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Review

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Historical and mechanistic perspective on ABP1-TMK1-mediated cell surface auxin signaling

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The plant hormone auxin regulates growth and development through at least two distinct signaling pathways. The nuclear pathway, involving TIR1/AFB receptors, mediates transcription; whereas the cell surface ABP1-TMK1 auxin perception triggers global ultrafast phosphorylation response. Here, we revisit the rich history of the disputed ABP1 auxin receptor, highlighting recent findings of the involvement of TMKs and other molecular components and focusing on their role in auxin canalization-mediated development.

Auxin is a central hormone in plant development, governing processes ranging from cell elongation to pattern formation and stress responses[1,2]. Understanding how auxin is perceived and translated into cellular responses has been a cornerstone of plant biology research. Within this context, Auxin-Binding Protein 1 (ABP1) has played a pivotal role in shaping our understanding of auxin signaling in plants.

Discovered over 50 years ago as the first potential hormone receptor in plants, ABP1 was initially celebrated as an auxin receptor responsible for orchestrating auxin-mediated growth and development[3]. After detailed biochemical and structural characterization and a later disproved embryo lethality of the apparent *abp1* knock-out mutant[4], a period of dormancy set in, as insights into ABP1's signaling mechanisms remained limited and were overshadowed by the successful characterization of the canonical Transport Inhibitor Response 1/Auxin Signaling F-box (TIR1/AFB)-dependent pathway[5]. Nonetheless, studies using conditional *abp1* knock-down lines[6] have linked ABP1 function to the regulation of endocytosis and trafficking, particularly of PIN auxin transporters[7]. A major blow came in 2015, when the identification of new *abp1* CRISPR-based null mutants with no obvious developmental defects[8], along with the failure of the *ABP1* gene to rescue the embryo-lethal phenotype[9], raised serious doubts about the physiological and developmental relevance of ABP1. At that time, it seemed that the saga of ABP1 had come to an end.

Nonetheless, after seven dormant years, ABP1 was resurrected as a cell surface auxin receptor[10], which, in cooperation with Transmembrane Kinase 1 (TMK1), mediates an ultrafast phosphorylation response to auxin[10]. This redefined ABP1's role in extracellular auxin perception and highlighted its crucial involvement in auxin transport-mediated canalization.

This review aims to trace the historical journey of ABP1, exploring its function, molecular interactions, and role in auxin perception and downstream signaling. Special attention is given to its partnership with TMKs. By revisiting the long and exciting history of ABP1 and integrating recent findings, we aim to provide a cohesive narrative of how ABP1, in conjunction with TMK1 and other molecular components, functions as a linchpin in auxin signaling and plant development.

The historical journey of ABP1: from discovery to resurrection

ABP1 has a rich, 50-year history, rising to prominence as a key auxin-binding protein, initially thought essential for plants' survival[4], only to see its role devalued[8,9] and, finally, regained within the complex landscape of cell surface auxin signaling[10]. This epic journey is well worth exploring in detail (Fig. 1).

After the discovery of auxin and the multitude of its effects on plant growth and development, scientists became keen to identify its receptor for a deeper understanding of the action of this prominent hormone. To achieve this, a radiolabeled azido-assay to identify auxin binders from corn coleoptiles (*Zea mays*) was conducted, resulting in the identification of ABP1 in 1972[3]. In the following 17 years, ABP1's primary sequence was finally revealed along with its predominant localization in the endoplasmic reticulum (ER)[11]. This was a key step, opening doors for physiologists, biochemists, and geneticists to test a possible role for ABP1 as an auxin receptor. The use of ABP1 agonistic and antagonistic peptides and antibodies has suggested a role for ABP1 in auxin-induced activation of the proton pump ATPase[12], along with potassium channels[13] and voltage-dependent anion channels[14,15]. The same approach established a role for ABP1 in auxin-induced protoplast swelling[16], further linking ABP1 to auxin signaling. ABP1's role in auxin-induced cell expansion was demonstrated in tobacco plants over-expressing ABP1[17]. Furthermore, this was supported by the reduced cell growth observed in tobacco cultured cells constitutively expressing antisense ABP1[4].

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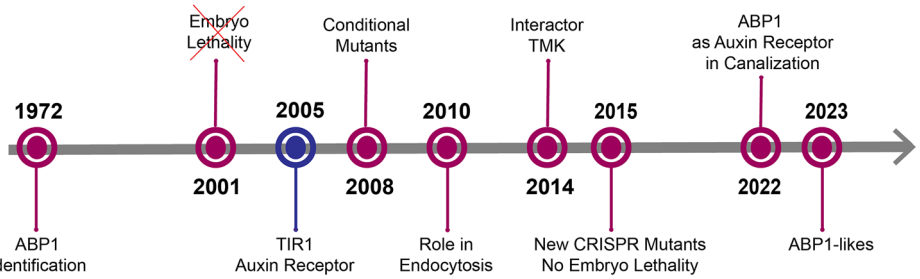

Fig. 1 | Graphical timeline of ABP1 research progress from its identification to recent discoveries. The key milestones in ABP1 history are highlighted in purple, while the blue marker indicate the establishment of TIR1 as an auxin receptor.

After decades of detailed physiological and biochemical analyses[18], the apparent embryo-lethal phenotype of the *abp1* null mutant strengthened the hypothesis on ABP1's importance for plant's survival[4]. Further studies using conditional knockdowns[6] confirmed its involvement in post-embryonic plant growth and development[19]. In particular, ABP1 was proposed to regulate plasma membrane (PM) processes, including endocytosis, endocytic trafficking, and thus PM-incidence of PIN auxin efflux carriers[20], thereby feedback regulating polar auxin transport[7,21].

Much of the ABP1 history revolved around the identification of the so-called "docking protein" that would transmit the signal from the cell surface, where ABP1 was proposed to act, to the cell interior. This proposal was spectacularly fulfilled by the identification of ABP1's interaction partners[22], the Transmembrane Kinase (TMK) family, which belongs among the Leucine-rich repeat receptor-like kinases (LRR-RLKs).

ABP1 was long considered a "red herring" in plant hormone research[23]. A persistent debate centered on its predominant presence in the endoplasmic reticulum (ER), where the pH is not favorable for auxin binding[24]. Furthermore, two major setbacks to ABP1's reputation occured simultaneously in 2015: (i) the identification of two new *abp1* null alleles similar to wild-type plants[8]; and (ii) the failure of the *ABP1* genomic fragment or coding sequence to rescue the embryo-lethal phenotype[9]. These findings led part of the community to doubt ABP1's significance in auxin signaling and plant development, despite the studies of the gain-of-function mutants continuing to suggest its role in the regulation of auxin transport and development[7,9,25].

It took a full seven years before ABP1 regained its position on the map of auxin signaling. This renaissance came when *Arabidopsis* ABP1 was characterized, its auxin-binding ability and dual localization in the ER and extracellular space were reconfirmed, and its crucial importance in auxin canalization and vasculature regeneration was identified genetically[10]. This work demonstrated that both ABP1 and TMK1 are essential for auxin-induced global, ultrafast phosphorylation as well as for several other cellular processes, including PM-ATPase activation and PIN regulation[26,27]. Subsequent research found that ABP1-like (ABL) proteins share ABP1's functional role, interacting with TMK1 to form a similar complex that senses extracellular auxin[27,28]. These exciting discoveries put ABP1-TMK cell surface auxin signaling back into a research focus.

ABP1-TMK1 cell surface auxin perception for global, ultrafast phosphorylation

ABP1 is a single-copy gene in most species, including *Arabidopsis*, and is distantly related to the cupin superfamily[29], specifically to the Germin-like protein (GLP) family, which is found in all plants. The GLP family shares with ABP1 a conserved tertiary structure—a stable, barrel-like fold—despite the limited similarity in the overall primary sequence[30]. In ABP1, this conserved β-barrel structure, known as the cupin fold, forms a binding pocket for auxin. Within this cupin domain, ABP1 contains a conserved germin box motif, composed of about 20 amino acid residues, which play a key role in coordinating a metal ion, typically zinc[29].

While ABP1 resides predominantly in the ER, a portion is secreted to the cell surface, where transmission electron microscopy (TEM) revealed that it forms aggregates in the apoplast[10]. Maize ABP1 was identified based on its strong binding to 1-naphthylacetic acid (1-NAA) in radiolabeled binding assays[3]. This binding was later confirmed also for tobacco ABP through purification and biochemical assays, while the crystallization was achieved with maize ABP1[29]. Notably, the auxin binding assays repeatedly demonstrated preferential auxin binding at a low pH of 5 and 5.5[29], which corresponds to the pH of the apoplast. New binding assays were conducted with the natural auxin indole-3-acetic acid (IAA) to demonstrate that also *Arabidopsis* ABP1 binds to IAA preferentially at the apoplastic pH[10]. At this pH, ABP1 binds auxin and forms clusters in the apoplast[10], facilitating its interaction with the extracellular domain (ECD) of TMK1. ABP1 can act redundantly with ABL1 and ABL2 in aerial parts and with ABL3 in roots; all these structural homologues of ABP1 bind auxin and act as co-receptors with TMKs[27,28]. Auxin binding assays indicate that ABP1/ABLs and TMKs bind auxin synergistically[28].

ABP1 and/or TMK1 have been mentioned in numerous studies associated with various signaling and developmental processes. However, it was the discovery of the global, ultrafast auxin phosphorylation response[10] that provided a clearer mechanistic understanding of how ABP1 with TMKs activates the downstream responses.

A phosphoproteomic approach in *Arabidopsis* roots revealed that, within just two minutes, auxin triggers the phosphorylation of close to a thousand proteins, independently of the canonical TIR1/AFB pathway[10]. Subsequent findings showed that auxin-induced phosphorylation can occur within as little as 30 s and is deeply conserved across the plant kingdom[31]. Notably, the phosphorylation response was found to be highly specific to the natural auxin IAA, as no similar response was observed with the synthetic auxins, benzoic acid, or formic acid[31]. Of particular interest, this phosphorylation response in *Arabidopsis* requires ABP1 and TMK1, with an overlap of many phospho-sites[10].

One of the interesting targets of ABP1-TMK1-triggered phosphorylation is the motor protein Myosin XI and Myosin binding (adaptor) protein (MadB2). Together, Myosin XI and MadB2 regulate the trafficking and dynamic distribution of PIN proteins, playing a crucial role in the feedback loop between auxin signaling and auxin transport across various developmental stages[32]. PIN proteins themselves are prominent phosphorylation targets; this regulation will be discussed in detail later. Another key target of the ABP1-TMK1 complex is the *Arabidopsis* H^+-ATPase (AHA), which regulates proton pumping across membranes, contributing to cellular pH changes and influencing processes such as cell expansion[33,34]. The role and mechanism of AHAs in the context of auxin will be elaborated upon in the TMK1 section. An important, conserved downstream target of the auxin phosphorylation response is the Rapidly Accelerated Fibrosarcoma (RAF)-like kinases[31]. These B4 RAF-like kinases in *Arabidopsis* and *Marchantia* are essential for the auxin phosphoresponse and play a pivotal role in growth and development. As both ABP1 and TMK1 are required for the auxin-induced phosphorylation of RAF-like kinases, they provide a link between the ABP1-TMK1 cell surface auxin signaling and the global, ultrafast auxin phosphoresponse[31].

TMKs and their downstream signaling in plant growth and development

In *Arabidopsis thaliana*, the TMK family includes four members[35]. They are composed of: (i) an ECD consisting of leucine-rich repeats and adopting a

horseshoe shape where ligands bind; (ii) a transmembrane domain that anchors the protein in the PM and connects the ECD to the cytosolic domain; and (iii) an intracellular domain, consisting of a kinase domain, responsible for all downstream signaling pathways[36] (Fig. 2).

TMK1 protein was structurally and biochemically characterized in *Arabidopsis thaliana* for the first time in 1992, highlighting that it encodes a receptor-like kinase and proposing its potential role in transmembrane signaling in plants[37]. Later, in 2000, TMK1 homologue in *Nicotiana tabacum* was isolated showing high homology with the one of *Arabidopsis* as well as with rice[38]. The initial role of TMK1, along with its three subfamily members (TMK2, TMK3, and TMK4), in plant growth was only demonstrated in 2013, especially in regulating cell expansion and cell proliferation[35]. Since then, research on the role of the TMK subfamily in signaling, specifically in the context of auxin, has proliferated. The TMK receptor kinases are involved in multiple developmental processes at different stages and in various organs during plant growth; most of which are linked to auxin action.

Starting with the emergence of the seedling from the soil, TMK1 modulates the formation and the maintenance of the apical hook of the young seedling in order to protect the cotyledons and the shoot apical meristem from any injury[39–41]. Auxin concentrations at the concave (inner) side of the apical hook are higher than at the convex (outer) side. This accumulation of auxin leads to the cleavage of TMK1 kinase domain by the DA1 family of peptidases, followed by its translocation to the nucleus where it phosphorylates two non-canonical transcriptional repressors, called Auxin/ Indole-3-Acetic Acid (Aux/IAA). Consequently, the ubiquitination of these IAA32 and IAA34 by the E3 ubiquitin ligases Cytokinin Induced Root Waving 1 (CKRW1)/ Wavy Growth 3 (WAV3) is prevented, resulting in their stabilization and the subsequent repression of the transcription factors; Auxin Response Factor (ARF), thereby inhibiting the cell elongation at the inner side of the apical hook.

In the hypocotyl, TMK1 regulates the elongation process through the acid growth theory mechanism[42]. In response to auxin, TMK1 interacts with and phosphorylates AHA H^+-ATPases at the PM[33]. This phosphorylation activates AHAs, promoting H^+ export, which leads to the acidification of the apoplast. The resulting acidic environment activates cell wall-modifying enzymes, causing the cell wall to loosen and soften, ultimately facilitating cell expansion[43].

On the other hand, in the root, the auxin-dependent phosphorylation of AHAs by TMK1 also occurs; however, it is antagonized and dominated by the increase in H^+ influx, which is regulated by the intracellular canonical TIR1/AFB, leading to the alkalization of the apoplast and thus inhibiting the root growth[34]. These two antagonistic processes presumably play a crucial role in fine-tuning root navigation through the soil[44].

TMK1 and TMK4 are also involved in the development of lateral roots by controlling the cell division pattern through activating Mitogen-Activated Protein Kinase (MAPK) signaling[45]. TMK1 and TMK4 phosphorylate MKK4 and MKK5 in an auxin-dependent manner and thus activate MPK4 and MPK6 signaling. Therefore, the development of the lateral root requires also coordination between the two auxin signaling pathways: the TIR1/AFB-dependent nuclear transcriptional pathway and the presumed posttranscriptional regulation by TMK1 and TMK4.

TMK1, acting together with ABP1/ABL3 auxin co-receptors, is also a major contributor to the root bending response to gravistimulation[27,46]. Gravistimulation triggers the sedimentation of dense, starch-filled organelles called statoliths in the root tip cells, leading to the relocalization of PIN auxin transporters in these cells. This relocalization correlates with auxin redistribution towards the lower root side, resulting in its accumulation in the epidermal cells at the lower side of the root. With increased auxin accumulation, the Membrane-Associated Kinase Regulator2 (MAKR2), which normally inhibits TMK1, translocates from the PM into the cytosol[46]. Consequently, TMK1 is activated, interacts with, and phosphorylates the hydrophilic loop of the PIN2 auxin transporter. This phosphorylation stabilizes PIN2, enhancing auxin asymmetry and promoting differential cell elongation[27]. This process ultimately causes the root to bend downward in response to gravity. It is an example of a feedback regulation between cell-surface auxin signaling and auxin transport, which also acts between TMK1 and PIN1 during organogenesis and vascular tissue development[26].

In *Arabidopsis* leaves, TMK1 is crucial for the interdigitation of epidermal pavement cells (PCs), mediated by auxin via ABP1/ABL1/2, as it activates Rho of plant GTPases (ROP) signaling pathways[28,47]. The jigsaw-puzzle appearance of PCs results from their spatially coordinated insertion between neighboring cells. At the lobe site, the activated ROP2, through its effector ROP-interactive CRIB motif-containing protein 4 (RIC4), promotes the formation of cortical F-actin microfilaments. Whereas in the opposing indenting site, ROP6, through activating its effector RIC1, promotes the microtubules and suppresses ROP2 activation. These two antagonistic mechanisms lead to the formation of lobes and indentations. To gain further insight, the relationship between local cell coordination and global tissue-wide coordination was examined in detail[48]. This investigation highlighted an additional layer of interaction between cell-surface auxin signaling and the canonical, nuclear auxin pathway. In this process, TIR1/

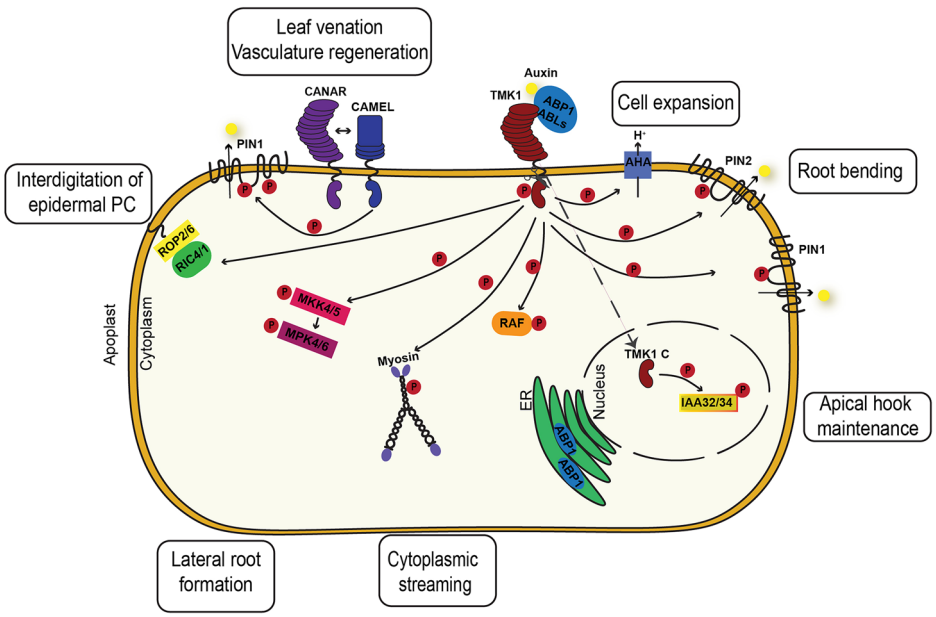

Fig. 2 | Cell-Surface Auxin Signaling Mediated by the ABP1/ABLs-TMK1 Complex. The auxin-induced interaction between ABP1 (Auxin-Binding Protein 1)/ABLs (ABP1-like proteins) and TMKs (Transmembrane Kinase) leads to TMK, initiating a downstream phosphorylation cascade. The specific roles of the ABP1/ABLs-TMK complex-based auxin perception in different developmental and cellular processes are outlined in labeled boxes placed outside the cell representation. Arrows represent the direction of signaling flow, and phosphorylation events are indicated with a "P" inside a red circle. Additionally, the CAMEL-CANAR complex is depicted due to its role in phosphorylating PIN auxin transporters.

AFB activates the expression of auxin biosynthesis genes, leading to the formation of a global auxin gradient with the maxima at the leaf tips. Auxin then locally activates the TMK mechanism across the entire tissue, which subsequently triggers downstream ROP signaling pathways, leading to the interdigitated growth of individual leaf epidermal cells.

So far, all the aforementioned functions stress the role of TMK in auxin signaling and the downstream developmental processes. However, it is important to emphasize the specific involvement of TMK4 in the inhibition of auxin biosynthesis[49]. This regulation is post-transcriptional, where TMK4, activated by auxin, phosphorylates Tryptophan Aminotransferase of Arabidopsis 1 (TAA1)—a key enzyme in auxin biosynthesis—at threonine 101 (T101), which is essential for TAA1 enzymatic activity. When phosphorylated, this site renders TAA1 inactive, thereby reducing auxin levels. Another role of TMK4 in auxin biosynthesis regulation involves its auxin-dependent phosphorylation of the FKBP12-Interacting Protein 37 (FIP37). This modification enhances FIP37 interaction with RNA, increasing the N6-methyladenosine (m6A) modification and thus the mRNA decay of the Nitrilase 1 (*NIT1*) gene which is involved in auxin biosynthesis[50].

Another interesting regulation is the involvement of TMK1 in the crosstalk between auxin and another phytohormone, abscisic acid (ABA)[51]. When auxin levels are high, TMK1 targets and phosphorylates ABA Insensitive 2 (ABI2), inhibiting its phosphatase activity. This inhibition releases SNF1-related protein kinases 2 (SnRK2s), which activate ABA responses. In contrast, low auxin concentrations do not trigger ABA signaling. Another hypothesized role of TMK1 and TMK4 is mediating the crosstalk between auxin and brassinosteroids during hypocotyl elongation[52]. Auxin promotes the elongation by activating MPK3 and MPK6, which phosphorylate general regulatory factor 4 (GRF4) leading to the accumulation of brassinazole-resistant 1 (BZR1), a key transcription factor in brassinosteroid signaling. Although TMK1 and TMK4 have already been shown to activate MAPK signaling in lateral root development, their involvement in hypocotyl elongation requires further investigation.

Above-mentioned examples likely represent only snippets of the more general and broader roles of the members of the TMK family in various cellular and developmental processes. It remains to be seen whether all these TMK functions will be matched by similar functions of ABP1 and ABLs, or whether TMKs or ABP1/ABLs also have independent functions.

The role of ABP1-TMK1 auxin signaling in auxin canalization

Among all the different developmental roles that have been so far associated with ABP1 and TMKs, the best characterized is their role in auxin canalization. We cannot truly discuss the concept of auxin canalization without acknowledging the foundational contributions of Tsvi Sachs, whose pioneering work has shaped our understanding of this critical process in plant development. Through this process, plants optimize their development by flexibly connecting new organs with preexisting vasculature network, establishing, and regenerating their vasculature. Auxin canalization is a self-organizing process in which auxin establishes narrow transport channels, known as auxin 'canals' [53], by a feedback mechanism between auxin signaling—where the auxin source is sensed—and the directional auxin transport, primarily mediated by PIN auxin transporters. The subcellular positioning (polarity) of these PINs is adjusted in such a way that auxin is transported away from the source towards a sink, thereby forming the transport channels[1]. To achieve this, individual cells within the tissue synchronize their polarizations, allowing them to work together to establish a unified pattern of auxin flow. This coordinated behavior enables the tissue to form a well-organized structure, ensuring the efficient formation of auxin transport routes that will provide a positional signal for the formation of new vasculature. The role of auxin channels in adjusting development can be illustrated through several examples. For instance, the prearranged positioning of PIN channels plays a crucial role in leaf venation, guiding the formation of veins[54]. Similarly, during vascular regeneration following wounding, PIN-expressing channels guide the new connection to circumvent the wound[55]. Another notable example is the integration of new organs formed at the shoot apical meristem[56] or lateral buds breaking dormancy[57], where PIN-expressing channels play an essential role in establishing connections with the existing vascular network.

Besides experimental studies, the computational models have also been developed to help explain the mechanisms behind the coordination of PIN polarity during canalization. These models typically focus on two mechanisms: (i) the impact of auxin on cellular growth and the transmission of mechanical stresses through the cell wall, which influences microtubule orientation and governs PIN polarity; and (ii) the role of both intracellular and extracellular auxin perception in regulating PIN expression and PIN endocytic trafficking[58]. In the second mechanism, the nuclear auxin signaling affects *PIN* transcription, while the extracellular perception regulates PIN endocytosis, affecting its incidence at the PM[59].

Recent studies have enriched the auxin canalization model, shedding light on the molecular players and cellular processes involved[58]. These discoveries have advanced our understanding of cell surface auxin signaling, highlighting its role in auxin canalization and its influence on directional auxin transport. Among the prominent molecular components, a PM-localized complex formed by two LRR-RLKs, the Canalization-related Auxin-regulated Malectin-type RLK (CAMEL) and the Canalization-related Receptor-like Kinase (CANAR), has been described[60]. CAMEL contains a malectin domain in its extracellular region, while CANAR acts as a pseudokinase and serves as a negative regulator of CAMEL. CAMEL phosphorylates PIN1, influencing its polarization. This complex is required for vascular tissue regeneration after wounding and leaf venation, with knockout mutants of these receptors exhibiting defects in both. While PIN1 phosphorylation is also regulated by the AGCIII-type kinases like PINOID and D6PK[61], CAMEL uniquely phosphorylates new sites in the hydrophilic loop of PIN1, distinct from those dependent on other kinases. This complex operates downstream of the transcription factor WRKY23[62], a key node in transcriptional auxin signaling mediated by TIR1/AFB. By linking this auxin signaling to PIN1 polarization, the CAMEL-CANAR complex serves as a mediator of auxin-driven developmental processes.

As introduced earlier, the ABP1-TMK1 auxin perception complex at the cell surface plays a key role in auxin canalization, which underscores the concept of diverse auxin perception mechanisms, converging on the regulation of the directional auxin transport. Both *abp1* and *tmk1* loss-of-function mutants exhibit defects in de novo vasculature formation and its regeneration in the inflorescence stems[10]. As noted earlier, Myosin and its binding partner MadB2 serve as downstream targets of rapid phosphorylation by the ABP1-TMK1 complex in response to auxin. This phosphorylation event plays a critical role in coordinating PIN subcellular localization during canalization. Furthermore, as mentioned, TMK1 can directly target and phosphorylate PIN hydrophilic loops, linking TMK1 activity to the modulation of PIN polarity and auxin distribution, also during canalization[26,27]. These elements represent a few of the possible intermediaries, through which the ABP1-TMK1 complex exerts its role in auxin canalization, although further research is needed to fully elucidate their precise contributions.

Conclusion and open questions

Auxin is a versatile hormone that orchestrates a wide range of processes in plants, operating at varying time scales[1,2]. This complexity arises from auxin's reliance on at least two distinct perception mechanisms and their branched downstream signaling cascades tailored to mediate specific responses[5,63,64]. The TIR1/AFB-mediated canonical pathway operates in the nucleus by modulating transcription, while the non-transcriptional branch of this pathway operates in the cytoplasm[65,66]. The ABP1-TMK-dependent pathway acts at the cell surface mediating both transcriptional and non-transcriptional responses[39]. These pathways likely act in coordination, to harmonize diverse physiological responses across the plant. Such connections are exemplified in the apical hook maintenance, where the TMK1 kinase domain is cleaved and translocated from the PM to the nucleus, regulating IAA32/34 and altering the expression of auxin-responsive genes[39–41]. Similarly, during lateral root development, the regulation of

lateral root initiation by TIR1/AFB must coordinate with the post-transcriptional regulation by TMKs[45]. Another partly characterized example of auxin's dual regulatory modes is observed in root growth inhibition, where the rapid phosphorylation and activation of the PM H^+-ATPases at the cell surface[10], promoting cell wall acidification[33], is antagonized by TIR1/AFB-mediated alkalinization[34]. This dual auxin action ensures precise spatial and temporal balance of root growth.

Unlike other plant hormones, auxin is unique in its ability to be transported directionally[20]. This directional transport is crucial for the formation of the asymmetric auxin distribution (local maxima and gradients), which dictate many aspects of plant development[67]. The PIN-dependent auxin network can integrate many endogenous and environmental signals, and the resulting auxin flow redirections and modifications enable plants to adapt and optimize their growth patterns in response to environmental and developmental cues[20]. Among the most important endogenous signals converging on PIN polarity is auxin itself. This tight feedback regulation between auxin signaling and transport is the key prerequisite for auxin canalization and the resulting vasculature formation[53].

A key player in the feedback regulation is the ABP1-TMK1 auxin sensing complex, which, following auxin binding, mediates downstream phosphorylation events[10] essential, among others, for regulating auxin transport. By triggering downstream phosphorylation of myosin[32], PIN1[26] and PIN2[27], ABP1-TMK1 modulate trafficking, polarization, and stability of auxin transporters, influencing distinct developmental processes. Despite significant progress in understanding the roles of ABP1 and TMK1, a major question remains: what are the other downstream players involved in this signaling cascade? For example, the involvement of the CAMEL-CANAR complex, which also regulates auxin transport through the phosphorylation of PIN1[60], adds another layer of complexity to this regulation. Exploring their potential connection to the ABP1-TMK1 pathway would provide valuable insights into how auxin signaling is integrated across different PM sub-compartments and developmental processes. Recent studies have also identified ABL proteins, which, like ABP1, form surface complexes with TMK1 to sense auxin[27,28]. This finding expands our understanding of cell surface auxin perception and underscores the need to investigate the mechanistic and structural details of the ABP1/ABLs complex and how these proteins contribute to the regulation of developmental processes beyond canalization. It remains a challenge for future research to determine whether other members of the large superfamily of > 30 GLPs besides ABL1-ABL3 can act as auxin receptors and how different combinations of ABP1/ABLs and TMKs can trigger distinct downstream responses[68].

Despite over 50 years of ABP1 research, its role within the ER where the majority of ABP1 and ABLs are found, remains enigmatic. This is just one example of the many mysteries of the ABP1/ABL-TMK pathway waiting to be exposed.

Data availability

No datasets were generated or analysed during the current study.

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Author contributions

A.M. wrote the manuscript and prepared the figures. J.F. revised and contributed to the manuscript writing.

Competing interests

The authors declare no competing interests.

Additional information

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