP1 protein itself. Thus a transmembrane docking protein such as receptor-like kinase (RLK) was speculated to act with ABP1

as auxin co-receptor on the cell surface to modulate auxin-mediated activation of ROP GTPase signaling.⁹

Our group recently addressed this question. We demonstrate that the transmembrane kinase (TMK) subfamily of receptor-like kinases with four closely related members (TMK1-TMK4) play a critical role as a transmembrane partner of ABP1 (**Fig. 1**). TMKs contain an extracellular leucine-rich-repeat domain, a single transmembrane region, and a cytoplasmic kinase domain. We first assessed whether TMKs function in auxin- and ABP1-mediated processes. While single loss-of-function mutants of TMKs do not display discernable phenotypes, unique double, triple, and quadruple mutants exhibit increasing auxin-related defects, respectively, with shorter roots and hypocotyls, smaller leaves, and stamen filaments that are due to the inhibition of cell growth and cell proliferation.¹⁸ In multicellular plants, polar auxin transport and the formation of auxin gradients are mediated by the auxin efflux carriers PIN-FORMED (PIN) protein.¹⁹ Emerging evidence suggests that ABP1- and ROP GTPase-dependent auxin signaling modulates trafficking and polarization of PINs.^{14,20–23} Here we found that the *tmk* quadruple mutant displays *pin1–1* like leaf phenotype and altered PIN1 localization. Embryo lethality was also found in *tmk* quadruple mutant, as in *abp1* null mutants. Furthermore, the

PCs of the quadruple mutant showed fewer and shorter lobes compared with wild type PCs, a defect similar to but stronger than that observed in an *abp1* allele (*abp1–5*), which harbors a point mutation (H94Y) in the auxin binding pocket of the ABP1 protein.¹⁴ Notably, the PC defects in either *tmk* quadruple mutant or *abp1–5* were not rescued by auxin. We conclude that ABP1 and TMKs genetically interact with each other and may function together to regulate auxin mediated PC interdigitation and other processes.²⁴

As the rapid activation of the antagonizing ROP2 and ROP6 pathways require ABP1-dependent auxin perception,¹⁴ we examined whether TMKs are also essential for auxin-mediated activation of ROP2 and ROP6 GTPases, as is ABP1.¹⁴ We performed a ROP activity assay, and found auxin-induced promotion of ROP2 and ROP6 activation was compromised in the *tmk* quadruple mutant, as in the *abp1–5* mutant.¹⁴ These results indicate that TMKs, like ABP1, function in the pathway that leads to auxin activation of ROP GTPase signaling pathways. In leaf PCs, the ROP2-RIC4 (Rop-interacting CRIB-containing proteins) pathway localizes to lobe tips and activates the accumulation of cortical actin microfilaments there, whereas the ROP6-RIC1 pathway that organizes cortical microtubules (MTs) at the indenting regions (**Fig. 1**).¹⁴ Thus, GFP-RIC4 was used as an in vivo marker for active ROP2, and YFP-RIC1's association with cortical MTs as an indicator for ROP6 activation in PCs. In *tmk* quadruple mutant, subcellular distribution of RIC1 and RIC4 and that of their targets were all abolished just as in the *abp1–5* mutant as well,¹⁴ further confirming that TMKs, like ABP1, are required for the activation of ROP2 and ROP6 in *Arabidopsis* PCs.²⁴

Visualization of TMK1 and ABP1 distribution by confocal microscopy analysis of GFP-tagged TMK1 or ABP1 and immunogoldhistochemistry in conjunction with transmission electron microscopy (TEM) confirms that both TMK1 and ABP1 are localized to the cell surface, where both ROP2 and ROP6 are

being activated. To further determine how TMKs and ABP1 function together to activate PM-localized ROP2 and ROP6, we next isolated TMK1-GFP protein complex from TMK1-GFP transgenic plants by immunoprecipitating using an anti-GFP antibody. ABP1 was then found present in this complex using the ABP1 antibody, indicating that ABP1 associates with the TMK1 protein complex. Co-immunoprecipitation in *Nicotianabenthiana* tobacco leaves further confirmed that ABP1 associates with the extracellular domain of TMK1 at the cell surface, but not with the cytoplasmic kinase domain of TMK1. Moreover, not only the natural auxin (Indole-3-acetic acid, IAA), but also the synthetic auxin (1-naphthaleneacetic acid, NAA), can enhance the interaction between ABP1 and TMK1 in a concentration-dependent manner, similar to those required to activate ROP2 and ROP6.^{14,24} In addition, the ABP1-5 mutation greatly compromised auxin-induced association of ABP1 with extracellular region of TMK1.²⁴ These results demonstrate that auxin promotes the interaction between ABP1 and the extracellular domain of TMK1 and that ABP1 sensing of auxin plays a pivotal role in the complex formation. Furthermore, overexpression of a dominant negative form of TMK1, in which the intracellular region is deleted, displayed a similar PCs phenotype as in *abp1-5* and *tmk* quadruple mutants and almost abolished auxin activation of ROP2 and ROP6 as well, suggesting that this truncated TMK1 blocks the function of ABP1 and endogenous TMKs.²⁴

Taken together, our group proposes an exciting model for ABP1- and TMK-based auxin perception and signaling at the cell surface. Upon perceiving extracellular auxin, secreted ABP1 becomes associated and/or more tightly associated with TMKs, leading to the activation of TMKs and their downstream signaling components such as ROP2 and ROP6 GTPases (**Fig. 1**). These findings provide strong evidence for ABP1 acting as a cell surface auxin receptor, which is also consistent with previous observations that ABP1 regulates auxin responses such

as PIN1 endocytosis and ROP GTPases activation at the cell surface.^{14,20} TMKs are the first trans-PM functional partners of ABP1 known to transmit auxin signal to downstream signaling, and their associations with ABP1 may be direct, but further work is needed to test the potential direct interaction. We do not exclude other possible interacting proteins such as GPI-anchored proteins recently described.^{9,25} Since auxin binding does not induce apparent 3D-structural changes in ABP1, auxin may act as a molecular glue to stabilize the interaction between ABP1 and TMKs, as shown for the TIR1-AUX/IAA co-receptors.²⁶ Beside the TMK-type RLKs clade, some additional RLKs have recently been well documented to participate in auxin signaling. For instance, a number of *Arabidopsis* T-DNA lines for root-expressed LRR-RLKs exhibit altered root growth response to the auxin.²⁷ OsRPK1 is identified as an auxin-related LRR-RLK as well, which affects polar auxin transport and the activity of OsPINs, implying its potential implication with the RAC/ROP signaling in rice.²⁸

The auxin-activated ABP1-TMK complex most likely induces the autophosphorylation of TMKs. In addition, TMKs activation on downstream substrates such as PRONE-type plant specific RopGEFs (guanine nucleotide exchange factors that activate ROPs)²⁹ would be prerequisite for the RopGEF activity toward different ROPs. ABP1 may exhibit different auxin binding affinities when forming co-receptor complexes with four functionally overlapping TMKs in different tissues, which could subsequently activate with two distinct RopGEFs, leading to respective activation of ROP2 and ROP6 (**Fig. 1**).⁹ It is also possible that auxin directly inhibits the endocytosis of TMKs to allow the recruitment of RopGEFs to the plasma membrane through their docking to the TMK intracellular domain that may be independent of its kinase activity. Several recent studies on the mechanism of pollen tube growth and ROS-mediated polarized root hair development showed that diverse RLKs can directly interact with and phosphorylate RopGEFs, and/or act

upstream of ROPs.³⁰⁻³⁴ A yeast two-hybrid screen showed that the tomato pollen-specific RLKs LePRK1 and LePRK2 interact with a tomato RopGEF homolog, which led to further work on a physical and functional interaction between the pollen-specific *Arabidopsis* RopGEF12 and a LePRK2 homolog AtPRK2a.^{30,31} A series of biochemical and overexpression experiments suggest that this interaction recruits RopGEF12 to the plasma membrane and releases the inhibition of the C-terminal region on the GEF activity via PRK2a-mediated phosphorylation.³¹ These results imply that AtPRK2 may act upstream of RopGEF12 to activate ROP signaling pathways in pollen tubes. More recent work regarding AtPRK2 uncovers that this pollen-specific RLK regulate both RopGEF1 and ROP1 activities via forming a complex with them, which makes a conclusion that RopGEF1 bridges the signaling event from AtPRK2 to ROP1 to control polarized pollen tube growth in *Arabidopsis*.³⁴ Also, several members of the *Catharanthusroseus* RLK (CrRLK)-related family were identified as upstream regulators of RopGEFs.^{32,35} CrRLK FERONIA (FER) acts as an upstream regulator of RAC/ROP2 signaling pathway most likely by interacting with RopGEF1. Loss of the CrRLK FER leads to reduced levels of active RAC/ROPs and severe root hair defects.³² This also suggests that RLK-mediated signaling event is involved in transmit cell surface signaling to ROP GTPases through the mediation of RhoGEFs. A recent study on one RLK candidate for ROP activation during the polarization of plant cell division and cell growth has been reported in maize.³³ Importantly, the PANGLOSS1 (PAN1) RLK is crucial for the accumulation of a membrane associated phosphoprotein and actin patches,³⁶ which in turn supports the conclusion that a RLK-ROP interaction play the role in the polarity establishment of plant cells. Besides the RLK-RopGEF-ROP cascade, active form of a tomato ROP (LeROP) has been proven to directly interact with LePRKs, indicating LePRKs function as ROP effectors as well.³⁷ Whether the PRK activation on RopGEFs requires the signaling feedback through the interaction between activated ROP

and the PRKs will be an exciting point to uncover.

Similar to the action of RLKs in plants, receptor tyrosin kinases (RTKs) that comprise the majority of animal receptor kinases activate Rho GTPases through RhoGEFs as well,³⁸ suggesting that receptor kinase signaling to Rho proteins is a general signal transduction mechanism. There is no precedence in related signaling events concerning the direct association between RTKs and Rho GTPases in animals and fungi, suggesting the specificity for the direct interaction between RLKs and ROPs in plant. Like AtPRK2 activating two distinct RopGEFs as RopGEF1 and RopGEF12, a single RTK is also capable of phosphorylating multiple Rho GEFs.^{31,34,38} And similar to the activation of Ras and the MAPK

pathway by RTKs, Rho GTPases are typically activated within minutes of RTK stimulation.³⁸ This also happens when auxin-induced ABP1-TMK1 complex rapidly activates downstream ROP2 and ROP6 GTPases. The *Arabidopsis* genome encodes more than 400 RLKs, the abundance of which is likely due to a lack of RTKs and scarcity of GPCRs in plants. This leads to a speculation that RLKs could be a primary mechanism by which extracellular signals activate ROPs in plants.^{17,39} Having a RLK cell surface receptor like the TMKs family to perceive a ROP activating signal from adjacent cells could be a common mechanism for cell-cell coordination for the spatial control of cellular processes in multicellular plant tissues consisting of walled cells. Therefore further identifying

specific RopGEFs regulated by different TMKs will be a fertile field for future studies of ROP signaling in connection to developmental mechanisms in plants.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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