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Review

The interplay between extracellular and intracellular auxin signaling in plants

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ABSTRACT

The phytohormone auxin exerts control over remarkable developmental processes in plants. It moves from cell to cell, resulting in the creation of both extracellular auxin and intracellular auxin, which are recognized by distinct auxin receptors. These two auxin signaling systems govern different auxin responses while working together to regulate plant development. In this review, we outline the latest research advancements in unraveling these auxin signaling pathways, encompassing auxin perception and signaling transductions. We emphasize the interaction between extracellular and intracellular auxin, which contributes to the intricate role of auxin in plant development.

Introduction

Auxin stands as a pivotal hormone in orchestrating a wide array of developmental processes in plants, ranging from the cellular level to the organism as a whole. The action of auxin is characterized by several critical features: self-organization, concentration-dependent and tissue-specific effects, and temporal responsiveness (Bhalerao and Bennett, 2003; Bennett et al., 2014; Hajný et al., 2022; Li et al., 2022; Cui et al., 2024). These characteristics underscore the intricate role of auxin in plant development and its adaptability to environmental cues.

Auxin possesses the capability to regulate its distribution within plant tissues, creating concentration gradients that guide developmental patterns through a self-organization mechanism. This self-organizing property ensures that auxin can independently establish localized areas of influence, directing growth and differentiation processes accordingly (Leyser, 2005). In this process, auxin autonomously organizes the establishment of polarized auxin-transporting channels, providing positional cues for the subsequent development of intricate vasculature during organogenesis, leaf venation, shoot branching, and vascular regeneration (Hajný et al., 2022). Through this "auxin canalization" mechanism, auxin promotes the formation of self-organizing channels that originate from a broad field of auxin-transporting cells and gradually refine into well-defined channels with enhanced auxin transport capacity (Hajný et al., 2022). These channels emerge from an auxin source and extend toward an auxin sink, with individual cells undergoing coordinated polarization by integrating signals from the auxin source, sink, tissue context, and the status of surrounding cells (Bennett et al., 2014; Hajný et al., 2022).

Auxin operates its versatile functionality across a spectrum of concentrations, triggering distinct developmental outcomes. At lower concentrations, auxin predominantly promotes cell elongation, enhancing the growth of certain plant tissues. Conversely, higher levels of auxin can inhibit cell elongation and stimulate cell division, underpinning the formation of new tissues and organs (Bhalerao and Bennett, 2003). Auxin also regulates plant development in a tissue-specific manner. Different plant tissues exhibit varied sensitivities to auxin, which allows for the differential growth and developmental responses that are essential for plant architecture and adaptability. Typically, roots and shoots respond differently to auxin due to their distinct sensitivities and roles in plants. Exogenous auxin at concentrations as high as 10 μM promotes the elongation of the hypocotyl in Arabidopsis in light conditions (Adamowski et al., 2019), whereas auxin at concentrations as low as 5 nM inhibits root growth (Fendrych et al., 2018). After gravity stimulation, auxin accumulation at the lower side promotes cell growth in the stem while inhibiting growth in the root, leading to different responses to gravity in stems

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and roots (Rakusová et al., 2016; Pařízková et al., 2017). The time taken for bending to occur in response to gravity stimulus also varies between roots and stems. The hypocotyl of Arabidopsis begins to bend within 1 h–2 h (Rakusová et al., 2011), whereas roots start bending significantly within about 10 min (Bailly et al., 2008; Taniguchi et al., 2017). The differences in response time and concentration suggest distinct mechanisms underlying auxin-induced cell growth in stems and roots.

Auxin also triggers both rapid and slow cellular responses, depending on the developmental or environmental context. Rapid responses typically occur within seconds to minutes, often in less than 1 min, including cell membrane depolarization, hydrogen ion flux, calcium ion oscillations, protoplast swelling, remodeling of the cytoskeleton, regulation of endocytic trafficking of PIN-FORMED (PIN) auxin transporters, etc. (Bates and Goldsmith, 1983; Monshausen et al., 2011; Barbez et al., 2017; Narasimhan et al., 2021; Serre et al., 2021; Fiedler and Friml, 2023; Zhou et al., 2024). Slow cellular responses unfold gradually over a period of hours to days, characterized by sustained changes in gene expression and protein synthesis. These changes influence cell growth, division, and differentiation, ultimately leading to associated developmental processes (Reinhardt et al., 2003; Heisler et al., 2005; Dubrovsky et al., 2008). This feature allows plants to quickly adjust to changing conditions while also supporting longer-term developmental strategies.

These properties together facilitate a complex and dynamic regulatory system in which auxin serves as a master regulator, swiftly and specifically guiding plant development and environmental response.

Extracellular auxin signaling pathways mediated by ABP1/ABLs-TMKs

The functions of TMK in auxin signaling

The TRANSMEMBRANE KINASE (TMK), identified as one of the first leucine-rich repeat receptor-like kinases (RLKs) in Arabidopsis three decades ago, is considered a key player in cell surface-based auxin signaling (Chang et al., 1992; Xu et al., 2014; Cao et al., 2019; Yu et al., 2023a). Comprising four distinct but functionally overlapping members in Arabidopsis, the TMK subfamily of RLKs is instrumental in plant development (Dai et al., 2013). Mutants lacking both TMK1 and TMK4, as well as higher-order *tmk* mutants, display a range of altered auxin-related phenotypes throughout their life cycle (Xu et al., 2014; Huang et al., 2019). Prior research has established that the kinase domain of activated TMKs can phosphorylate substrates like plasma membrane (PM)-localized AUTOINHIBITED H^+-ATPASE (AHA), mitogen-activated protein kinase kinase 4/5 (MKK4/5), tryptophan aminotransferase of Arabidopsis 1 (TAA1), and abscisic acid insensitive 1 and 2 (ABI1/2), leading to rapid auxin responses and influencing various developmental stages (Huang et al., 2019; Wang et al., 2020; Li et al., 2021; Lin et al., 2021; Yang et al., 2021; Zhang et al., 2023) (Fig. 1). However, how the TMK proteins perceive auxin is unclear, and has only recently been elucidated.

It has been proposed that the AUXIN BINDING PROTEIN1 (ABP1) binds auxin and interacts with extracellular domain of TMK1 in an auxin-dependent manner, initiating Rho of plant (ROP) GTPases ROP2/6 signaling that affects cytoskeletal organization and leaf cell morphology in mere seconds (Xu et al., 2010, 2014). Recent findings

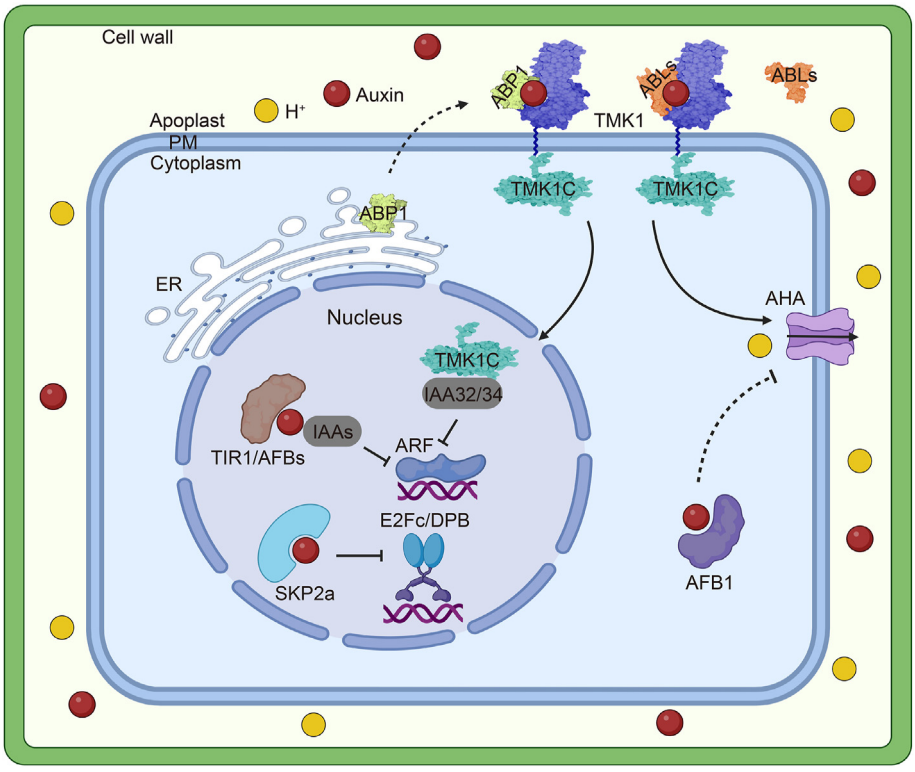

Fig. 1. The extracellular and intracellular auxin signaling pathways in plants. The apoplastic-localized ABLs and ABP1 secreted from the endoplasmic reticulum physically interact with the receptor-like kinase TMK1, forming co-receptor complexes at the plasma membrane to perceive extracellular auxin. The ABP1/ABLs–TMKs complex activates H^+-ATPases to induce apoplast acidification through direct phosphorylation. High auxin also promotes the cleavage of the TMK1 C-terminus domain, which enters the nucleus, phosphorylates, and stabilizes IAA32/34 to control ARF-mediated gene expression. The cytosolic auxin receptor AFB1 has been shown to regulate apoplast alkalinization, but the detailed mechanism has yet to be clarified. Meanwhile, auxin binds nucleus auxin receptors TIR1/AFBs to induce their interaction with Aux/IAA repressors, which mediates the degradation of Aux/IAA, freeing transcription factors ARF to regulate gene expression. Direct binding of auxin to nucleus SKP2a promotes its interaction with E2Fc and DPB and degrades them to facilitate the auxin-induced expression of cell cycle-related genes. PM, plasma membrane; ER, endoplasmic reticulum.

indicate the ABP1-TMK1 complex is essential for quick phospho-responses, cytoplasmic streaming, and the development of vascular structures after auxin treatment (Friml et al., 2022). Nevertheless, considering ABP1's low cell surface presence and primary endoplasmic reticulum (ER) localization (Henderson et al., 1997; Napier, 2021; Friml et al., 2022), coupled with the absence of discernible morphological changes in new *abp1*-null mutants (Gao et al., 2015), new evidence suggests ABP1 and structurally related, apoplastic localized ABP1-like proteins (ABL1 and ABL2) are co-receptors with TMKs to regulate rapid auxin responses and various plant developmental processes (Yu et al., 2023a).

The discovery of ABP1-like proteins

The auxin-binding protein ABP1 was first identified in the maize coleoptile membranes over 50 years ago (Hertel et al., 1972). Phylogenetic analyses indicate that ABP1 is an evolutionarily ancient and conserved protein present from algae to higher land plants (Woo et al., 2002; Tromas et al., 2010). ABP1 proteins from various species show similar auxin-binding affinities at physiological concentrations (Löbler and Klämbt, 1985; Kim et al., 1998; Shimomura et al., 1999; da Costa et al., 2017). In Arabidopsis, ABP1 exists as a unique gene with no close homologs based on sequence similarity and is expressed during all growth and developmental stages (Feng and Kim, 2015; Napier, 2021). Initial studies reported that the knockdown *abp1* mutants such as SS12S, SS12K, and *abp1-AS*, which were raised from immunization and RNAi against ABP1, exhibited several auxin-related phenotypes (Braun et al., 2008; Xu et al., 2010; Chen et al., 2014). Weak alleles, such as *abp1-5* which contains a point mutation in the auxin-binding site, led to the suppression of auxin responses (Robert et al., 2010; Xu et al., 2014; Dahlke et al., 2017; Yu et al., 2023a). This is controversial to the phenotype in *abp1*-null alleles, suggesting a dominant negative effect of ABP1-5 on other auxin-binding proteins that might functionally overlap with ABP1 by collaborating with TMKs as co-receptors for extracellular auxin.

Recent years have underscored a critical need to identify other ABPs or ABP1-like proteins that might function redundantly or in tandem with ABP1 as co-receptors in TMK-mediated auxin response at the cell surface, especially given the absence of notable phenotypes in *abp1*-null mutants (Napier, 2021; Yu et al., 2023a; Kuhn et al., 2024). Interestingly, structural analyses reveal that ABP1 is akin to ancient germin or germin-like proteins (GLPs), all members of the functionally diverse but sequence-wise divergent cupin superfamily (Woo et al., 2002). Studies have shown that certain *Prunus persica* GLPs, specifically ABP19 and ABP20, along with Arabidopsis GLP4, can bind auxin, although the biological relevance of this binding is yet to be elucidated (Ohmiya et al., 1998; Yin et al., 2009). Given that GLP4 is predominantly localized in the Golgi apparatus, it is unlikely to act as a co-receptor in the same manner as ABP1 for extracellular auxin signaling.

Research conducted in Arabidopsis has identified two additional GLPs, ABL1 and ABL2. Despite their low sequence similarity to ABP1, they contain a motif reminiscent of the A-box (auxin-binding box) found in ABP1 and are therefore designated as ABP1-like proteins. Comprehensive biochemical, cellular, and genetic analysis has shown that ABL1 and ABL2 overlap functionally with ABP1 and physically associate with TMKs to form cell surface auxin co-receptor complexes. These complexes are crucial for both rapid auxin responses and various developmental processes (Yu et al., 2023a). The discovery of ABL proteins marks a key advancement in the study of plant auxin signaling through cell surface co-receptors. As members of the large GLP family, which includes over 30 proteins with the conserved auxin-binding box-like motif, the potential for further interactions between these GLPs, TMKs, and auxin presents a promising avenue for uncovering unknown functions of GLP or ABL proteins in plant development (Murphy and Jones, 2023; Tena, 2023; Kuhn and Weijers, 2024; Sheen, 2024).

Intracellular auxin signaling pathways

TIR1/AFBs-based auxin signaling

The TRANSPORT INHIBITOR RESPONSE 1 (TIR1)/AUXIN SIGNALING F-BOX (AFBs)-mediated transcriptional regulation is known as the classical auxin signaling pathway, is pivotal for auxin responses (Kubeš and Napier, 2019) (Fig. 1). Auxin synthesized within or imported into the cell enhances the interaction between auxin/indole-3-acetic acid proteins (Aux/IAAs) and the TIR1/AFBs receptor family (comprising TIR1 and AFB1-5), triggering the ubiquitination and subsequent degradation of Aux/IAA transcriptional repressors (Dharmasiri et al., 2005; Kepinski and Leyser, 2005; Tan et al., 2007). The classical Aux/IAA proteins inhibit the auxin-responsive factors (ARFs), thereby modulating gene expression (Tan et al., 2007). The diversification and intricacy of regulatory roles of auxin in development are achieved by various Aux/IAA-ARF transcriptional regulatory combinations (Chapman and Estelle, 2009; Weijers and Wagner, 2016; Leyser, 2018; Jing et al., 2023).

Some members of the TIR1/AFBs family are also found in the cytoplasm, suggesting a role for these receptors in non-transcriptional auxin signaling. Among these members, AFB1 plays a primary role in rapid root growth inhibition within seconds via a non-transcriptional auxin signaling pathway (Dubey et al., 2023). The N-terminal region of AFB1 is not only essential and sufficient for its specific role in the rapid response but also indispensable for auxin-triggered calcium influx, which is a prerequisite for rapid root growth inhibition (Dubey et al., 2023). TIR1/AFBs receptors are also hypothesized to possess adenylate cyclase and guanylate cyclase motifs, producing cAMP and cGMP in vitro, which in turn modulate intracellular cAMP and cGMP levels and facilitate rapid auxin responses like Ca^{2+} signaling and root growth inhibition (Qi et al., 2022, 2023). Therefore, TIR1/AFBs also mediate the non-transcriptional auxin signaling pathway, which may be involved in regulating some rapid response to auxin.

Aux/IAAs, crucial to the classical nuclear auxin pathway, are upregulated by auxin and act as immediate early response genes. The 29-member Aux/IAA protein family is mostly characterized by four conserved domains (I–IV), with six IAAs (IAA20, IAA30, IAA31, IAA32, IAA33, and IAA34) lacking domain II. Domain II mediates ubiquitination and subsequent TIR1-triggered degradation of Aux/IAAs (Abel et al., 1994). Hence, its absence in atypical Aux/IAAs prevents this regulatory degradation. In contrast to auxin-dependent degradation of canonical Aux/IAA proteins, auxin stabilizes these atypical Aux/IAAs by phosphorylation through protein kinase. For instance, IAA32 and IAA34 (IAA32/34) can be phosphorylated by the cleaved TMK1 by DA1 peptidases under high concentrations of auxin, enhancing their stability and modulating ARF-regulated gene transcription (Cao et al., 2019; Gu et al., 2022). Another atypical Aux/IAA IAA33 can also be stabilized by auxin via mitogen-activated protein kinase 14 (MPK14), negatively regulates auxin signaling by interacting with ARF10 and ARF16 and protects them from IAA5-mediated inhibition (Lv et al., 2020). Furthermore, atypical ARFs like ETT/ARF3, which lack the PB1 domain for Aux/IAA interaction, suggest the existence of Aux/IAA-independent auxin signaling pathways (Guilfoyle, 2015). ETT/ARF3 can bind IAA directly to activate downstream gene expression (Kuhn et al., 2020). Transcriptomic analyses of wild-type and *ett-3* mutants imply that ETT/ARF3 modulates auxin signaling even without exogenous auxin application (Simonini et al., 2017). ETT/ARF3 directly binds to AuxREs in gene promoters, regulating their transcription (Franco-

Zorrilla et al., 2014; Simonini et al., 2016). ETT/ARF3 has been demonstrated to orchestrate gene expression in response to auxin, recruiting topless (TPL) and histone deacetylase 19 (HDA19) at low auxin levels to condense chromatin and repress transcription, with higher auxin levels leading to dissociation of this repressive complex and gene activation (Gallei et al., 2020; Yu et al., 2022). The branches of both atypical Aux/IAAs and ARFs suggest multiple levels of regulation in auxin responses, contributing to the complex functions of auxin in plant development and environmental adaptations. However, many mechanisms related to these atypical members remain unknown, including both upstream regulatory mechanisms and downstream functional outputs.

Other auxin signaling pathways

SKP is a key component of the SCF E3 ubiquitin ligase complex (SKP, Cullin, F-box containing complex) in mammals. It influences the stability and function of important cell cycle proteins, such as p27, cyclin E, and E2F1 (Marti et al., 1999; Tsvetkov et al., 1999; Nakayama et al., 2000; Tedesco et al., 2002; Kamura et al., 2003; Li et al., 2003). In plants, two SKP homologs, SKP2A and SKP2B, have been characterized. SKP2A, a cell cycle-regulated receptor, binds auxin to facilitate the degradation of the cell cycle-related protein DPB (del Pozo et al., 2002; del Pozo et al., 2006; Jurado et al., 2008). Despite sharing a high degree of similarity with SKP2A, SKP2B does not interact with auxin. Instead, it targets the CDK inhibitor kip-related protein1 (KRP1) for degradation, underscoring the functional specificity between the two homologs (Ren et al., 2008; Jurado et al., 2010). Auxin signaling through SKP2A directly links to cell cycle control (Fig. 1), complementing other known auxin-mediated pathways and highlighting the hormone's influence on cell division and growth.

The plant mitogen-activated protein kinase (MAPK/MPK) cascades are integral signaling mechanisms, highly conserved and implicated in a multitude of growth and developmental processes (Tena et al., 2011; Xu and Zhang, 2015). These cascades are generally composed of a sequence of three protein kinases from MAPKKK and MAPKK to MAPK. There is evidence of crosstalk between MAPK signaling and auxin signaling, with exogenous auxin eliciting activation of various MAP kinases, including MPK1, MPK2, MPK3, MPK6, and MPK14, suggesting the involvement of MAPK cascades in auxin-mediated responses (Mockaitis and Howell, 2000; Huang et al., 2019; Lv et al., 2021). Nicotiana protein kinase 1 (NPK1), a plant MAPKKK, activates an unidentified MAPK cascade, whereas MEKK1 collaborates with downstream MPK4, modulating the expression of early auxin response genes in either inhibitory or promotive roles, illustrating the fine-tuning of auxin sensitivity through distinct MAPK pathways (Kovtun et al., 1998; Nakagami et al., 2006). However, the mechanisms by which auxin influences NPK1 or MEKK1 activities remain elusive. Recent findings have implicated B4 rapidly accelerated fibrosarcoma (RAF)-like kinases of the MAPKKK family as being activated by auxin through the ABP1-TMK1 complex, linking rapid, ancient signaling to cellular auxin responses across various species (Kuhn et al., 2024). TMK1 and TMK4 are also required for the activation of MKK4/5-MPK3/6 cascades by auxin during lateral root development (Huang et al., 2019). The identification of downstream targets for these kinases remains to be explored.

Recent extensive studies have cast light on the vital connections between MPK3/MPK6 and auxin signaling pathways. Auxin has been found to stimulate MPK3/MPK6, which in turn phosphorylates and modulates the stability of proteins such as PIN1, IAA15, DPa, STOP1, and GRF4, thereby influencing lateral root development, root distal stem cell maintenance, and hypocotyl elongation (Jia et al., 2016; Kim et al., 2022; Wang et al., 2023; Yu et al., 2023b; Liu et al., 2024). Additionally, auxin can activate MPK14, which phosphorylates the non-canonical Aux/IAA protein IAA33 and ERF13, thereby affecting root distal stem cell fate and lateral root formation, respectively (Lv et al., 2020, 2021). Investigating whether the auxin-activated MPK3/MPK6 or MPK14 signaling pathways operate through the ABP1/ABLs-TMKs co-receptor complex could further elucidate the molecular intricacies of auxin responses in plant development and diversify our understanding of these signaling networks.

Interplay of extracellular and intracellular auxin signaling pathways

The distinct auxin perception and signaling pathways are coordinated in response to auxin to target the same signaling components and play overlapping, antagonizing, or specific roles through the intracellular TIR1/AFBs receptors and the extracellular ABP1/ABLs-TMKs co-receptors, which are important to control diverse auxin associated processes in plant development, including acid growth, dosage effects, slow and fast responses, and canalization.

Acid growth in shoots and roots

The acid growth theory, proposed in 1971, illuminated how auxin modulates cell elongation through cell wall acidification (Hager et al., 1971). Despite its initial introduction, the intricate molecular mechanisms underlying auxin-induced wall acidification remained elusive for a long time. Subsequently, it was discovered that this acidification correlates with the regulation of the PM H^+-ATPase (AHA) pump. Auxin facilitates the phosphorylation at the C-terminus of AHA, relieving self-inhibition and leading to enhanced proton efflux and acidification (Takahashi et al., 2012). It has been found that the phosphatase PP2C.D dephosphorylates this region, maintaining the inhibition of AHA. Auxin upregulates SAUR19 through the primary TIR1/AFBs signaling pathway, counteracting the effect of PP2C.D, thereby promoting AHA activity (Spartz et al., 2014). The trigger for auxin-induced AHA phosphorylation was also dependent on the TMK-AHA interaction, which occurs rapidly within 30 s of auxin application, indicative of a non-transcriptional response (Lin et al., 2021). Auxin induces TMK1/4-dependent phosphorylation and activation of AHA, triggering cell expansion (Lin et al., 2021; Li et al., 2021) (Fig. 2). These discoveries have enriched our understanding of the acid growth framework. Interestingly, recent studies have shown that although apoplastic acidification typically promotes growth, elongation is inhibited when the pH falls below a certain threshold. This effect of auxin over-dosage can be mitigated by deactivating AHA activity. Auxin and light together modulate the apoplastic pH suitable for hypocotyl growth by antagonistically regulating the SAUR-PP2C.D-AHA pathway (Wei et al., 2023).

Although auxin promotes acidification and growth in hypocotyls, it induces alkalization and growth inhibition in roots, even at low concentrations, suggesting the contrasting roles of auxin in different tissues (Fendrych et al., 2016; Barbez et al., 2017; Fendrych et al., 2018; Li et al., 2021; Serre et al., 2021). In roots, auxin promotes H^+ influx and subsequent apoplast alkalization via the cytoplasmic AFB1 receptor. However, the mechanism by which auxin activates the TMK1-AHA pathway for H^+ efflux, counteracting the alkalization induced by TIR1/AFBs, remains to be elucidated (Li et al., 2021). Notably, in auxin-mediated root growth inhibition, the alkalization pathway predominates (Barbez et al., 2017; Li et al., 2021) (Fig. 2). The acid growth theory also sheds light on gravitropic responses; a horizontal orientation leads to asymmetric auxin distribution, lower pH, and accelerated growth on the lower side of stems, resulting in upward curvature. In roots, the same auxin gradient inhibits growth on the lower side, causing downward growth. This theory coherently

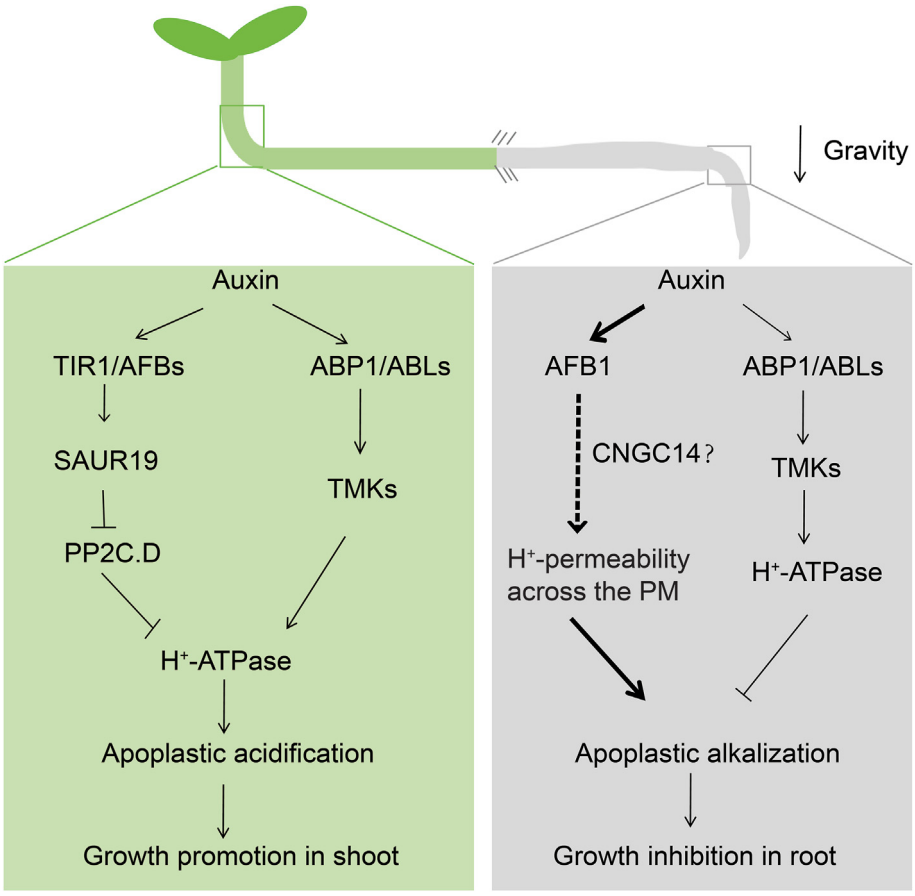

Fig. 2. Coordination of auxin signaling pathways in acid growth. In the shoot, auxin promotes transcription of SAUR through the TIR1/AFBs pathway, thereby inhibiting PP2C.D and promoting H^+-ATPase activation to increase apoplastic acidification. Simultaneously, auxin activates the proton pump through the ABP1/ABLs-TMKs pathway to further enhance acidification and promote stem growth or growth of lower-layer cells under gravity. In the root, the mechanism of rapid phosphorylation activation of the proton pump by ABP1/ABLs-TMKs still exists, but the predominant role is played by massive influx of H^+ mediated by cytoplasmic AFB1, ultimately leading to apoplastic alkalinization, inhibiting root growth or growth of lower-layer cells under gravity. The auxin-induced acid growth mechanism mediated by both TIR1/AFBs and ABP1/ABLs-TMKs leads to the tissue specificity of plant responses to auxin.

explains the differential tissue sensitivity to auxin in plant development (Li et al., 2021, 2022) (Fig. 2).

Dosage effects of auxin responses in apical hook development

In both animals and plants, the cellular concentrations of signaling molecules play a pivotal role in which varied activities at different concentrations contribute to the functional versatility of these molecules. In plants, auxin is often characterized by its morphogen-like behavior, forming gradients within tissues and acting in a concentration-dependent manner to influence development, although the specifics of how these gradients translate to diverse developmental processes are not fully elucidated (Teale et al., 2006).

In plant morphogenesis, the concentration of auxin plays a crucial role. For example, in the root meristem, high levels of auxin promote cell division, whereas lower concentrations in the elongation zone encourage cell elongation (Perrot-Rechenmann, 2010). Auxin gradients are essential for forming the apical hook, a structure enabling seedlings to break through the soil surface. High levels of auxin on the inner side of the hook initiate the cleavage of TMK1's C-terminal kinase domain, leading to its relocation to the nucleus and subsequent augmentation of the stability of transcriptional inhibitors, IAA32/34. Conversely, on the outer side of the hook, low auxin levels are insufficient to activate the TMK1-IAA32/34 pathway (Cao et al., 2019), thus promoting cell growth, particularly in sustaining the apical hook structure (Fig. 3). During this process, the low concentration of auxin in the outer cells may promote apoplastic acidification through the activation of AHA activity via TMKs. Concurrently, TIR1/AFBs may regulate AHA transcription or activity at multiple levels to contribute to apoplastic acidification. TIR1/AFBs not only increase AHA transcription levels but also relieve the inhibition of AHA activity by PP2C.D through SAURs (SAUR9/19/40/72). Additionally, TIR1/AFBs ensure AHA activity to promote apoplastic acidification by reducing SYP132 levels (Xia et al., 2019). On the other hand, TIR1/AFBs may induce the expression of cell wall remodeling genes and turgor pressure-related genes to promote cell expansion (McQueen-Mason et al., 1992; Philippar et al., 2004; Monshausen et al., 2009; Hocq et al., 2017; Yu et al., 2022) (Fig. 3).

TIR1/AFBs receptors and the non-canonical ARF transcription factor ETT/ARF3 respond positively to varying auxin concentrations, modulating downstream gene expression in accordance (Bargmann et al., 2013; Kuhn et al., 2020). These components are thought to discern different auxin levels, translating them into proportional signaling responses. TMKs, in contrast, may facilitate the promotion of growth at low concentrations of auxin through rapid phosphorylation of membrane-associated substrates. When auxin levels are high, the cleavage and subsequent nuclear activities of TMK1 exert a restrictive influence on growth, curbing excessive auxin signaling during the critical phase of apical hook maintenance (Cao et al., 2019).

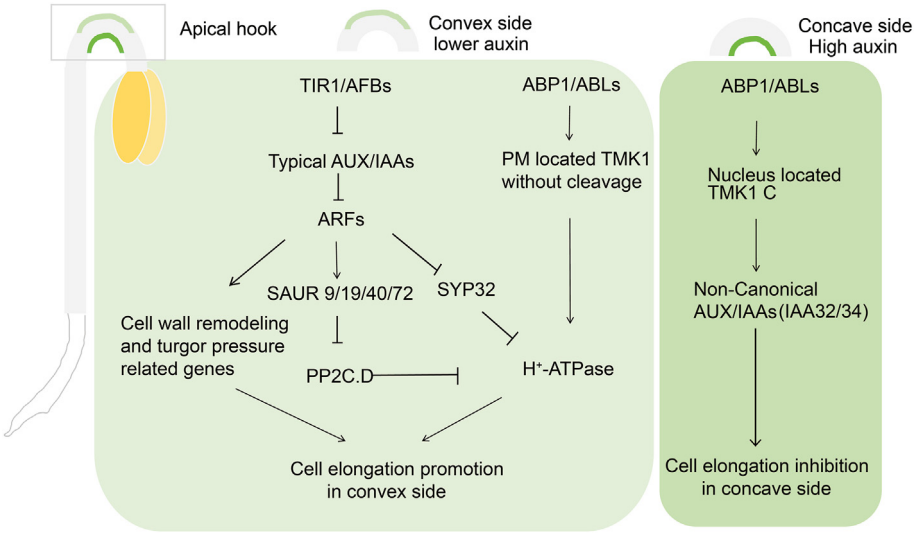

Fig. 3. Apical hook formation based on the dosage effects of auxin responses. The maintenance of apical hook curvature is crucially dependent on the concentration effect of auxin. On convex side of the hook, auxin promotes the transcription of cell wall remodeling and turgor-related genes through TIR1/AFBs, induces SAUR9/19/40/72 transcription to relieve PP2C.D inhibition of proton pump, and inhibits transcription of SYP32 to activate the proton pump; on the other hand, it may promote convex side cell elongation by phosphorylating important proteins such as H^+-ATPase on the membrane through ABP1/ABLs-TMK1. On the concave side of the hook, high concentrations of auxin primarily inhibit cell elongation by stabilizing the non-canonical Aux/IAA IAA32/34 through TMK1 cleavage into the nucleus.

Fast and slow auxin responses

The cellular responses triggered by auxin are divided into two types: fast and slow (Fiedler and Friml, 2023; Zhou et al., 2024). The transcriptional-dependent slow auxin responses are mediated by both the canonical TIR1/AFBs-Aux/IAAs-ARFs and the non-canonical TMK1-IAA32/34-ARFs auxin signaling pathways. The transcriptional-independent rapid auxin responses often occur in seconds. A key rapid response to auxin is the depolarization of the plasma membrane within seconds after the application of auxin to various plant tissues from studies conducted during the late 1970s (Cleland et al., 1977; Göring et al., 1979). More recently, plasma membrane depolarization, coinciding with cytosolic CNGC14-dependent Ca^{2+} transients and apoplast alkalinization, is crucial for the auxin-induced rapid inhibition of root growth. These changes are too fast to be transcriptionally regulated. Mutations in TIR1/AFBs disrupt these responses, indicating a fast non-transcriptional signaling branch via the TIR1/AFBs receptors. Cytoplasmic AFB1, in particular, seems integral to initiating these rapid auxin effects, although the precise mechanisms were not well understood (Chen et al., 2023; Dubey et al., 2023). Recent studies suggest that auxin-induced cGMP production, facilitated by TIR1/AFBs with guanylate cyclase activity, is key to these non-transcriptional reactions (Qi et al., 2023). The auxin-induced plasma membrane depolarization, calcium signaling, and rapid root growth inhibition do not seem to depend on ABP1 (Shih et al., 2015; Paponov et al., 2018), as shown by intact responses in *abp1*-null lines; the potential role of ABLs in this process alongside ABP1 remains to be clarified in the near future.

Further studies have illuminated additional rapid, non-transcriptional effects of auxin, including ROP GTPases activation, protein phosphorylation, cell wall acidification, protoplast swelling, and increased cytoplasmic streaming (Xu et al., 2010; Dahlke et al., 2017; Friml et al., 2022; Kuhn et al., 2024) (Fig. 4). These processes are stimulated by cell surface ABP1- or ABLs-TMKs signaling, yet the specific contributions of the novel non-transcriptional TIR1/AFBs signaling branch are still being explored. The latest studies unveiled that in the presence of auxin, the extracellular domain of TMKs engages with ABP1 and ABLs in the apoplast, acting as an extracellular auxin signal essential for the rapid activation of plant-specific ROP GTPase and ultrafast global protein phosphorylation (Friml et al., 2022; Yu et al., 2023a). Auxin induces a swift global phosphorylation response, affecting over 1700 proteins within 30 s through the extracellular ABP1/ABLs-TMKs signaling pathway and independently of the nuclear TIR1/AFBs pathway (Yu et al., 2023a; Kuhn et al., 2024). Besides phosphorylating plasma membrane H^+-ATPases target for acid growth, auxin-induced rapid phosphorylation of myosin XI and myosin-binding proteins in the regulation of cytoplasmic streaming and post-endocytic trafficking are mediated by the ABP1-TMK1, further supporting their importance in auxin signaling (Friml et al., 2022). A recent notable study reported that the auxin phos-response is ancient and conserved, stretching from land plants to at least basal *Streptophyte algae*. The B4 RAF-like protein kinases, standing out for their remarkable conservation across various plant species, link this rapid phosphorylation event to broader cellular auxin responses, such as accelerated cytoplasmic streaming (Kuhn et al., 2024). How the ultrafast auxin phos-response mediated by the ABP1-TMK1 complex at the cell surface interacts with the TIR1/AFBs-mediated pathway and contributes to plant growth, development, and environmental adaptation will be an interesting direction for future research.

Although both ABP1/ABLs-TMKs and TIR1/AFBs mediate rapid auxin responses, analysis of the evolutionary history of auxin response systems suggests that the sister group closest to land plants, *Streptophyta algae*, does not carry nuclear auxin pathway mediated by TIR1/AFBs and in some cases even lacks all of its components. It is thus proposed that the rapid phosphorylation events mediated by ABP1/ABLs-TMKs originated earlier than the nuclear auxin response pathway mediated by TIR1/AFBs (Mutte et al., 2018). Since the rapid system is conserved in algal species that lack genes used by land plants for auxin synthesis, this suggests that the primary role of the rapid system may be to sense and respond to exogenous auxin, whereas the nuclear system mainly mediates responses to endogenous auxin. Many organisms, including bacteria and fungi, produce auxin and secrete it into the surrounding environment. Given the close interactions between plants and microbial communities, it is speculated that the rapid auxin response system may mediate the ecological roles of exogenous auxin (Kuhn et al., 2024).

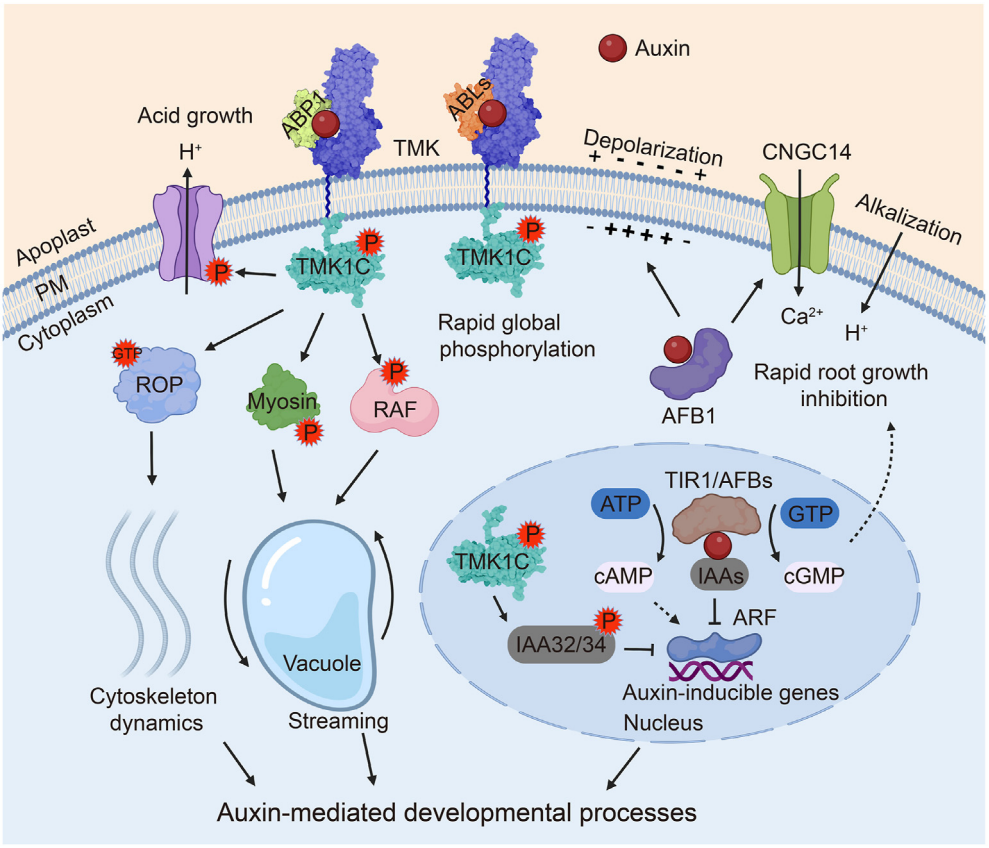

Fig. 4. The rapid and slow auxin signaling and response in plants. The rapid auxin responses are mainly regulated by the ABP1/ABLs-TMKs co-receptor complex at the cell surface and RAF-like kinases in the cytosol. The ABP1/ABLs-TMKs complex triggers auxin-induced rapid global phosphorylation to regulate several fast responses, such as apoplast acidification, the activation of ROP, and cytoplasmic streaming. The cytosolic receptor AFB1 activates rapid plasma membrane depolarization, CNGC14-mediated Ca^{2+} increase, and apoplast alkalization, together with auxin-induced cGMP produced by TIR1/AFBs to control rapid root growth inhibition. These rapid non-transcriptional responses and slow transcriptional reactions involving gene expression and protein synthesis work further together to control several auxin-mediated developmental processes in plants.

Auxin canalization and self-organization

The "auxin canalization" mechanism facilitates auxin self-organization in various processes, including vein formation, stem branching growth, and vascular regeneration after injury. The hypothesis of "auxin canalization" was first proposed by Sachs (1968, 1981), suggesting that auxin plays a polarization role through directed flow between cells and feedback between auxin signaling and transport. Research has shown that the TIR1/AFBs-mediated intracellular auxin perception pathway plays a key role in auxin canalization. Auxin induces PIN transcription in a tissue-specific manner (Vieten et al., 2005). Mutants related to this pathway display defects in auxin-induced changes in PIN polarity in roots and in vascular formation driven by canalization, suggesting that this pathway influences both transcription and activity of factors regulating PIN polarity and auxin canalization (Sauer et al., 2006; Prát et al., 2018; Mazur et al., 2020). Recent studies have shown that TIR1/AFBs mediate auxin canalization by indirectly influencing the phosphorylation of PIN proteins. Initially, auxin enhances the expression of the transcription factor WRKY23 via the TIR1/AFBs signaling pathway. WRKY23 then binds directly to the promoter of the auxin-regulating receptor CAMEL (Canalization-related, auxin-regulated male hormone-type RLK), stimulating its transcription. CAMEL subsequently forms a complex with CANAR on the plasma membrane, which affects the polarity of PIN proteins through direct phosphorylation, thereby playing a crucial role in auxin canalization (Prát et al., 2018; Hajný et al., 2020) (Fig. 5).

Recent studies have reported the pivotal role of the cell surface auxin signaling mediated by ABP1-TMKs in auxin canalization. Utilizing PIN1-GFP and DR5 markers, along with Toluidine blue O staining, it was observed that mutant alleles *abp1-c1* and *abp1-TD1* were unable to form auxin canalization channels, whereas their corresponding complementation lines (*comp-c1* and *comp-TD1*) successfully rescued these defects. During vascular regeneration, *tmk4-1* exhibited the strongest defect in channel formation, followed by *tmk3-1* and *tmk1-1*. This indicates that ABP1-TMKs mediate auxin canalization during injury-induced vascular formation (Friml et al., 2022). Interestingly, ABP1 and TMKs are implicated in regulating PIN localization (Robert et al., 2010; Xu et al., 2010; Gelová et al., 2021; Yu et al., 2023a). Recent studies reported that TMKs directly interact with PIN1 and PIN2, respectively, that regulates PIN localization (Rodriguez et al., 2022; Wang et al., 2022), suggesting a direct self-organization mechanism between auxin signaling and auxin transport (Fig. 5).

Perspectives

The field of auxin research is on the brink of a paradigm shift, driven by emerging evidence that is reshaping our understanding of how this essential plant hormone orchestrates development and environmental responsiveness. The discovery of rapid, non-transcriptional signaling mechanisms complemented the long-standing view that auxin primarily exerts its effects through the modulation of gene expression. These recent insights into both

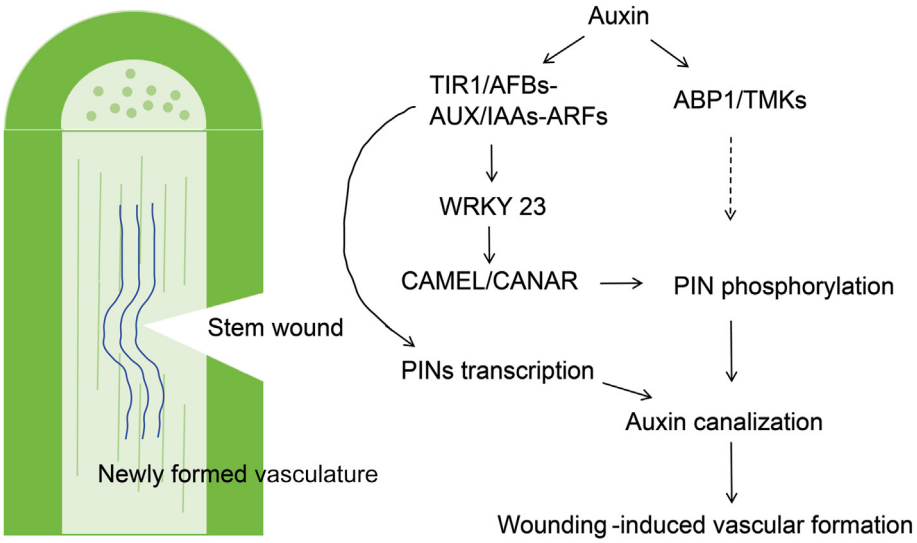

Fig. 5. Interplay between TIR1/AFBs and ABP1-TMKs in auxin self-organization. The formation of auxin canalization induced by plant stem wounding relies on the coordinated action of TIR1/AFBs and ABP1-TMKs, which regulate the phosphorylation of PIN proteins to alter auxin transport direction. TIR1/AFBs promote the transcription of WRKY23, leading to increased phosphorylation of the CAMEL/CANAR complex on PIN proteins, whereas ABP1-TMKs may directly phosphorylate PIN proteins. Additionally, TIR1/AFBs also directly regulate the transcription of PIN proteins to facilitate auxin canalization.

extracellular and intracellular pathways of auxin signaling, which are rapid and extend beyond transcriptional control, depict auxin as a far more versatile and dynamic hormone than previously recognized.

Despite this, many questions about auxin perception, signal transduction, and biological functions are yet to be explored. How do ABP1/ABLs activate TMKs after binding auxin? Because ABLs belong to a large family, do other ABLs interact with TMKs to mediate extracellular auxin signal perception? What are the biological functions of TMKs phosphorylating different substrates in the ancient species? What is the evolutional drive of the formation of TIR1/AFBs-based signaling pathway? How does it collaborate with ABP1/ABLs-TMK at multiple levels in different developmental stages? Answers to these questions will help us further understand how the complex auxin signaling network is integrated to relay and control diverse biological processes in plants. Moreover, the potential conservation of auxin signaling mechanisms from ancient algae to modern angiosperms hints at a universal regulatory mechanism, an evolutionary thread that might offer clues to the success and adaptability of the plant kingdom. It is ready to harness the full spectrum of auxin's roles in plant life to answer basic scientific questions.

Conflict of interest

The authors declare no competing interests.

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