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Pho-view of Auxin: Reversible Protein Phosphorylation in Auxin Biosynthesis, Transport and Signaling

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ABSTRACT

The phytohormone auxin plays a central role in shaping plant growth and development. With decades of genetic and biochemical studies, numerous core molecular components and their networks, underlying auxin biosynthesis, transport, and signaling, have been identified. Notably, protein phosphorylation, catalyzed by kinases and oppositely hydrolyzed by phosphatases, has been emerging to be a crucial type of post-translational modification, regulating physiological and developmental auxin output at all levels. In this review, we comprehensively discuss earlier and recent advances in our understanding of genetics, biochemistry, and cell biology of the kinases and phosphatases participating in auxin action. We provide insights into the mechanisms by which reversible protein phosphorylation defines developmental auxin responses, discuss current challenges, and provide our perspectives on future directions involving the integration of the control of protein phosphorylation into the molecular auxin network.

Key words: *Arabidopsis*, auxin, protein kinase, phosphatase, phosphorylation

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INTRODUCTION

Live organisms represent a fluctuating continuum of highly ordered matter, energy, and information. Compared with animals, plants exhibit an even higher plasticity during postembryonic development, likely reflecting an adaptation to their sessile life style. Developmental plasticity is ensured by complex molecular frameworks, among which signals triggered by the phytohormone auxin (indole-3-acetic acid [IAA]) play fundamental roles in various essential activities of living plants (Figure 1). This is exemplified by the key role auxin plays in shaping plant organogenesis, tropic responses, and plant morphogenesis in general (Vanneste and Friml, 2009). To date, core molecular components have been characterized for auxin biosynthesis (Zhao, 2014), directional transport (Adamowski and Friml, 2015), and signaling frameworks (Salehin et al., 2015). These key molecular players include transcription factors, transporters, and enzymes, among which protein kinases and phosphatases, catalyzing reversible protein phosphorylation, are emerging to participate as key regulators of virtually all steps of auxin action (Figure 1).

Most proteins are post-translationally modified, either constitutively or conditionally in response to internal or external signaling cues. Among the various post-translational modifications, reversible protein phosphorylation plays essential roles in almost every aspect of cellular activity. Biochemically, reversible protein phosphorylation is catalyzed antagonistically by the phosphate "writer" kinase and the "eraser" phosphatase. Many kinases serve as molecular hubs in signal transduction, e.g., the largest plant family of kinases, receptor-like kinase (RLK), can perceive extracellular signaling molecules (ligands), and further amplify and transduce this signal to downstream substrates by phosphorylation (Gish and Clark, 2011). In the model plant *Arabidopsis thaliana* there are over 1000 genes encoding protein kinases, and 112 genes encoding catalytic subunits of phosphatases (Wang et al., 2007). Experimentally, 642 (±55) kinases and 119 (±6) phosphatases can be detected by mass spectrometry across tissues (Mergner et al., 2020). In the *Arabidopsis* proteome, 47% of the expressed proteins are phosphorylated in at least one instance, as revealed by recent data (Mergner et al., 2020).

Multiple kinases and phosphatases have been identified to be critical components essential for generating and transmitting

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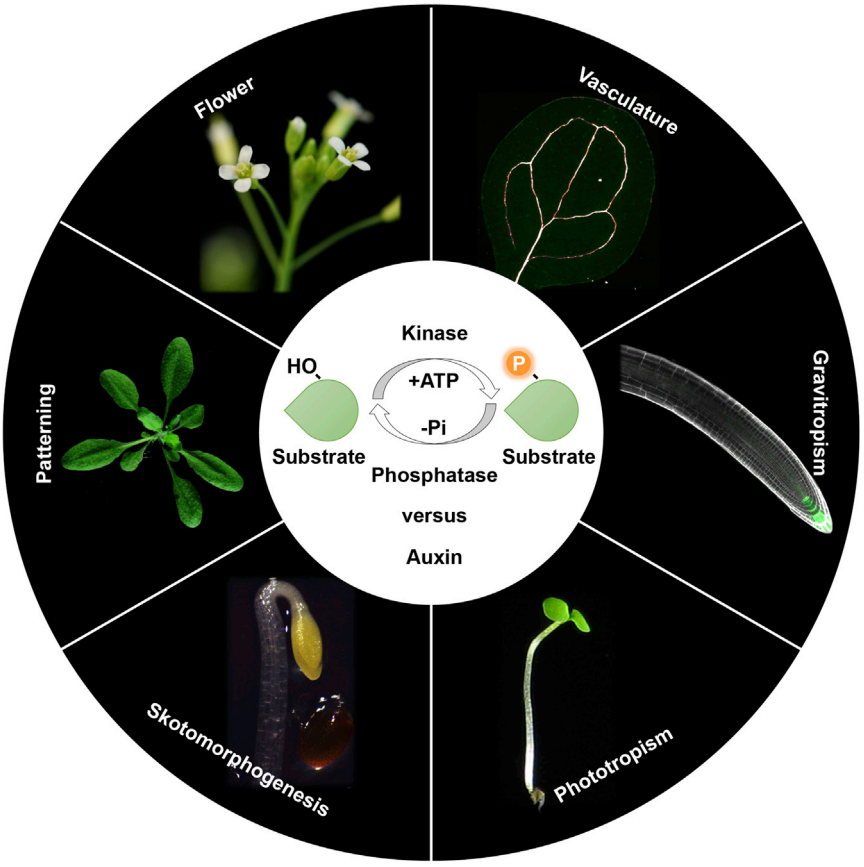

Figure 1. Auxin and Reversible Protein Phosphorylation Are Involved in Various Processes in Plants.
Auxin participates in various developmental patterning processes as well as distinct tropic responses, for which reversible protein phosphorylation serves as a common regulatory mechanism.

the auxin signal. This type of signaling components transduces specific upstream signals, which selectively activate certain kinases or phosphatases and, by specific enzyme–substrate interactions, transduce the signal to downstream components. Notably, those studies not only include a functional characterization of these enzymes, but also, most importantly, identify pairwise enzyme–substrate interactions (Table 1), illustrating the molecular frameworks underlying the action of auxin.

Here, we review studies characterizing the phosphorylation-based regulation of auxin biosynthesis, transport, or signaling, although it is likely that numerous additional phosphorylation events influence the auxin pathway in a less direct manner. We discuss past and recent advances and the remaining challenges, and offer our perspectives on protein phosphorylation in auxin research.

PROTEIN PHOSPHORYLATION IN AUXIN BIOSYNTHESIS

A major auxin biosynthesis pathway is the indole-3-pyruvic acid branch, catalyzing tryptophan-to-auxin conversion by two stepwise enzymes: TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS (TAA1)/TRYPTOPHAN AMINOTRANSFERASE-RELATED PROTEINs (TARs) and YUCCAs (YUCs), with 3 and 11 paralogs, respectively, in *Arabidopsis* (Zhao, 2014). To date, auxin biosynthesis has been reported extensively to be regulated by both intrinsic developmental signals and external environmental cues, with dynamic transcript levels for both *TAA1/TARs* and *YUCs* under control of distinct transcription factors (Brumos et al., 2018; Robert et al., 2018; Zhao, 2018). Intriguingly, TAA1 is phosphorylated *in planta*, at a conserved and essential phospho site threonine 101 (T101) across species, as detected by mass spectrometry (Wang et al., 2020).

Recently, it has been shown that this phosphorylation is mediated by a member of the RLK family. RLKs are the largest family of kinases in various plant species. Members of this protein family are characterized by an extracellular domain of variable length, a short single transmembrane domain, and an intracellular kinase domain, an archetypical structure that functions in the perception of various extracellular cues and translates them into distinct substrate phosphorylation events. Intriguingly, compared with other organismal groups, there is an obvious expansion of the RLK family in plant species, further highlighting the importance of this particular signal-transducing machinery in plants. Based on sequence analysis, there are more than 600 genes encoding RLKs and receptor-like cytoplasmic kinases in the genome of the model plant *A. thaliana* (Shiu and Bleecker, 2003) and more than 1000 in *Oryza sativa* (Shiu et al., 2004). Based on the predicted structure of the extracellular domain, *Arabidopsis* RLKs can be grouped into more than 21 subfamilies, among which leucine-rich repeat (LRR)-RLKs are the most prominent. There are approximately 220 LRR-RLKs in *Arabidopsis* (Shiu and Bleecker, 2003; Wu et al., 2016). Several of those have been reported to participate in the auxin action, with the TRANSMEMBRANE KINASE (TMK1 to TMK4) family functioning as key regulators impacting on auxin biosynthesis, transport, and signaling.

Indeed, TMK4 is the kinase responsible for T101 phosphorylation resulting in suppression of TAA1 enzymatic activity, and impacting on root meristem size and root hair development in *Arabidopsis* (Wang et al., 2020). Importantly, this TMK4-mediated phosphorylation was found to be auxin-responsive, representing a negative feedback loop that links auxin homeostasis and signaling in root patterning (Wang et al., 2020), but the nature of auxin perception for regulating TMK4 remains unclear. By analogy, when searching available proteomics databases, it turned out that members of the YUC family are subject to phosphorylation as well (Willems et al., 2019). The kinase responsible for such YUC phosphorylation and its mechanistic significance, however, remains to be characterized.

		Substrate	Enzyme	Upstream regulator	Reference
Auxin biosynthesis	TAA	TAA1	TMK4	auxin	Wang et al., 2020
	YUCs	YUCs	unknown	unknown	–
Auxin transport	PINs	PINs	PID/WAGs	PA, NPH3 MELs	Friml et al., 2004; Cheng et al., 2008; Dhonukshe et al., 2010; Huang et al., 2010; Zhang et al., 2010; Furutani et al., 2011, 2014; Grones et al., 2018
		PINs	D6PK/D6PKLs	PDK1	Zegzouti et al., 2006a, 2006b; Zourelidou et al., 2009, 2014; Weller et al., 2017; Tan et al., 2020a
		PINs	PAX	PDK1 BRX PIP5Ks	Marhava et al., 2018, 2020; Xiao and Offringa, 2020
		PINs	MPK6	TMK1?	Jia et al., 2016; Dory et al., 2018; Huang et al., 2019
		PINs	CRK5	Calcium	Rigó et al., 2013; Baba et al., 2018, 2019a, 2019b
		PINs	CAMEL	Auxin	Hajný et al., 2020
		PINs	PP2A	SA	Michniewicz et al., 2007; Tan et al., 2020b
		PINs	PP2A	PYR/PYLs, ABA	Li et al., 2020a, 2020b
		PINs	PP6	unknown	Dai et al., 2012
		PINs	PP1	unknown	Guo et al., 2015
	ABCBs	ABCB1	PID	unknown	Henrichs et al., 2012
		ABCB	unknown	unknown	–
	PIN LIKES	PILS	unknown	unknown	–
	WAT1		unknown	unknown	–
	AUX1/LAXs	AUX1/LAXs	unknown	unknown	–
TIR1 auxin signaling	TIR1/AFBs	TIR1/AFBs	unknown	unknown	–
	AUX/IAA	AUX/IAA	unknown	unknown	–
	ARFs	ARF2	BIN2	BR	Vert et al., 2008
		ARF2	unknown	K^+	Zhao et al., 2016
		ARF7	BIN2	TDIF	Cho et al., 2014
Non-canonical signaling	AUX/IAA	IAA32/34	TMK1	auxin	Xu et al., 2014; Cao et al., 2019
		IAA33	MPK14	unknown	Lv et al., 2020
	MPK	MKK4/5	TMK1	auxin	Huang et al., 2019

Table 1. Pairwise Enzyme–Substrate Characterization.

PROTEIN PHOSPHORYLATION IN AUXIN TRANSPORT

Intercellular auxin transport, which is essential for the establishment and maintenance of local auxin concentrations, is regulated by multiple transporters, including PIN-FORMED (PIN) (Adamowski and Friml, 2015) and ATP binding cassette (ABC) transporters (Zhu et al., 2016), as well as AUXIN1 (AUX1)/LIKE AUXIN1 (LAX) importers (Marchant et al., 1999). Besides, there are also intracellular auxin transporters, such as the endoplasmic reticulum-localized PIN-LIKES (PILS) (Barbez et al., 2012) and tonoplast-associated WALLS ARE THIN1 (WAT1) (Ranocha et al., 2013), presumably involved in the subcellular compartmentalization of auxin. PIN auxin efflux carriers are essential for maintaining local morphogenetic auxin concentrations and variations therein (hormonal minima and maxima as well as concentration gradients established between such extreme values), thereby regulating auxin-mediated organ patterning and tropisms (Adamowski and Friml, 2015). PINs are subject to multifaceted regulation, defining their activity in a spatiotemporal context. Notably, PIN activities are regulated, often post-translationally, by multiple hormonal pathways, including cytokinins (Marhavý et al., 2014), gibberellins (Löfke et al., 2013; Salanenka et al., 2018), jasmonic acid (Sun et al., 2011), salicylic acid (SA) (Du et al., 2013; Tan et al., 2020a), abscisic acid (ABA) (Li et al., 2020a), brassinosteroids (BRs) (Li et al., 2005; Retzer et al., 2019), strigolactones (Shinohara et al., 2013; Zhang et al., 2020a), and auxin itself (Paciorek et al., 2005; Pan et al., 2009; Robert et al., 2010; Baster et al., 2013; Platre et al., 2019), involved in various developmental processes. This suggests that PIN-dependent directional auxin transport serves as a major hub, integrating

multiple endogenous and environmental signals. Among these regulatory processes, protein phosphorylation appears central for controlling directionality and flux rate of PIN-mediated auxin transport, with multiple kinases and phosphatases implicated in the PIN phosphorylation status (Barbosa et al., 2018).

PID/WAGs-MELs as Regulators of Apical-Basal PIN Polarity

The AGC (named after the cAMP-dependent [PKA] and cGMP-dependent protein kinases [PKG], and protein kinase C [PKC]) protein kinase family plays critical roles in various life activities in different organisms (Pearce et al., 2010). Notably, the AGCVIII subclade is plant specific (Galván-Ampudia and Offringa, 2007; Zhang and McCormick, 2009; Garcia et al., 2012), and members of this subset of kinases represent essential regulators of auxin transport, several of which have been reported to be involved in the direct phosphorylation of PIN proteins (Figure 2). Originally, a loss-of-function mutant affecting AGCVIII kinase PINOID (PID) has been found to exhibit pin-like inflorescences, strikingly resembling *pin1* mutant phenotypes (Bennett et al., 1995; Christensen et al., 2000; Benjamins et al., 2001). Further genetic and biochemical analyses then revealed that PID and its close homologs, WAVY ROOT GROWTHs (WAGs), acts as a binary phosphoswitch directing the apico-basal polarity of PIN1, PIN2, and PIN4 proteins and thus the directionality of intercellular auxin transport in both shoots and roots (Friml et al., 2004; Dhonukshe et al., 2010; Huang et al., 2010; Zhang et al., 2010). The *pid wag1 wag2* triple mutant exhibits agravitropic root growth, most likely due to the observed defect in the PIN2 apical localization; however, the *35S::PID* overexpression line also shows defects in root gravitropism but, due to the basal-to-apical switch of PIN1 and PIN4 in stele and PIN2 in cortex, which depletes the auxin maxima in root tips (Friml et al., 2004; Dhonukshe et al., 2010). Besides, PID/WAGs also regulates the re-localization of PIN3 protein in phototropism in hypocotyls (Ding et al., 2011; Grones et al., 2018), and gravitropism in both shoots and roots (Rakusová et al., 2016; Grones et al., 2018). Biochemically, PID directly phosphorylates residues in the PIN cytoplasmic domain, also referred to as the hydrophilic loop (PIN-HL), of PIN proteins (Michniewicz et al., 2007; Dhonukshe et al., 2010). Notably, there is a direct correlation between phosphorylation by PID and apical localization of PINs in both shoots and roots, whereas reduced phosphorylation brought about by PP2A-catalyzed dephosphorylation coincides with basal localization of PIN proteins. This is indicated by a preferential apical localization of PIN1, PIN2, and PIN4 proteins in roots of *35S::PID* gain-of-function lines and *pp2aa1 pp2aa3* mutants (Michniewicz et al., 2007).

The role of PID/WAGs in the apical-basal PIN polarity regulation is supported by multiple lines of evidence: (1) the basally localized PIN proteins switch to apical localization quite rapidly after induced PID overexpression (Friml et al., 2004); (2) mutants defective in antagonistically acting phosphatases PP2A or PP6 show similar PIN apicalization (Michniewicz et al., 2007; Dai et al., 2012); (3) complementarily, in *pid* mutants, apically localized PINs (as PIN1 in the shoot apical meristem or PIN2 in root epidermis) switch to more basal localization (Friml et al., 2004; Dhonukshe et al., 2010); (4) similar polarity switches occur in plants expressing the engineered phosphomimic (leading to apicalization) and phosphodead (leading to basal localization) versions of PIN proteins (Huang et al., 2010; Zhang et al., 2010). Given the biochemical demonstration that PID phosphorylates PIN proteins (Dhonukshe et al., 2010; Barbosa et al., 2014), the most straightforward interpretation of this set of consistent observations was that PID-mediated phosphorylation of PINs led to their preferential apical delivery (or retention), whereas dephosphorylated PINs end up preferentially at the basal cell side.

This simple model is not consistent with the recent detection of phosphorylated PIN1 protein at the basal cell sides using an antibody specifically recognizing the PID/D6 PROTEIN KINASE (D6PK) phosphorylated PIN1 (Weller et al., 2017). This suggests a more complex mode for PID/WAGs regulating PIN polarity and activity, which is also supported by no detectable decrease in PIN1 phosphorylation in either *pid wag1 wag2* or *d6pk d6pkl1 d6pkl2* mutants (Weller et al., 2017). Besides, the phosphomimic version of PIN1, PIN1S3E, could not rescue the inflorescence defects of the *pin1* mutant (Huang et al., 2010), further supporting that the dynamic phosphorylation status, rather than constitutive phosphorylation, is essential for PIN function. Thus, additional insights are needed to reconcile these observations.

Notably, apart from the role of directing PIN apico-basal polarity, PID-mediated phosphorylation also increases the transport activity of PINs (Zourelidou et al., 2014; Weller et al., 2017). Given the evidence that there is a dramatic change of PIN polarity in *pid* loss- or gain-of-function mutants, which is exactly paralleled by PIN polarity changes observed for phosphodead and phosphomimic PIN variants, PID-conducted phosphorylation might indeed have dual roles in regulating both polarity and activity of PIN proteins. Notably, during cytokinesis, WAG1 AGC kinase exhibits a predominant presence at the cell plate, where it co-localizes with PIN2, possibly reflecting ongoing phosphorylation of PIN2 in cytokinetic cells, as a prerequisite for its correct polar targeting (Glanc et al., 2018). Thus, apart from their evident function in the regulation of PIN activity, several experimental lines of evidence convincingly demonstrate a key role for PID/WAGs in polarity acquisition of the PIN proteins.

Genetic approaches identified MACCHI-BOU4 (MAB4)/ENHANCER OF PID (ENP)/NAKED PINS IN YUCCA-LIKE1 (NPY1) protein and homologous MELs (MAB4/ENP/NPY1-LIKEs) as additional determinants of polar PIN distribution. MAB4 is mainly expressed in shoots, apparently antagonizing depolarization of PIN plasma membrane distribution, acting synergistically with PID in the regulation of organogenesis (Furutani et al., 2007, 2011). Strikingly, a *mel1 mel2 mel3 mel4* quadruple mutant defective in ENP/MAB4 homologs exhibits agravitropic root growth strongly resembling *pid wag1 wag2*, with both mutant combinations characterized by a decreased apical PIN2 abundance in root epidermis cells, very likely contributing to the severe defects in gravitropic root growth (Furutani et al., 2011, 2014). MAB4 and its MEL homologs exhibit a polar localization at the plasma membrane, almost completely overlapping with co-expressed PIN proteins in a range of tissues/cell files (Cheng et al., 2007; Furutani et al., 2011, 2014). The function for MAB4/MELs in PIN polarity establishment remained difficult to

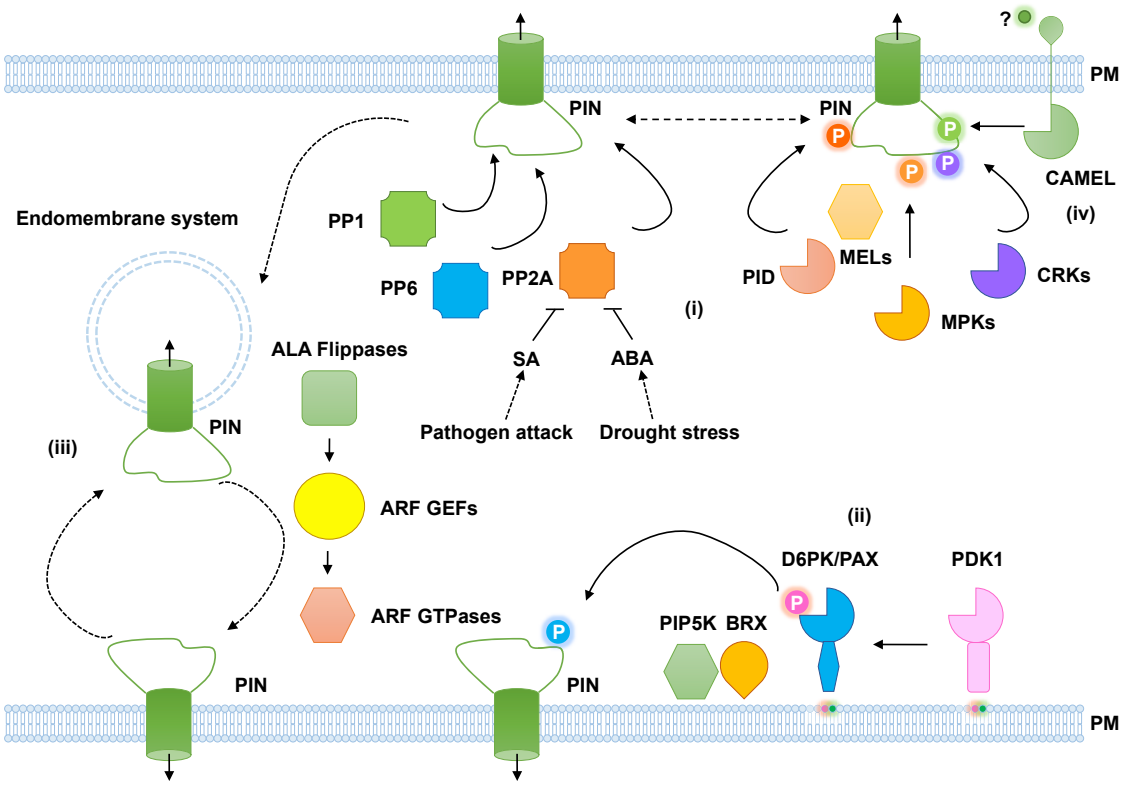

Figure 2. A Current Model Showing Protein Phosphorylation Involved in PIN Regulation.
There are four major, possibly overlapping pathways: (i) PID versus PP2A in apico-basal targeting of PIN proteins through modulating the phosphorylation status of PIN hydrophilic loops. (ii) Basal PDK1-D6PK/PAX regulating PIN activity. (iii) ARF GEF and ARF GTPase-centric endomembrane system for polar delivery of PIN proteins. (iv) CAMEL RLK phosphorylates the hydrophilic loop of PINs in the PM.

reconcile, as localization studies are consistent with MAB4/MELs acting on PIN polarity control and, vice versa, PINs acting on polarity of MAB4/MELs. The molecular interactions among these proteins and the detailed underlying biochemical events require further characterization. One proposed mechanism is that a different phosphorylation status of PIN proteins affects its apico-basal targeting by the ADP-ribosylation factor (ARF) GTPase pathway. This is evident by the observation that long-term treatment with the ARF GEF inhibitor, brefeldin A (BFA), leads to the apicalization of basal PIN1 proteins, whereas there is decreased BFA-induced internalization for apical PIN2 or in the *PINOID* overexpression plants compared with normal PIN1 in wild-type plants (Kleine-Vehn et al., 2009). Similar PIN polarity changes have also been observed for genetic perturbations of components from the ARF GTPase pathway, such as the ARF GEF GNOM or the flippase ALA3 (Geldner et al., 2003; Kleine-Vehn et al., 2009; Zhang et al., 2020b), as well as chemical perturbations, such as Endosidin 4 (Kania et al., 2018). Future research might elucidate how exactly PID-mediated PIN phosphorylation directs its subcellular trafficking.

Apart from PINs, PID can also phosphorylate and activate the ABCB1 auxin transporter, which is antagonized by quercetin treatment, a natural compound that inhibits the kinase activity of PID (Henrichs et al., 2012). Furthermore, a recent study demonstrates that PID phosphorylates CONSTITUTIVE PHOTOMORPHOGENESIS1 (COP1), participating in controlling the molecular interplay between seedling skoto- and photomorphogenesis, which underlines additional functions of PID, beyond phosphorylating PINs (Lin et al., 2017). It is noteworthy that the *pid wag1 wag2* triple mutant highly resembles *pin* mutants, raising questions about the significance of PID phosphorylating additional proteins next to PINs. Perhaps, some of these phosphorylation events influence joint activities of ABCB and PIN proteins in auxin transport (Mravec et al., 2008), which is indicated by their possible interaction (Titapiwatanakun et al., 2009). COP1, on the other hand, has been linked to light-dependent regulation of PIN expression and proteolytic turnover, thereby modulating auxin flow (Sassi et al., 2012). However, molecular mechanisms connecting these highly diverse regulators by AGC VIII kinase-mediated protein phosphorylation remain to be unveiled.

D6PKs and PAX as Regulators of PIN Activity

Unlike nonpolar PID, D6PKs are AGC kinases that accumulate at the basal plasma membrane domain in various cell files and tissues, where they appear to phosphorylate co-localizing PINs across multiple developmental processes (Zourelidou et al., 2009, 2014; Willige et al., 2013). Biochemical analysis reveals that D6PKs and PID recognize and phosphorylate three of five overlapping phosphosites within the TPRNS motifs found in the hydrophilic loop of canonical PIN proteins (Zourelidou et al., 2014). Somewhat surprisingly, when probing distribution of

PIN1 phosphorylated at PID/WAG and D6PK phosphorylation sites, in either *pid wag1 wag2* or *d6pk d6pkl1 d6pkl2* seedlings, no major differences to wild-type controls were detected (Weller et al., 2017). These results suggest that phosphorylation of the TPRNS motifs per se is not sufficient for apicalization of PINs and furthermore imply that neither D6PKs nor PID/WAGs can fully account for PIN loop phosphorylation *in planta*. Together with transport assays from *Xenopus* oocytes showing increased PIN transport activity when co-expressed with either D6PK or PID (Zourelidou et al., 2014), these results suggest that PID-mediated phosphorylation has a more complex role in modulating PIN, by both activity and polarity. The different phenotypes of *pid wag1 wag2* and *d6pk d6pkl1 d6pkl2* suggest a distinct function of these two subclades of AGCVIII kinases, although they modify overlapping phosphosites in PIN proteins. This combinational regulatory mode of action might reflect a certain dynamics in PIN phosphorylation, apparently contributing to PIN localization and activity control.

Similar to D6PKs, PROTEIN KINASE ASSOCIATED WITH BRX (PAX), another basally (rootward) localized AGC homolog, is specifically expressed in the protophloem, where PAX phosphorylates and activates PIN proteins to regulate local auxin transport and ultimately vascular development (Marhava et al., 2018). BREVIS RADIX (BRX) interacts with and inhibits PAX activity, and thus PIN1 phosphorylation, acting together as a molecular rheostat to execute protophloem development and root patterning (Figure 2). Intriguingly, PIN1 localizes at a specific "donut-like" domain in developing sieve elements, surrounding a complementary "muffin-like" domain-localized enriched for PAX and BRX. PAX and BRX, together recruit PtdIns(4)P 5-kinases (PIP5Ks), which produce phosphatidylinositol-4,5-bisphosphates (PtdIns(4,5)P_2) at the outer margin of the "muffin-like" domain, with a local increase of PtdIns(4,5)P_2 levels likely promoting PIN1 endocytosis and thereby defining its localization at the plasma membrane (PM) (Marhava et al., 2020). This mode of BRX/PAX, PIP5K, PtdIns(4,5)P_2, and PIN interactions, represents an elegant example for a self-reinforcing mechanism that evolved to maintain PIN distribution, and ultimately auxin fluxes controlling morphogenesis. Notably, PtdIns(4,5)P_2 exhibits asymmetric distribution in the PM (Tejos et al., 2014) and regulates PIN2 clustering at lipid nanodomains (Li et al., 2020b).

BRX Proteins as Regulators of PAX and D6PKs

BRX proteins appear to function as key components in the regulation of PIN polarity, and, in more general terms, represent mediators of cell polarity in various developmental contexts. In stomatal development, BRX LIKE4 (BRXL4) and additional BRX family members interact with BASL (BREAKING OF ASYMMETRY IN THE STOMATA LINEAGE) acting as a central polar molecular scaffold complex, essential for guard cell lineage specification and transition (Rowe et al., 2019). In rice (*Oryza sativa* L.), LAZY1 (LZY1), a component acting in the transmission of signals triggered by gravity-sensing statolith relocation and downstream PIN-mediated auxin transport, interacts with and changes the subcellular localization of BRXL4 (Li et al., 2019). Recently, it has been reported that LZYs (LAZY LIKEs; LZY; also called NEGATIVE GRAVITROPIC RESPONSE OF ROOTS, NGR, or DEEPER ROOTING, DRO) interact with RLD (RCC1-like domain) proteins, a BRX domain protein family, together establishing a gravity-sensitive complex that regulates PIN3 relocation during gravitropism (Taniguchi et al., 2017; Furutani et al., 2020). However, the underlying mechanism by which the LZY-RLD complex regulates PIN3 localization is unclear. Given that BRX inhibits the kinase activity of PAX and D6PK (Marhava et al., 2018), it would be interesting to test if additional BRX domain-containing proteins, such as RLDs, exhibit similar inhibitory functions, and if LAZY proteins regulate PIN-mediated auxin transport by BRX domain proteins and D6PK.

Additional AGC Kinases Involved in Auxin Transport or PIN Regulation

Phototropins are proteins that function as blue-light receptors, regulating phototropic growth responses of plants (Christie, 2007). However, while sharing biochemical attributes with further AGC kinases, the kinase domain of phototropin1 (PHOT1) does not phosphorylate PIN-HL *in vitro* (Ding et al., 2011). In contrast, a directional light source is sufficient to trigger phototropic hypocotyl bending, leading to differential expression of PID, and—as a likely consequence—to asymmetric phosphorylation and thus asymmetric distribution of PIN3 proteins in hypocotyl cells (Ding et al., 2011). Related PID-mediated effects on PIN3 relocation have been described in columella root cap cells in response to gravistimulation (Kleine-Vehn et al., 2010; Grones et al., 2018).

While the PHOT1 kinase domain appears insufficient for PIN phosphorylation, the blue light receptor readily phosphorylates ABCB19, locally interfering with its transport activity as a likely prerequisite for asymmetric auxin flux during phototropism (Christie et al., 2011). Apart from the ABCB19 auxin transporter, phototropins phosphorylate NPH3 (NON-PHOTOTROPIC HYPOCOTYL3) and RPT2 (ROOT PHOTOTROPISM2), proteins from the BTB/POZ (bric-a-brac, tramtrack, and broad complex/pox virus and zinc finger) family, which all have been linked to the regulation of ABCB (auxin) transporter activity (Suetsugu et al., 2016). Detailed phenotypic analysis reveals that PIN3 and ABCB19 participate in distinct steps in the progression of phototropic growth (Christie et al., 2011; Haga et al., 2018), and thus these kinase regulators might have more complex functions beyond what has been revealed.

Emerging Role of Lipids in Regulating AGC and PIN Phosphorylation

3′-PHOSPHOINOSITIDE-DEPENDENT PROTEIN KINASE1 (PDK1) represents a master regulator of AGC kinases in eukaryotes. Consistent with this role, it was long believed that PDK1 phosphorylates PID to govern PIN polarity and thus PIN-mediated auxin transport. However, recent studies revealed that true *pdk1* null alleles exhibit pleiotropic developmental defects (Tan et al., 2020b; Xiao and Offringa, 2020), which appear unrelated to PID functionality, thus contradicting earlier biochemical studies (Zegzouti et al., 2006a, 2006b). In fact, the *pdk1.1 pdk1.2* (also called *pdk1 pdk2*) double mutants, exhibited phenotypes and growth defects that strongly resemble those of *d6pk d6pkl1 d6pkl2* triple mutants (Tan et al., 2020b; Xiao and Offringa, 2020). Consistently, PDK1 was characterized as an activator of D6PK-type AGC kinases, and it participates in embryogenesis, lateral root formation, apical hook formation, gravitropism, phototropism, and so forth (Tan

et al., 2020b). Besides, PDK1 phosphorylates and activates PAX to regulate protophloem development (Xiao and Offringa, 2020). Furthermore, association of PDK1 and AGC kinases with membrane domains was found to depend on lipid compositions, establishing the PDK-AGC kinase regulatory module as a lipid-dependent phospho-switch, controlling activity, rather than polarity, of PIN proteins (Tan et al., 2020b). This resembles regulation of plasma membrane accumulation of D6PK, which likely involves interactions between positively charged amino acids and lipids (Stanislas et al., 2015; Barbosa et al., 2016; Tan et al., 2020b). However, truncated PDK1 protein with a disrupted PH domain (0–408 aa) keeps most of its functionality, although without the ability to localize to the PM, calling into question the importance of this domain and its membrane association (Xiao and Offringa, 2020). It is noteworthy that the kinase domain of PDK1 (0–399 aa) has retained the ability to bind lipids, but characterization of the exact lipid binding residues that is indispensable for understanding modes of PDK1 lipid association is still elusive. Furthermore, phosphatidic acid has been demonstrated to modulate localization and activity of PP2AA1 (RCN1) and PID, respectively, underlining an importance of (phospho)-lipid signaling in the regulation of intercellular auxin transport (Gao et al., 2013; Laetitia et al., 2016; Wang et al., 2019). Two recent studies (Tan et al., 2020a; Xiao and Offringa 2020) corroborate these observations and once again highlight the power of genetic analyses, which are often indispensable for an accurate interpretation of pure biochemical results.

In summary, PIN phosphorylation was found to be regulated by multiple sub-clades of AGC kinases, predominantly by PID/WAGs, D6PK/D6PKLs, and PAX/PAXL. While all of these kinases share similar phosphosites and the ability to boost PIN transport activity by their kinase activity, only PID/WAGs exhibit additional functional attributes required for the dynamic regulation of PIN polarity. Modes of action that led to this functional diversification in AGC kinase-dependent regulation of PINs remain to be determined. One possibility is that, among AGC kinases, PID/WAGs exclusively function together with additional molecules that are essential for establishing and/or maintaining PIN polarity. Such interaction partners may involve MAB4/MELs proteins, seemingly required for correct sorting and polar plasma membrane deposition of PINs (Furutani et al., 2011).

Flexibility in PIN phosphorylation is likely a result of adjustable enzymatic activities. The distribution of various kinases and phosphatases, as well as their regulators, might be variable in different cell types at different stages. For example, D6PKs locate at the basal side of cells, where it most likely will not affect the activity of apically localized PIN2. Therefore, neither *d6pk d6pkl1 d6pkl2* nor *pdk1.1 pdk1.2* exhibit strong agravitropic root growth phenotypes, which are a hallmark feature of *pin2* alleles. *pid/wag* mutant combinations, in contrast, exhibit pronounced defects in root gravitropism likely as a result of PIN2 mis-localization due to phosphorylation deficiencies (Dhonukshe et al., 2010). Differences in the recognition of PIN phospho sites by distinct AGC kinases thus might produce subtle but potentially relevant variations in the overall PIN phosphorylation pattern. Perhaps such variations contribute to the establishment of a PIN phosphorylation code, dynamically controlling directionality and rates of intercellular auxin transport.

Other Protein Kinases Involved in PIN Phosphorylation

Apart from the AGC kinases, additional types of kinases have been reported to participate in PIN phosphorylation control as well. This includes MITOGEN-ACTIVATED PROTEIN KINASE (MPK) kinases and CDPK-RELATED KINASE5 (CRK5). Because of its role in the regulation of shoot branching, PIN1 phosphorylation by the MKK7–MPK6 module appears essential, as it modulates PIN intracellular distribution to adjust polar auxin transport. Strikingly, the conserved motifs harboring PIN phosphorylation sites recognized by MPKs appear to overlap with those of AGC-type kinases, arguing for co-evolution of distinct kinase signaling events converging in the regulation of PINs (Jia et al., 2016; Dory et al., 2018). In this context, it has to be stressed that MPK signaling does not impact on polar PIN sorting, therein resembling the effects of AGC kinases, with the notable exception of the PID/WAG subclade. Thus, different protein kinases seemingly exert quite distinct effects on the function of PINs, providing indirect evidence for the evolution of a phosphorylation code that defines polar auxin transport by variable modification of PINs. However, the upstream part of the MPK pathway awaits further characterization, which will be further discussed in the TMK1 session.

CRK5 encodes another protein kinase, exhibiting a "U"-shaped subcellular localization on certain PM domains in lateral root cap cells. Intriguingly, CRK5 was found to phosphorylate PIN2, which might explain its function in the regulation of root gravitropism (Rigó et al., 2013; Baba et al., 2018). Recent studies have revealed that CRK5 also participates in apical hook development by directly phosphorylating PIN3 protein (Baba et al., 2019a) and in embryonic development by catalyzing PIN1 phosphorylation (Baba et al., 2019b). These studies illustrate a conserved function for CRK5-catalyzed PIN phosphorylation in various developmental contexts across different PIN proteins. In addition, BR signals, transduced by a phosphorylation cascade, suppress PIN endocytosis (Retzer et al., 2019). Genetic or pharmacological perturbation of the BR signal transduction supports that BR governs the subcellular sorting of endocytic PIN2, and thus modulates polar auxin transport during root gravitropism. Besides, the availability of an external nitrogen source shapes root system architecture, also by modulating the phosphorylation status of PIN2 protein (Vega et al., 2020; Ötvös et al., 2020); however, the underlying kinase or phosphatase responsible for the regulation of this phosphorylation requires further dissection. Apart from these cytosolic (or non-transmembrane) kinase activities, PM-resident RLKs recently emerged as another class of kinase capable of phosphorylating PINs at the PM. Firstly, CAMEL, an RLK identified as a downstream target of the auxin-responsive transcription factor WRKY23 (Prát et al., 2018), phosphorylates and directs PIN repolarization during vascular development in leaf venation (Hajný et al., 2020).

All these diverging observations on different kinases and different effects on different aspects of PIN activities puts forward an urgent question: How do all these various kinase activities communicate with each other to provide coherent and coordinated regulation of PIN activities? This reoccurring theme in the biology of signal perception/transmission clearly represents one of the most urgent issues when it comes to an understanding role of PIN-mediated auxin transport in different aspects of plant development.

Dephosphorylation of PIN Proteins by Multiple Phosphatases

Contrary to protein kinases, protein phosphatases dephosphorylate PIN proteins by hydrolyzing the covalently linked phosphate group. Several protein phosphatases have been reported to be involved in PIN dephosphorylation, including protein phosphatase 2A (PP2A) (Michniewicz et al., 2007), PP1 (Guo et al., 2015), and PP6 (Dai et al., 2012).

PP2A antagonizes PID by dephosphorylating PIN proteins, and both of them jointly determine the apico-basal polarity of PIN proteins. PP2A functions as a heterotrimeric phosphatase complex, composed of a scaffolding A subunit (encoded by three loci in the *Arabidopsis* genome), a regulatory B subunit (15 in *Arabidopsis*), and a catalytic C subunit (5 in *Arabidopsis*). Additional analysis of *Arabidopsis* mutants affected in the catalytic C subunits, PP2AC3 (also named PP2A-3) and PP2AC4, further confirmed an involvement of PP2A in regulating PIN-dependent auxin transport (Ballesteros et al., 2013).

A recent study revealed that PP2A C subunits also interact with members of the PYRABACTIN RESISTANCE1 (PYR)/PYRABACTIN RESISTANCE1-LIKE (PYL)/REGULATORY COMPONENT OF ABA RECEPTOR (RCAR) family of ABA receptors, forming a protein complex independent of the presence of ABA, which regulates PIN-mediated polar auxin transport and root adaptive development (Li et al., 2020a). Apparently, the PYR/PYL-PP2A C complex functions as a molecular hub, mediating the crosstalk between auxin and ABA. Intriguingly, another phytohormone, SA, binds directly to PP2A A subunits, to suppress PP2A phosphatase activity, thereby regulating PIN polarity and root development (Tan et al., 2020a). These studies pinpoint the essential role of PP2A, regulating PIN-dependent auxin transport by integrating hormonal crosstalk in the control of adaptive growth responses.

Interestingly, the PP2A A subunits, PP2AAs, also function as scaffolding proteins for the PP6 protein phosphatase complex, by interacting with SAPS DOMAIN-LIKE (SAL, SAL1 to SAL 4) regulatory subunits and phytochrome-associated serine/threonine protein phosphatase (FyPP1/3) catalytic subunits. PP6 is also involved in the apico-basal polarity regulation of PIN proteins, by directly dephosphorylating PINs (Dai et al., 2012). The *fypp1 fypp3* double mutant, as well as lines expressing enzymatically inactive dominant-negative *FYPP* alleles (*F1DN* or *F3DN*), exhibit severe gravitropic defects in roots, consistent with a key role in the regulation of polar auxin transport. In addition, a single member of the PP1 family, TYPE-ONE PROTEIN PHOSPHATASE4 (TOPP4), regulates the patterning process of leaf pavement cells by dephosphorylating PIN proteins (Guo et al., 2015).

PROTEIN PHOSPHORYLATION IN AUXIN SIGNALING

Protein Phosphorylation Modulates the Canonical TIR/AFBs Pathway

The canonical TRANSPORT INHIBITOR TRANSPORT1 (TIR1)/AUXIN SIGNALING F-BOX (AFBs)-mediated auxin pathway has been thoroughly characterized for decades by groundbreaking genetic and biochemical studies. Auxin activates TIR1/AFB E3 ligase-type receptors to degrade the AUXIN/INDOLE ACETIC ACID (AUX/IAA) co-receptor repressors, thereby releasing AUXIN RESPONSE FACTOR (ARF) transcription factors that control downstream gene expression programs. Furthermore, TIR1/AFB-related signaling has recently been demonstrated to impact on non-transcriptional responses by so far elusive molecular pathways (Figure 3) (Dindas et al., 2018; Fendrych et al., 2018). This core auxin signaling module seems to mediate most of the characterized auxin responses, and phenotypes of the *tir1/afb* mutants can account for most of published auxin outputs, reported for auxin biosynthesis or transport mutants (Prigge et al., 2020). However, some aspects of auxin responses and mechanisms involved remains to be characterized. This involves pathways by which auxin activates acidic growth and canalizes its own transport (Gallei et al., 2020). Notably, a transcriptional response is thought to be relatively "slow," while additional comparably "rapid" hormonal responses transmit auxin signals by so far poorly characterized pathways (Fendrych et al., 2018).

Auxin exhibits contrasting effects on shoot and root development, i.e., promoting cell growth in shoots, whereas it inhibits it in roots. In accordance with the classical acid growth theory (Rayle and Cleland, 1970, 1992), auxin swiftly induces expression of SMALL AUXIN UP RNA proteins by the TIR1/AFB-AUX/IAA pathway, causing inhibition of PP2C-D. Consequently, activity of PM H^+-ATPase AHAs is increased, resulting in elevated extracellular proton concentrations to stimulate cell elongation in shoots (Figure 3) (Spartz et al., 2014; Fendrych et al., 2016). In contrast to this primarily transcriptional response, further studies demonstrated involvement of the TIR1/AFB pathway in rapid auxin-induced root growth inhibition, but by a transcription-independent, essentially enigmatic pathway (Fendrych et al., 2018). These studies highlight a central role of the canonical TIR1/AFB pathway in auxin-regulated growth in both roots and shoots. However, the non-transcriptional sub-branch requires additional uncharacterized components.

FERONIA (FER), an RLK-perceiving extracellular RAPID ALKALINIZATION FACTOR peptides, seems to be involved in root gravitropism and auxin-induced lateral pH gradients associated with control of such differential root growth (Barbez et al., 2017). Nonetheless, whether this FER-mediated signaling acts through a TIR1/AFB-dependent pathway, and how auxin induces local variations in pH, remains unclear. Evidently, an answer to these questions would largely expand our knowledge on mechanisms underlying auxin-mediated growth regulation. Notably, AFB1, one member of the six *Arabidopsis* auxin co-receptor homologs, plays a prominent role mediating this rapid response (Prigge et al., 2020). It remains to be tested whether or not such swift responses are mediated by a phosphorylation switch that acts on the activity of PM H^+-ATPase proton pumps.

Given the biochemical properties, i.e., defined enzymatic activities recognizing and modifying downstream substrates of protein kinases and phosphatases, it is not surprising that many such enzymes serve as molecular hubs for signaling crosstalk. For instance, the TRACHEARY ELEMENT DIFFERENTIATION INHIBITORY FACTOR (TDIF) signaling peptide, recognized

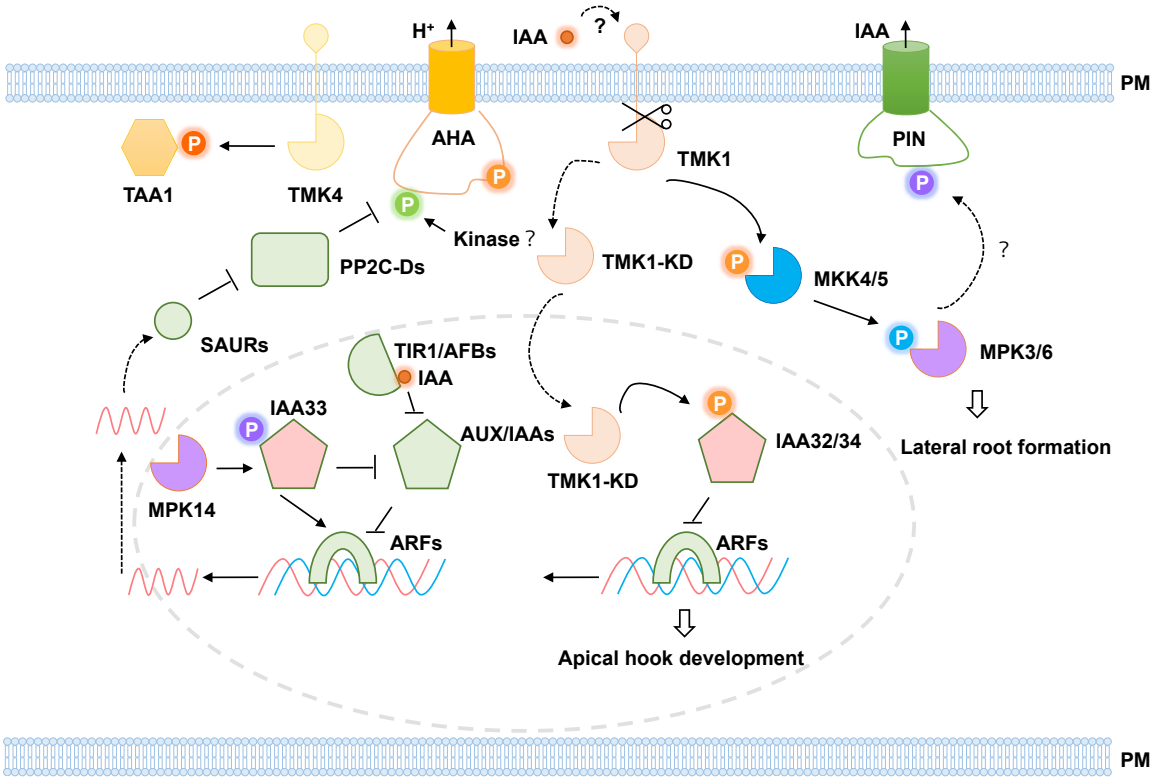

Figure 3. Protein Phosphorylation in Auxin Signaling.
The TMK family of RLKs plays key roles in auxin signaling: firstly, the kinase domain of TMK1 translocates into the nucleus in an auxin-responsive manner, interacting and phosphorylating non-canonical AUX/IAA proteins to shape apical hook development; secondly, TMK-mediated auxin signaling phosphorylates MKKs from a MAPK cascade, which further transduces this auxin signal to the phenotypic output, lateral root formation; thirdly, given the studies showing phosphorylation of PIN proteins by MPK, it would be interesting to test if this is regulated by the upstream auxin-TMK1 pathway; fourthly, MPK14 phosphorylates non-canonical AUX/IAA proteins to regulate root distal stem cell identity. On the other hand, the core auxin-TIR1/AFB signaling pathway activates AHA via PP2C-D phosphatases, meanwhile multiple protein kinases have been demonstrated to be involved in this pathway via phosphorylating certain components.

by RLK receptor kinase TDIF RECEPTOR, can activate BRASSINOSTEROID-INSENSITIVE2 (BIN2) kinase, which further phosphorylates ARF7 and ARF19, two key transcription factors in the auxin signaling pathway, to regulate the organogenesis of lateral roots (Cho et al., 2014). Notably, this type of regulatory mode not only represents a common pattern in hormonal crosstalk, but also serves in signal amplification triggered by protein de/phosphorylation (i.e., one kinase or phosphatase can phosphorylate or dephosphorylate multiple substrate molecules). Some additional phosphorylation of auxin signaling components has been demonstrated, e.g., BR-regulated BIN2 kinase phosphorylates ARF2 mediating its crosstalk with auxin signaling (Vert et al., 2008); and ARF2 was implicated in the control of K^+ homeostasis (Zhao et al., 2016), and it appears quite likely that related events, acting in a variety of developmental and physiological processes, will be identified in a foreseeable future.

The TMK1-Mediated Auxin Signaling Pathway and the MPK Pathway

TMK1 was one of the earliest RLKs to be identified in *Arabidopsis* (Chang et al., 1992). Members of the TMK1 family of RLKs have been proposed to act as PM-resident receptors or part of a receptor complex, perceiving extracellular auxin followed by signal translation into various phosphorylation events. The *tmk1 tmk4* double mutant as well as higher-order *tmk* mutants exhibit strong auxin-related defects in growth and development throughout the life cycle of *Arabidopsis* (Dai et al., 2013; Xu et al., 2014; Huang et al., 2019).

Apart from the canonical TIR1/AFB pathway functioning within the cellular boundaries, a complementary PM-associated auxin receptor activity has long been proposed (Kubeš and Napier, 2019; Du et al., 2020). Abolishment of PM protein internalization following short-term auxin (1-NAA) treatment, for example, has been linked to such an alternate mode of auxin perception (for reviews, see Adamowski and Friml, 2015; Gallei et al., 2020; Semeradova et al., 2020). Recent evidence has revealed that auxin stabilizes RHO OF PLANT (ROP) GTPase at the PM, inhibiting endocytosis and PIN internalization (Platre et al., 2019). A similar auxin-induced clustering behavior at the subcellular level was observed for TMK1 (Pan et al., 2020), stressing a role of TMK1 and ROP signaling in the regulation of PIN subcellular distribution. Auxin exhibits dual effects on PIN localization: on one hand, short-term auxin treatment inhibits PIN internalization (Paciorek et al., 2005); on the other hand, long-term auxin exposure destabilizes PIN proteins at PM (Abas et al., 2006; Baster et al., 2013).

The *tmk1* single mutant shows defects in the apical hook formation process, suggesting participation of the kinase (Cao et al., 2019). Intriguingly, the TMK1 kinase domain was found to be cleaved by the activity of uncharacterized proteases in different tissues following auxin activation. Thereafter, the cleaved kinase domain translocates into the nucleus to regulate transcriptional responses by interacting with and phosphorylating non-canonical AUX/IAA proteins, IAA32 and IAA34 (Figure 3) (Cao et al., 2019). In addition, the *tmk1 tmk4* double mutant is deficient in initiating lateral roots, and this mutant turned out to be less responsive to auxin in control of root organogenesis (Huang et al., 2019). Furthermore, the auxin-TMK pathway activates a MPK pathway to govern lateral root patterning. TMK1 and TMK4 phosphorylate MITOGEN-ACTIVATED PROTEIN KINASE KINASE4/5 (MKK4/5), thus activating MPK3/6 and regulating cell division to promote lateral root formation (Huang et al., 2019).

It has long been proposed that auxin activates an MPK signaling pathway in plant cells (Kovtun et al., 1998), although without evidence showing whether it is perceived intracellularly or extracellularly or what the signaling mechanism may be. As mentioned above, TMK1 transduces this auxin signal to downstream MKK4/5–MPK3/6 to activate this MAP kinase cascade for further lateral root formation (Huang et al., 2019). Together with the notion that MPK phosphorylates PIN proteins directly (Jia et al., 2016; Dory et al., 2018), it appears plausible that TMK-mediated MPK signaling modulates PIN-mediated auxin transport as well. Moreover, INFLORESCENCE DEFICIENT IN ABSCISSION, an organ abscission peptide, and its receptors HAESA (HAE) and HAESA-LIKE2 (HSL2), activate a MKK4/5–MPK3/6 kinase cascade, which further regulates lateral root emergence via the cell wall remodeling-mediated cell separation pathway, specifically in those outer layer root cells covering lateral root primordia. Furthermore, *ida* and *hae hsl2* mutants exhibit decreased sensitivity to auxin treatment, suggesting participation of the corresponding gene products in mediating auxin effects in the regulation of lateral root emergence (Zhu et al., 2019). Collectively, the MAPK pathway serves an essential molecular hub, participating in at least two pathways; auxin/TMK1-controlled cell division in lateral root primordia and HAE/HSL2-mediated cell separation in the outer covering cells, to coordinate distinct cell files for lateral root development. In parallel, there is also evidence showing that MPK14, activated by auxin, interacts with and phosphorylates non-canonical IAA33, an internal competitor of the canonical AUX/IAA repressor IAA5, to regulate root development (Lv et al., 2020). These studies establish critical roles of TMKs and MPKs in auxin-mediated developmental processes, including both apical hook formation and lateral root initiation along with insights into biological functions and modes of action of non-canonical AUX/IAAs.

Notably, it has been reported that AUXIN BINDING PROTEIN 1 (ABP1) acts as a hormone co-receptor in an ABP1-TMK1 complex to regulate ROP GTPase and cytoskeleton dynamics in epidermal cells (Xu et al., 2014). However, the true *abp1* null alleles show no apparent phenotypes (Gao et al., 2015; Michalko et al., 2015, 2016), calling into question the validity of such co-receptor complex models. This, together with the fact that solely co-immunoprecipitation data provided support for an interaction between ABP1 and TMK1 (Xu et al., 2014), makes it highly desirable to address the role of a hypothetical ABP1-TMK complex in auxin perception in further detail.

In summary, reversible protein phosphorylation plays multiple roles in regulating the various key elements of the auxin signaling pathway. There is a collective evidence for auxin signaling at the cell surface, likely involving the phosphorylation cascade by the TMK family of RLKs. Given the fact that the *tmk1 tmk4* double mutant exhibit severe growth defects, it is not surprising that it shows distorted response to a range of exogenous pharmacological treatments, including auxin; therefore, it is paramount to verify those signaling outputs. Importantly, it remains entirely unclear to date how the auxin is perceived by this mechanism. Specifically, while there is compelling evidence for a TMK-dependent auxin signaling branch, it remains to be demonstrated whether or not such TMKs might act as auxin (co-)receptors. Currently, it cannot be ruled out that TMKs perceive certain growth-promoting cues (ligands), which might involve members of the small peptide family. The GOLVEN (GLV, also called ROOT GROWTH FACTOR LIKE) small peptide (Whitford et al., 2012; Fernandez et al., 2013, 2020) was proposed as a possible candidate (Armengot et al., 2016), supported by the similar phenotypes observed in *tmk* and *glv* mutants. However, a confirmation of such a hypothesis needs further investigation. Additional external cues, such as mechanical stimuli, were recently proposed to get integrated by TMK1-mediated auxin signaling during apical hook development (Baral et al., 2020). Also, a potential crosstalk between TMKs and the canonical TIR1/AFB signaling network remains to be explored.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

With the outcome of several decades of auxin research as solid support, it is now widely accepted that spatiotemporal variations in auxin responses are under stringent control by local auxin metabolism and transport and hormone perception. Different auxin concentrations cause distinct auxin responses, which cannot be fully explained by the canonical TIR1/AFB transcriptional pathway. Thus, both transcriptional regulation as well as post-translational processes represent central topics in the field of "auxin regulation." When it comes to protein phosphorylation, pairwise kinase/phosphatase–substrate interactions in different developmental contexts await their concise characterization. Furthermore, it will be necessary to investigate the phosphoproteomes in different genetic backgrounds, with the help of cutting-edge mass spectrometry approaches. Ultimately, establishment of molecular networks using molecular system biology approaches should produce essential insights into the various regulatory loops and is likely to reveal so far still unknown modes of action of the auxin signaling network.

In addition, along with the development of novel techniques, studies in the coming decades will present a more detailed picture that will be extremely helpful for the development of novel tools highly beneficial for both future basic science and agriculture. For example, multiple auxin analogs are widely used agri-pharmaceuticals. To date, most of our knowledge comes from the model plant *Arabidopsis thaliana*. Research in organisms from different evolutionary clades may help elucidating how

plants have evolved the molecular auxin network. Besides, it will be interesting to learn if all these mechanisms can be translated into other species, especially those with agronomic value.

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