RESEARCH HIGHLIGHT

The new horizon of plant auxin signaling via cell-surface co-receptors

Jen Sheen [1]✉

Cell Research (2023) 0:1–2; https://doi.org/10.1038/s41422-023-00921-0

Auxin is a central hormone regulating plant growth and development, but the cellular sites and molecular mechanisms of auxin perception and signaling remain incompletely understood for decades. A recent *Cell* paper by Yu et al. provided new compelling evidence that multiple auxin-binding proteins, ABP1 and related ABL1 and ABL2, bind auxin synergistically with plasma membrane co-receptors, TMK receptor kinases, in apoplast to trigger rapid global protein phosphorylation and diverse developmental processes.

Despite being the first promising candidate as an auxin receptor with a definitive auxin-binding pocket at 1.9 Å resolution, where and how auxin-binding protein 1 (ABP1) perceives and transmits auxin signals remained elusive for half a century.[1] Many assays were developed to indicate ABP1 receptor activity in cells and plants. However, as ABP1 contains a C-terminal KDEL signal sufficient for endoplasmic reticulum (ER) retention and is structurally related to the ancient germin, a metal-binding protein with oxalate oxidase and superoxide dismutase activities, and seed storage 7S protein superfamily in plants, its roles in auxin regulation seemed puzzling.[1]

Intriguingly, the *Arabidopsis* transmembrane kinase (TMK) subfamily of receptor-like kinases with extracellular leucine-rich repeat, a single transmembrane region, and a cytoplasmic kinase domain were suggested to be a component in auxin signaling based on growth phenotypes of double and high-order *tmk* mutants.[2] The findings with *tmk* mutants and the detection of a small amount of ABP1 protein in apoplast then led to a new hypothesis that ABP1 could bind extracellular auxin and activate TMKs to regulate plasma membrane or cytoplasmic responses not necessarily involving gene expression mediated by the nuclear TIR1/AFB F-box auxin receptors and AUX/IAA co-receptor families.[3] This innovative model gained support by experiments showing that a weak *abp1-5* mutant enhanced the various auxin-related phenotypes in the *tmk1–/+;tmk2,3,4* mutant and TMKs were required for auxin-mediated activation of ROP2/6 small GTPases in regulating cytoskeleton and leaf pavement cell shape. Importantly, auxin promoted the association of TMK1 and the extracellular domain of TMK1 with ABP1 as shown by co-immunoprecipitation (co-IP) in *Arabidopsis* and tobacco leaves.[3] However, these promising new findings were then shadowed by the analyses of two new *abp1* null mutants without overt growth phenotypes.[4]

Intensive research on TMKs continued nevertheless to uncover new molecular links to diverse auxin responses in various biological processes. For instance, high levels of auxin mediated the C-terminal cleavage of TMK1, and the cytoplasmic TMK1 kinase domain was translocated to the nucleus to stabilize non-canonical IAA32 and IAA34 transcription repressors in the concave side of the apical hook of germinating seedlings to regulate gene expression and inhibit cell expansion.[5] This breakthrough was followed by the discovery of TMK-based cell-surface auxin signaling that triggered phosphorylation and activation of the plasma membrane H^+-ATPases (PM AHAs) and cell-wall acidification, supporting the acid growth model in hypocotyl cell expansion.[6] In roots, although auxin activated the phosphorylation of PM AHA2 leading to apoplast acidification and promoted cell growth, it was also shown that IAA triggered H^+ influx and apoplast alkalinization via intracellular TIR1/AFB, which antagonized root elongation.[7] How TIR1/AFB and TMK1 converge on the opposite apoplast pH regulation in response to auxin remains to be resolved in specific or differential auxin regulation of root growth inhibition and promotion, respectively.

Interesting studies have recently emerged to shed new light on the roles of ABP1 in connection to TMK-mediated auxin signaling. It was shown that auxin could bind ABP1 at the apoplast pH of 5.5 with *K*d values of 15–22 μM. When overexpressed, ABP1-GFP was detected as clusters in the apoplast besides the ER. The *tmk1-1* and *abp1-TD1* mutants failed to support auxin-triggered global hyper-phosphorylation after 100 nM IAA stimulation for 2 min in roots and exhibited reduced auxin-regulated AHA1(T948), AHA2(T947), and myosin XIK(S1234) phosphorylation and activities. Moreover, *abp1-c1*, *abp1-TD1* as well as *tmk1*, *tmk3*, and *tmk4* mutants showed retarded vascular regeneration after stem wounding. The *abp1* and *tmk* mutants also failed to form auxin-transporting channels marked by *pPIN1::PIN1-GFP* auxin transporter and *DR5rev::GFP* auxin reporter. The ABP1 variant deficient in auxin binding was non-functional.[8]

Writing in *Cell* 2023, compelling evidence suggested that ABP1 and structurally related ABP1-like proteins (ABL1 and ABL2) were co-receptors with TMKs that directly bound auxin synergistically and controlled pavement cell shape, rapid hypocotyl elongation, leaf size and morphology, root gravitropism, and fertility.[9] Because plant ABP1 does not have closely related sequence homologs, IP-mass spectrometry analysis was conducted to investigate the interactome of TMK1-FLAG in transgenic *Arabidopsis* plants. Two

[1]Department of Molecular Biology and Centre for Computational and Integrative Biology, Massachusetts General Hospital, and Department of Genetics, Harvard Medical School, Boston, MA, USA. ✉email: sheen@molbio.mgh.harvard.edu

Published online: 05 January 2024

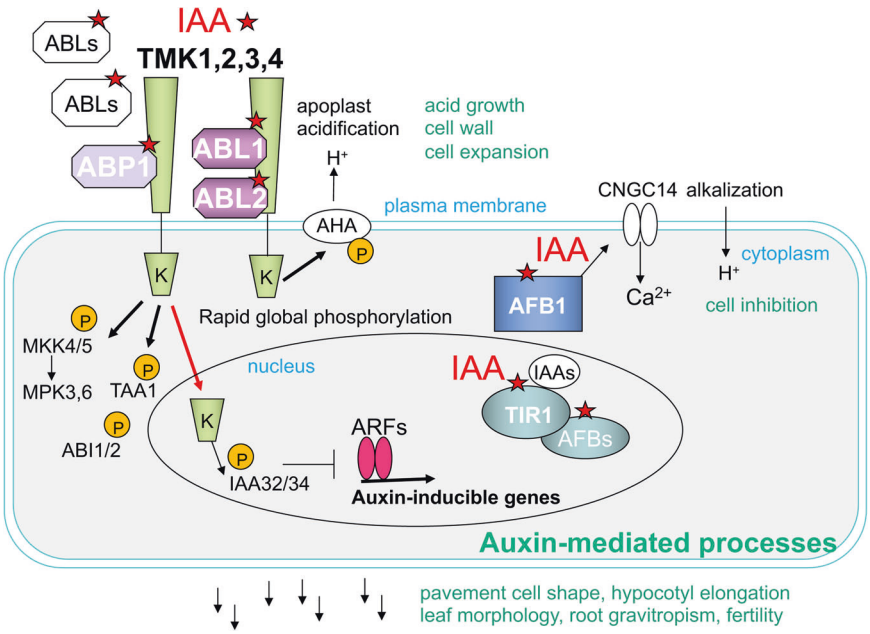

Fig. 1 Coordination of the distinct auxin perception and signaling pathways. In response to extracellular auxin (red stars) and intracellular auxin, the plasma membrane ABP1/ABL–TMK co-receptors and the nuclear TIR1/AFB–IAA co-receptor complex play overlapping, antagonizing, or specific roles to control diverse biological processes in plants. Known TMK phosphorylation substrates are marked (P). AFB1 is a newly discovered cytoplasmic auxin receptor. More ABLs could bind auxin and interact with TMKs. K protein kinase domain, ARFs auxin-responsive transcription factors, IAA indole-3-acetic acid, IAAs auxin-responsive repressors, AHA H^+-pump ATPase, CNGC14 calcium channel, TAA1 tryptophan aminotransferase of Arabidopsis 1, ABI1/2 ABA-insensitive PP2C.

germin-like proteins (GLPs) sharing the conserved metal-binding motif $PXHXH(X)_{11}G$ in the ABP1 auxin-binding pocket were identified as ABL1 and ABL2. Structure-based docking predicted similar auxin binding.[1,9] ABL1 and ABL2 contain predicted signal peptides and ABL1 was localized on the cell surface. ABL1 and ABL2 interaction with TMK1 was stimulated by auxin within minutes as shown by co-IP and fluorescence resonance energy transfer analysis.[9]

Thorough analyses of the double *abl1/2* and triple *abp1;abl1/2* mutants showed reduced ROP2/6 activation, hypocotyl elongation, and pavement cell size, as well as severely retarded leaf development, and abolished global protein phosphorylation by auxin within 1–5 min, supporting functional redundancy of ABL1 and ABL2 with ABP1 in regulating overlapping and distinct functions. Genetic and phenotypic analyses of *abp1;abl1;tmk1+/–;tmk4* confirmed functional interactions of ABL1, ABP1, and TMKs showing strikingly reduced primary root, shoot, inflorescence, and fertility. Comprehensive auxin-binding assays by the microscale thermophoresis method were carried out using ABP1-FLAG, ABL1-FLAG, or HA-tagged TMK1-ex proteins with active auxins and inactive analogs. Although all three proteins bound active auxin with high specificity, the combination of ABP1/TMK-ex or ABL1/TMK1-ex displayed the highest affinity for IAA (94−525 nM), suggesting that ABP1/ABL1 and TMK1 bound auxin synergistically and acted as co-receptors for extracellular auxin in plants. No auxin binding was detected in ABP1-5 and ABL1-M2 carrying HXH mutations in the predicted auxin-binding pocket.[1,9] Notably, proteins purified from *Arabidopsis* protoplast transient expression system showed much higher auxin-binding affinity than those purified from human 393T cells by unknown mechanisms. Importantly, ABL1-M2 failed to complement *abl1/2* with severe deficiency in leaf development.[9]

While enduring decades of the *abp1* controversy, these inspiring discoveries have laid a solid foundation for the ABP1/ABL1/2–TMK-mediated auxin signaling pathway on the cell surface. Future research will elucidate how co-receptors bind auxin synergistically and trigger distinct downstream responses. ABL1 and ABL2 belong to a large superfamily of > 30 GLPs with the conserved $PXHXH(X)_{11}G$ motif critical for high auxin affinity. It would be interesting to investigate their interactions with TMKs and auxin to uncover more new GLP functions.[9] The most fundamental question is how the distinct auxin perception and signaling pathways are coordinated in response to auxin and play overlapping, antagonizing, or specific roles via the nuclear TIR1/AFB receptors and the plasma membrane ABP1/ABL–TMK co-receptors (Fig. 1). Solving another puzzle, a recent finding revealed that AFB1 acted as a unique cytoplasmic auxin receptor to rapidly stimulate CNGC14 Ca^{2+} channel and inhibit root growth.[10] After 50 years, it is humbling but exciting to explore future surprises in the discovery of how the complex auxin signaling network is integrated to relay and control diverse biological processes in plants.

REFERENCES

1. Woo, E.-J. et al. *EMBO J.* **21**, 2877–2885 (2002).
2. Dai, N. et al. *PLoS One* **8**, e60990 (2013).
3. Xu, T. et al. *Science* **343**, 1025–1028 (2014).
4. Gao, Y. et al. *Proc. Natl. Acad. Sci. USA* **112**, 2275–2280 (2015).
5. Cao, M. et al. *Nature* **568**, 240–243 (2019).
6. Lin, W. et al. *Nature* **599**, 278–282 (2021).
7. Li, L. et al. *Nature* **599**, 273–277 (2021).
8. Friml, J. et al. *Nature* **609**, 575–581 (2022).
9. Yu, Y. et al. *Cell* **186**, 5457–5471 (2023).
10. Dubey, S. M. et al. *Mol. Plant* **16**, 1120–1130 (2023).

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Jen Sheen.