COMMENTARY

ABP1/ABLs and TMKs form receptor complexes to perceive extracellular auxin and trigger fast phosphorylation responses

Qiao Cheng,[1,2] Jiayang Li,[1,3] and Bing Wang[1,2,*]

[1]Key Laboratory of Seed Innovation, Institute of Genetics and Developmental Biology, Innovation Academy for Seed Design, Chinese Academy of Sciences, Beijing 100101, China
[2]University of Chinese Academy of Sciences, Beijing 100049, China
[3]Yazhouwan National Laboratory, Sanya 572024, China
*Correspondence: bingwang@genetics.ac.cn

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In 1880, Charles Robert Darwin and his son found that a substance (later known as auxin) moving from the tip of oat coleoptiles to the lower part can induce bending toward directional light. Over the past one and half centuries, auxin has been identified as an essential plant hormone involved in almost all aspects of plant growth and development. The biosynthesis, transport, and signaling of auxin have been hot topics in plant biology. Auxin is synthesized predominantly in rapidly dividing and growing tissues, such as shoot apical meristems, leaf primordia and root apical meristems. Short-range and long-range polar auxin transport (PAT) is critical for the spatiotemporal distribution of auxin and is mainly mediated by the influx carriers of AUXIN RESISTANT1/LIKE AUX1 (AUX1/LAX) and the efflux carriers of PIN-FORMED (PIN) and ATP-BINDING CASSETTE SUBFAMILY B (ABCB) families. The differential localization of PIN proteins at specific sites of the plasma membrane creates a directional auxin flow that eventually establishes a PAT stream through adjacent cells. The auxin concentration gradients are further perceived by auxin receptors, including the canonical nuclear receptor TRANSPORT INHIBITOR RESPONSE 1/AUXIN SIGNALING F-Boxes (TIR1/AFBs), the non-canonical nuclear receptor ETTIN/ AUXIN RESPONSE FACTOR 3 (ETT/ARF3), and the extracellular receptor complex Auxin Binding Protein 1–TransMembrane Kinases (ABP1–TMKs) (Figure 1).[1] However, the perception and signaling pathway of extracellular auxin has been controversial over the past few years.

TIR1/AFBS ARE CANONICAL INTRACELLULAR AUXIN RECEPTORS

The canonical intracellular auxin signaling is mainly mediated by three protein families: the auxin-binding F-box proteins TIR1/AFBs, the repressor proteins AUXIN/INDOLE-3-ACETIC ACID (Aux/IAA), and the transcription factors AUXIN RESPONSE FACTOR (ARF).[1] The efficient binding of auxin to TIR1 requires the assembly of a co-receptor complex consisting of TIR1 and Aux/IAA. Different nuclear auxin response apparatuses regulate the transcription of various downstream target genes, which are involved in embryogenesis, shoot and root architecture, leaf and stomata development, stress tolerance, and defense responses. In general, in the absence of auxin, AUX/IAAs repress the function of ARF proteins to regulate the expression of downstream genes by sequestering ARFs away from promoters of target genes and/or recruiting the transcriptional corepressors TOPLESS/TOPLESS - RELATED (TPL/TPR) to inhibit transcription. In the presence of auxin, the TIR1/AFB F-box proteins and Aux/IAA form a co-receptor complex, triggering ubiquitination and degradation of Aux/IAA and releasing transcription activities of ARF proteins on auxin-responsive genes (Figure 1).

WHETHER ABP1 IS AN EXTRACELLULAR AUXIN RECEPTOR HAS BEEN THE FOCUS OF CONTROVERSY

Although the nuclear auxin signaling is classical and powerful, its transcriptional regulation is relatively slow to mediate several rapid auxin responses, such as plasma-membrane hyperpolarization, cytosolic Ca^{2+} transients, protoplast swelling, activation of Rho of plant (ROP) GTPases, and the rapidly accelerated fibrosarcoma (RAF)-like kinase-dependent cytoplasmic streaming.[2] It is possible that a class of auxin receptors distinct from the nuclear TIR1/AFB receptors can regulate these rapid auxin responses and are presumably localized on the cell surface. The plasma-membrane-localized protein ABP1 is the first identified auxin-binding protein with high affinity and is a receptor candidate for extracellular auxin. The *abp1-1* and *abp1-1s* mutants are embryo lethal, but two *abp1* knockout alleles *abp1-c1* and *abp1-TD1*, generated by CRISPR/Cas9 and T-DNA insertion, respectively, showed no obvious phenotypes under normal growth conditions.[3] The lethality of *abp1-1* and *abp1-1s* mutants was later clarified to result from a T-DNA insertion influence of a tightly linked neighboring gene *BELAYA SMERT/RUGOSE 2* (*BSM/RUG2*) rather than from *ABP1* knockout, raising the debate on roles of ABP1 in auxin perception and early embryogenesis.[1,3]

Genetic and biochemical analyses indicated that ABP1 is important for rapid auxin response through association with TMKs on the cell surface. Both the inducible *ABP1* knockdown transgenic lines and the weak allele *abp1-5*, which contains a point mutation of H94Y in the auxin-binding pocket, exhib-

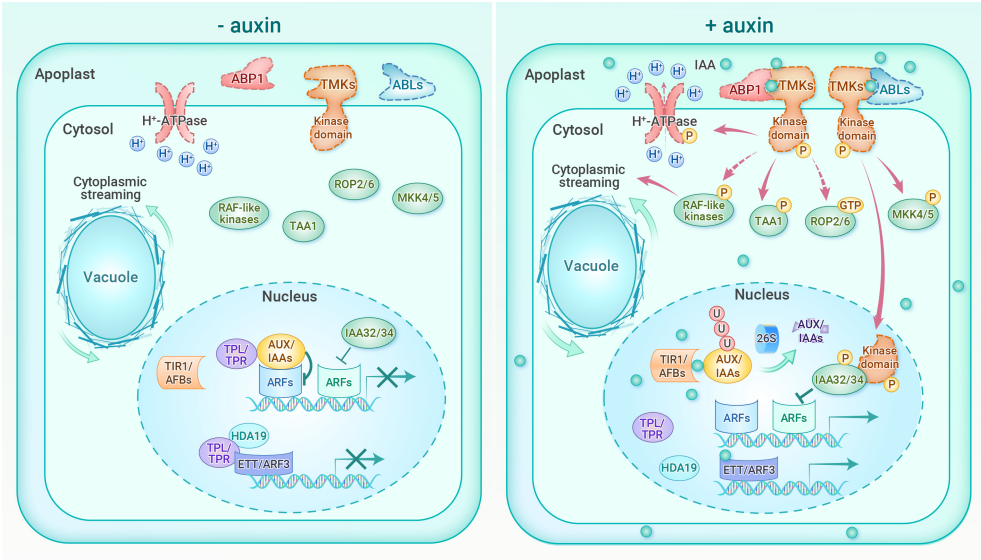

Figure 1. The extracellular and nuclear auxin signaling through ABLs/ABP1–TMKs and TIR1/AFB–AUX/IAA pathways, respectively Left, in the absence of auxin, the phosphorylation level of membrane-localized TMKs is low. AUX/IAAs in the nucleus repress transcriptional activation of ARFs by recruiting TPL/TPR, while the non-canonical IAA32/34 shows weak suppression on activities of ARFs. ETT/ARF3 restricts its own regulation on downstream genes through TPL/TPR and HDA19. Right, in the presence of auxin, extracellular auxin is perceived by ABP1/ABLs and the extracellular domain of TMKs, triggering the ABP1/ABLs–TMKs interaction and phosphorylation of TMKs. The activated TMK kinase domain further triggers fast phosphorylation of multiple effectors that promote rapid cellular auxin responses. Meanwhile, the intracellular auxin is perceived by TIR1/AFB which forms a co-receptor complex with Aux/IAA and triggers ubiquitination and degradation of Aux/IAA, releasing transcription activities of ARF proteins on auxin-responsive genes. The nuclear auxin also dissociates the ETT/ARF3–TPL–HDA19 complex to stimulate gene transcription. Interestingly, locally-high auxin level triggers cleavage of TMK C terminus that phosphorylates and stabilizes IAA32/34 to regulate nuclear gene expression.

ited cotyledon defects, growth arrest, pavement cell defects, and auxin insensitivity. In addition, overexpression of a tagged ABP1 that contains a substitution in the auxin binding pocket in the *abp1-1* background resulted in deficiencies of pavement cell development, auxin sensitivity and auxin transport, indicating essential roles of ABP1 in auxin responses. The TMK receptor-like kinases and ABP1 form a cell surface auxin perception complex that activates ROP signaling pathways and regulates global non-transcriptional cytoplasmic responses and associated fundamental processes.[2] However, whether ABP1 is an indispensable receptor for extracellular auxin is still controversial. In particular, it remains elusive why many TMK-dependent developmental processes and auxin responses are unaffected in the *abp1* null mutant.

ABP1/ABLS AND TMKS FORM EXTRACELLULAR AUXIN RECEPTOR COMPLEXES

A recent study revealed that new auxin-binding proteins, ABP1-like protein 1 (ABL1) and ABL2 physically interact with TMKs and form receptor complexes at the plasma membrane to perceive extracellular auxin. ABL1 and ABL2 regulate rapid auxin responses and various plant developmental processes through distinct and overlapping functions with ABP1.[4]

To verify roles of ABP1 in auxin responses, a dominant negative form of ABP1-5 was overexpressed in a null *abp1-TD1* mutant. Similar to the *tmk1 tmk4*, *tmk1 tmk2 tmk3 tmk4*, and *pin1* mutants, the *ABP1-5;abp1* transgenic line exhibits a series of developmental deficiencies and shows impaired auxin responses, indicating that accumulation of the mutant protein ABP1-5 suppresses TMK-modulated auxin responses.

To search for other extracellular auxin binding proteins that might act together with TMKs, immunoprecipitation (IP) and mass spectrometry (MS) analyses using the *TMK1-FLAG* transgenic line were performed and ABL1 was identified in the TMK complex. ABL1 and its close homolog ABL2 share low sequence similarity to ABP1 but exhibit a conserved auxin binding motif similar to ABP1 according to structural simulations using AlphaFold. ABL1 is almost exclusively localized to the apoplast, and both ABL1 and ABL2 form protein complexes with TMK1 in an auxin concentration-dependent manner. Interestingly, auxin induces the interaction between TMK1 and ABL1/2 within 1 min, indicating a rapid complex formation in plant cells. ABL1 directly interacts with the extracellular domain of TMK1 (TMK1-ex) in an auxin-inducible manner, showing that auxin promotes the formation of the ABL1–TMK1 complex on the cell surface.

To assess whether ABLs and ABP1 are functionally redundant or distinct from each other, the phenotypes and auxin responses of a series of mutants and complemented lines were analyzed carefully. The *abp1;abl1/2* triple mutant showed developmental deficiency and exhibited insensitivity to auxin treatment. Complementation analyses further indicated that both ABL1/2 and ABP1 are required for rapid auxin responses, and the redundant ABL1 and ABL2 have distinct and overlapping functions with ABP1 in plant growth and development. Moreover, the *abp1;abl1;tmk1-/+;tmk4* quadruple mutants presented stronger developmental impairments and more severe deficiencies in fast auxin responses than the *abp1;abl1* and *tmk1-/+;tmk4* double mutants, demonstrating that ABLs/ABP1 and TMKs mainly act in the same pathway in the regulation of auxin responses.

Finally, auxin-binding assays using the microscale thermophoresis (MST) method showed that ABL1 bound to active auxin but not inactive analogs, whereas the mutated protein ABL1-M2, which failed to complement defects of the *abp1;abl1/2* triple mutant, exhibited no binding with IAA, indicating that the auxin binding activity of ABL1 is essential for its physiological functions. Importantly, the TMK1-ex also performed specific auxin binding activity, and the mixture of TMK1-ex with ABP1 or ABL1 exhibited significantly greater IAA binding affinity than ABP1/ABL1 or TMK1-ex itself, demonstrating that TMK1 and ABP1/ABL1 are co-receptors of apoplastic auxin in plants.[4]

ABP1 AND TMKS ARE REQUIRED FOR CONSERVED AND RAPID PHOSPHORYLATION MEDIATED BY RAF-LIKE KINASES

ABP1 and TMK1 are required for the auxin-induced ultrafast global phospho-response and downstream processes, such as the activation of H^+-ATPase and accelerated cytoplasmic streaming. Interestingly, another recent study revealed that the phosphorylation-based signaling represents a highly conserved fast auxin response mechanism in the angiosperm Arabidopsis, the bryophytes *Marchantia polymorpha* and *Physcomitrium patens*, and the streptophyte algae *Klebsormidium nitens* and *Penium margaritaceum*.[5] A range of algae respond to externally applied auxin but do not activate the nuclear auxin pathway. How algae respond to auxin is a fascinating question. The authors found that 2 min of auxin treatment could trigger large shifts in the phospho-proteome in all species detected, and the core set of conserved phospho-targets represent a broad range of cellular responses to auxin. Further analysis demonstrated that the rapidly accelerated RAF-like protein kinases mediated the auxin-triggered phosphorylation and fast cellular responses across species. ABP1 and TMK1 are required for the phosphorylation of RAF-like kinases, indicating that the ABLs–TMKs auxin co-receptor system is presumably ancient and widespread in the green lineage.[5]

CONCLUSION AND PERSPECTIVES

These milestone studies demonstrate that ABLs and TMKs are co-receptors for extracellular auxin and trigger fast phosphorylation through RAF-like protein kinases. In general, extracellular auxin is perceived by apoplast-localized ABP1/ABLs and the extracellular domain of TMKs, triggering the direct interaction of TMKs with ABP1/ABLs and the phosphorylation of TMKs. The activated TMK domain further transfers extracellular signals to intracellular space through the fast phosphorylation of multiple effectors that promote cellular auxin responses, including the activation of H^+-ATPase and acceleration of cytoplasmic streaming (Figure 1). The ABP1/ABLs-TMKs co-receptor system represents a conserved mechanism mediating the ancient and widespread responses to extracellular auxin in the green lineage.

Since ABP1–TMKs and ABLs–TMKs function as co-receptors of extracellular auxin, it is worthwhile to explore the structures of these receptor complexes, to investigate the conformational changes triggered by auxin binding, and to elucidate the mechanism underlying activation and cleavage of the TMK1 intracellular kinase domain. It is interesting to determine whether ABP1, ABLs, and TMKs form a super complex and associate with PIN proteins and other receptors to mediate the integration of auxin perception, polar auxin transport and other important signaling processes. The discovery of ultrafast global protein phosphorylation triggered by extracellular auxin opens an avenue to characterize unknown posttranslational mechanisms underlying the rapid responses of other plant hormones and signaling molecules. The characterization of ABL1 and ABL2 as potential auxin receptors by AlphaFold-based structure simulations revealed the increasingly extensive application of artificial intelligence in scientific research, which will dramatically accelerate the course of exploration worldwide in the near future.

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DECLARATION OF INTERESTS

The authors declare no competing interests.