Cell Surface ABP1-TMK Auxin-Sensing Complex Activates ROP GTPase Signaling
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Here, we report that the plasma membrane Auxin-binding protein 1 (ABP1) was discovered nearly 40 years ago and was shown to be reversed in animals treated with 2NP, as marked by Ly-6G (Fig. 4A). Exposure to DNP and signaling systems (Fig. S6C). Immune cell infiltration was similarly increased in animals exposed to DNP or 2NP (Fig. S6D). By 12 hours after stimulation, the thickness of the dermis returned to DNP or 2NP (fig. S6D). By 12 hours after stimulation, the thickness of the dermis was similar at 3 hours with time (fig. S6B), and the increase in mast cell infiltration was still elevated relative to control mice (fig. S6A). Consistent with this result, more infiltrates were still elevated relative to control mice (fig. S6A). Consistent with this result, more infiltrates were elevated relative to control mice (fig. S6A). Consistent with this result, more infiltrates were still elevated relative to control mice (fig. S6F). Given that an inflammatory response was initiated by either DNP or 2NP, we explored the cell types involved. Gr-1+ CD11c+CD11b+ cells were distinguished on the basis of the myeloid marker 7/4 [Ly-6B.2, which is somewhat more highly expressed by recently generated inflammatory macrophages (20)] from neutrophils as marked by Ly-6G (Fig. 4A). Exposure to DNP caused increased numbers of neutrophils relative to inflammatory macrophages, whereas this ratio was reversed in animals treated with 2NP, consistent with the increased secretion of monocyte-or macrophage-attracting chemokines, such as CCL2, CCL3, and CCL4, after treatment of mast cells with 2NP (Fig. 1D). Whole-mount immunohistochemical analysis of the skin also revealed these differences (Fig. 4, B and C), and skin mast cells from 2NP-treated mice produced greater amounts of CCL2 than did those in the skin of DNP-treated mice (fig. S7). Thus, low-affinity stimulation of FcεRI results in an inflammatory response marked by a shift in the monocyte or macrophage/neutrophil ratio.

Collectively, our findings demonstrate that differences in the affinity of antigen and antibody interactions are discriminated by receptors through qualitative changes in molecular signals resulting in distinct outcomes. This discriminatory ability of receptors may extend beyond the immune system.

References and Notes

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Auxin-binding protein 1 (ABP1) was discovered nearly 40 years ago and was shown to be essential for plant development and morphogenesis, but its mode of action remains unclear. Here, we report that the plasma membrane–localized transmembrane kinase (TMK) receptor–like kinases interact with ABP1 and transduce auxin signal to activate plasma membrane–associated ROPs [Rho-like guanosine triphosphatases (GTPases) from plants] to the plasma membrane, activating ROP signaling pathways, regulating transmembrane cytoplasmic responses and associated fundamental processes.

Auxin-regulates nearly all aspects of plant development and behavior and impinges on a great variety of responses involving cell polarization, expansion, division and differentiation. Exactly how this small-molecule hormone achieves this multitude of diverse roles is largely unexplained, although it may be perceived by multiple functionally distinct auxin perception and signaling systems (1–6). Members of the nuclear TIR1/AFB F-box protein auxin receptor and AUX/IAA co-receptor families modulate nuclear gene transcription in response to various auxin concentrations (1–4).

Independently of the TIR1 family, auxin-binding protein 1 (ABP1) was proposed to perceive extracellular auxin to regulate a plethora of plasma membrane or cytoplasmic responses not necessarily involving gene transcription (6–18).

ABP1 may also coordinate with the TIR1/AFB pathway to regulate gene transcription (16, 19). ABP1 is essential for early embryogenesis, root development, leaf expansion, cell morphogenesis, and subcellular distribution of PIN auxin transporters (6, 8, 9, 12, 13, 15–18, 20, 21). ABP1 is required for the auxin-dependent activation of ROPs [Rho-like guanosine triphosphatases (GTPases) from plants] to the plasma membrane,


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Supplementary Materials
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which subsequently regulates cytoskeletal organization and clathrin-mediated endocytosis of PIN proteins (5, 17, 18, 22, 23). However, it is not known how ABP1 transmits the auxin signal to regulate these cytoplasmic responses. Here, we demonstrate that the transmembrane kinase (TMK) members of the receptor-like kinase family interact with ABP1 on the cell surface in an auxin-dependent manner and are required for the auxin-mediated activation of ROP GTPase signaling.

To transduce extracellular auxin to the cytoplasmic responses, secreted ABP1 is expected to communicate with the cytoplasm through a transmembrane docking protein (17, 18, 24). We hypothesized that TMKs serve as ABP1 docking proteins. TMKs belong to a clade of receptor-like kinases with four functionally overlapping members whose founding member is TMK1. They contain an intracellular kinase domain, a single transmembrane founding region, and are required for the auxin-mediated cell interdigitation. TMKs affect multiple auxin-cellular responses, secreted ABP1 is expected to communicate with the cytoplasm through a transmembrane domain and are required for the auxin-mediated activation of ROP2 and ROP6 pathways in leaf pavement cells, similar to ABP1.

We next assessed the effect of tmk mutations on ROP2 and ROP6 signaling targets in leaf pavement cells. The ROP2 effector RIC4, localized to the plasma membrane especially at the tip of the lobes, promotes the accumulation of cortical actin microfilaments (F-actin) (Fig. 2E) (18, 22, 23). In the tmk1−/−tmk234 mutant, the lobe tip and plasma membrane distribution of GFP-RIC4 was abolished, as in abp1-5 and rop2RNAi;rop4-1 mutants (Fig. 2F) (18, 22). Furthermore, cortical F-actin, which normally accumulates at lobe sites (fig. S3A), was absent from the cortical regions of tmk1−/−tmk234 mutant pavement cells, just as in abp1-5 mutants (18, 22) (fig. S3B). The ROP6 effector RIC1 associates with cortical microtubules and promotes their organization upon activation by ROP6. The association of yellow fluorescent protein (YFP)–RIC1 with cortical microtubules was abolished in the tmk1−/−tmk234 quadruple mutants, causing the disorganization of cortical microtubules, as observed in the abp1-5 and rop6-1 mutants (18, 23) (Fig. 2, G to H; and fig. S3, C and D). These results indicate that TMKs participate in auxin perception or signaling that activates both the ROP2 and ROP6 pathways in leaf pavement cells, similar to ABP1.

Given the occurrence of both ROP2 and ROP6 activation at the plasma membrane (18, 22, 23), their upstream signaling components are expected to localize to the cell surface as well. In both the leaf pavement cells and mesophyll cells of a pTMK1::TMK1-GFP transgenic line, TMK1-GFP was localized to the plasma membrane (fig. S4, A and B; and fig. S2, H and I). Although ABP1 is mostly found in the endoplasmic reticulum (ER), a fraction of ABP1 was observed on the cell surface in maize (8, 10, 11, 24, 29, 30). To determine ABP1 distribution, we performed immunogold histochemistry in conjunction with transmission electron microscopy (TEM) (fig. S5, B to H) and epifluorescence microscopy of GFP-tagged ABP1 (fig. S5I). The TEM analysis indicates that the majority of ABP1 localized to the ER, whereas ~22% ABP1 is detected on the plasma membrane in Arabidopsis root cells.

![Fig. 1. Transmembrane kinase genes are required for auxin-mediated pavement cell interdigitation.](image)

(A to C) The cotyledon phenotype in the wild type (A), tmk1−/−tmk234 mutant (B), and pin1-1 mutant (C). Scale bars, 500 μm. (D to H) pavement cell phenotype of the wild type with (D) or without (E) auxin (20 nM NAA) treatment, and tmk1−/−tmk234 quadruple mutant with (F) or without (G) auxin (20 nM NAA) treatment. Scale bars, 10 μm. The degree of pavement cell interdigitation was quantified by determining the average number of lobes per square micrometer of pavement cells (Ave Lobe No./μm²) (H). Error bars indicate SD.
consistent with ABP1 distribution in maize. ABP1-GFP was found both in the ER and on the plasma membrane in Arabidopsis pavement cells (fig. S5I). When these cells were plasmolyzed to detach the plasma membrane from the cell wall, ABP1 signal was observed in strands connecting the apoplast and the plasma membrane (fig. S5I). As a negative control, cytoplasmic dominant-negative ROP2 and ER-localized Calnexin did not show comparable apoplastic signal (fig. S5, K and L). These results support the hypothesis that TMK1 and ABP1 are localized to the cell surface suitably for the activation of ROP2 and ROP6.

Our data suggest that TMKs and ABP1 are both required for the activation of plasma membrane–localized ROP2 and ROP6 GTPases in leaf pavement cells (Fig. 2) (18). In addition, plasma membrane–localized TMKs are required for cell expansion in several other cell types, including mesophyll cells (fig. S2I) (25). ABP1 is also known to promote cell expansion in mesophyll cells; in particular, the cell surface–localized ABP1 has been implicated in the promotion of auxin–mediated expansion of mesophyll cells (12, 13). Thus, we hypothesized that secreted ABP1 and the plasma membrane–localized TMK1 physically form a complex in the perception and signaling of extracellular auxin. To test this, we used an antibody to GFP (anti-GFP) to immunoprecipitate the TMK1-GFP protein complex from the protoplasts of ptMK1::TMK1-GFP transgenic plants, which were isolated from expanding leaves and contained both mesophyll cells (92%) and epidermal cells (8%). The ABP1 antibody was used to detect the presence of ABP1 in the complex. ABP1 was detected in the TMK1-GFP protein complex but not in the control BRI1-GFP immunoprecipitates, indicating that ABP1 specifically associates with the TMK1 protein complex (Fig. 3A). Treatments with auxin NAA or IAA increased the amount of ABP1 detected in the TMK1-GFP protein complex (Fig. 3A). The auxin dosage response was similar to that for the activation ROP2 and ROP6 (18). Furthermore, the chosen range of concentrations was between 0.2 and 2 times the dissociation constant of NAA binding to ABP1 (7). In a reciprocal experiment using the ABP1 antibody to immunoprecipitate the ABP1 protein complex, we also found that auxin treatment increased the amount of TMK1 protein in this complex (Fig. 3B). These results demonstrate that auxin promotes the formation of the ABP1-TMK1 protein complex.

We next assessed whether auxin promotion of the ABP1-TMK1 complex formation involves ABP1 perception of auxin using the ABP1-5 mutant protein. The abp1-5 point mutation in the auxin-binding pocket of ABP1 greatly reduces its sensitivity to auxin for the activation of ROP2 and ROP6 (18). Anti-ABP1 detected the ABP1-5 mutant protein in the TMK1 complex only after extended exposure, suggesting that the ABP1-5 mutant protein is, at best, weakly associated with TMK1 (fig. S6, A and B). Moreover, auxin (both NAA and IAA) treatment only weakly enhanced the association of the ABP1-5 mutant protein with the TMK1 complex (Fig. 3C and fig. S6, A and B). These results indicate that ABP1 sensing of auxin is important for the ABP1-TMK1 complex formation.

On the basis of the above results, we propose that the secreted form of ABP1 associates with the extracellular domain of TMK1 at the cell surface. If the extracellular domain associates with ABP1, overexpression of truncated TMK1 (EX-TMK1, AA1–520), in which the intracellular domain is deleted, would act as a dominant-negative mutant (DN-TMK1) by trapping ABP1 and compromising the function of ABP1 and endogenous TMKs. The 35S::EX-TMK1 construct induced a similar pavement cell phenotype to that of tmk1−/−tmk234 and abp1-5 mutants (fig. S7, A and B). Furthermore, EX-TMK1 partially or totally blocked the activation of ROP6 and ROP2 by IAA treatment (fig. S7C).

We next tested the physical interaction between ABP1 and EX-TMK1 coexpressed in Nicotiana benthamiana tobacco leaves. Immunoprecipitation showed that ABP1 associated with both TMK1 and EX-TMK1 fused with HPB (HA-PreScission-Biotin; HA, GE Healthcare Life Sciences) in tobacco leaves (Fig. 4A). The kinase domain, a mutant with the N-terminal LRR repeats removed, or HPB alone did not associate with ABP1 (fig. S6C). ABP1 appeared to immunoprecipitate a greater amount of EX-TMK1 compared with full-length TMK1. Furthermore, the interaction between ABP1 and EX-TMK1 was induced by NAA or IAA in a concentration–dependent manner, very similar to that observed for the association of ABP1 with TMK1-GFP in Arabidopsis (Fig. 4A). Finally, the ABP1-5 mutant protein interacted weakly with EX-TMK1-HPB, and the interaction was not promoted by the addition of NAA or IAA (Fig. 4B). Therefore, auxin mediates the interaction between ABP1 and the extracellular domain of TMK1.

Our findings demonstrate that the plasma membrane–localized TMK1 receptor–like kinase...
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Fig. 3. Auxin promotes the association of TMK1 with ABP1 in Arabidopsis. (A and B) The association of ABP1 with TMK1-GFP in Arabidopsis leaves was determined by coimmunoprecipitation (Co-IP) assay. Plasma membrane–localized BR1-GFP was used as a negative control. The protein complex from leaf protoplasts treated with different concentrations of auxin (NAA and IAA) was immunoprecipitated by GFP antibody (A) or ABP1 antibody (B). ABP1 was detected in the TMK1-GFP complex in an auxin-dependent manner (A). TMK1-GFP was detected in the ABP1 complex, also in an auxin-dependent manner (B). Input ABP1 indicates the total amount of ABP1 in protein samples before coimmunoprecipitation. (C) The association of TMK1-GFP with an ABP1-5 mutant protein in the abp1-5;TMK1-GFP mutant was not induced by auxin addition. The signal shown here (B) was obtained by extended exposure, compared with that shown in (A) (see fig. 56, A and B).

Fig. 4. Auxin promotes the interaction of ABP1 with the extracellular domain of TMK1. (A) The association of ABP1 with TMK1 or EX-TMK1 was analyzed by coimmunoprecipitation in tobacco leaves that transiently expressed ABP1 and EX-TMK1 tagged with HPB. Streptavidin-coated magnetic beads were used to immunoprecipitate TMK1-HPB or EXTMK1-HPB protein complexes, which were immunoblotted with the ABP1 antibody. The same assay was carried out for ABP1-5 and EX-TMK1 (B).

is functionally and physically associated with ABP1 at the cell surface to regulate auxin- and ABP1-mediated activation of ROP GTPase signaling. TMK1 is at least one of the long-sough docking proteins coupling extracellular auxin and its perception by ABP1 to cytoplasmic signaling (6, 14, 24). This discovery solves the mystery of the cell surface–cytoplasmic auxin perception and signaling system and opens up a new horizon in auxin biology. Clearly, the TIR1/AFB-based nuclear pathways are essential for various auxin responses (1, 2). The pleiotropic phenotypes of the tmk and abp1 mutants also indicate an essential role for the extracellular auxin perception (12, 13, 15, 17, 18, 25). The functions of ABP1 and TMKs agree with their role in regulating PIN distribution but also point to unexplored roles for extracellular auxin in other pathways (6, 17, 18, 21, 26–28). Therefore, the discovery of the ABP1-TMK complex underlies many exciting prospects of elucidating the roles of cell surface auxin perception and its relation with the TIR1/AFB-based nuclear auxin perception.

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Table S1

Fig. 2. Auxin promotes the interaction of ABP1 with TMK1 in Arabidopsis leaves. (A) The association of ABP1 with TMK1 or EX-TMK1 was analyzed by coimmunoprecipitation in tobacco leaves that transiently expressed ABP1 and EX-TMK1 tagged with HPB. Streptavidin-coated magnetic beads were used to immunoprecipitate TMK1-HPB or EXTMK1-HPB protein complexes, which were immunoblotted with the ABP1 antibody. The same assay was carried out for ABP1-5 and EX-TMK1 (B).