Hormonal Control of Polar Auxin Transport

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Abstract Polar auxin transport (PAT) is required for the formation and maintenance of local auxin gradients, which is of crucial importance for many aspects of auxin-mediated plant development. Different plant hormones regulate plant growth and development mostly by modulating PAT, particularly, pointing to the PINFORMED (PIN) proteins. In this book chapter, we review recent advances on hormonal regulation of PAT.

1 Polar Auxin Transport

Polar auxin transport (PAT) has been found throughout the higher plant species. It is characterized by its strictly controlled directionality, as designated by the name, polar auxin transport. Briefly, the major polar flow of auxin can be traced from apical tissues towards the base of the plant and further to the root tip. Once auxin reaches the tip of the root, part of it is redirected back upwards (basipetally) through the root epidermis into the root elongation zone (Rashotte et al. 2000) where it can be recycled back into the vasculature stream (Blilou et al. 2005). A number of physiological and genetic studies have clearly demonstrated that PAT, which is required for the formation and maintenance of local auxin distribution patterns so-called local auxin gradients, is of crucial importance for many auxin-mediated developmental processes.

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R. Chen and F. Baluška (eds.), *Polar Auxin Transport*,
Signaling and Communication in Plants 17, DOI 10.1007/978-3-642-35299-7_6,

2 Subcellular Trafficking of PINs

The polar, subcellular localization of PIN auxin efflux carriers determines the direction of intercellular auxin flow, thus defining the spatial aspect of auxin response. The current model of polar targeting of PIN proteins towards different plasma membrane domains encompasses apolar secretion of newly synthesized PINs followed by endocytosis and recycling back to the plasma membrane (PM) in a polarized manner (Dhonukshe et al. 2008). The reversible phosphorylation of PIN proteins by the serine/threonine protein kinase PINOID (PID) and protein phosphatase 2A (PP2A) is important for the decision about apical or basal delivery of PIN proteins (Friml et al. 2004; Michniewicz et al. 2007).

The clathrin-dependent endocytosis is operational in plants and constitutes the predominant pathway for the internalization of numerous PM-resident proteins including PIN auxin efflux carriers (Dhonukshe et al. 2007; Kitakura et al. 2011). The *Arabidopsis* protein GNOM is a brefeldin A (BFA)-sensitive ARF-GEF that is required for the proper polar localization of PIN1 (Geldner et al. 2003). The apical and basal PIN targeting pathways are interconnected but molecularly distinct by means of ARF-GEF vesicle-trafficking regulators. The *Arabidopsis* ortholog of the yeast and mammalian vacuolar protein sorting 29 (VPS29), a member of the retromer complex, is required for endosome homeostasis, PIN protein cycling, and dynamic PIN1 repolarization (Jaillais et al. 2007). The SORTING NEXIN 1 (AtSNX1) retromer complex acts to retrieve PIN2 proteins from a late/pre-vacuolar compartment back to the recycling pathways (Jaillais et al. 2006; Kleine-Vehn et al. 2008).

3 Hormonal Control of PAT

The PAT-dependent auxin gradients mediate many aspects of plant growth and development. A large body of evidence shows that different plant hormones regulate plant growth and development partially by modulating PAT, in particular, pointing to the PIN proteins.

3.1 Auxin-Mediated Regulation of PAT

Auxin itself positively feeds back on PIN expression in a time- and concentration-dependent manner. Even when the protein synthesis was inhibited by cycloheximide, auxin effects on the expression of PIN still occur, demonstrating that the auxin-dependent PIN upregulation does not require *de novo* synthesis of any factors. Higher auxin concentrations, besides modulating *PIN* expression, posttranscriptionally downregulate the abundance of specific PIN proteins (Vieten et al. 2005). Moreover, local auxin application or accumulation during *de novo* organ formation

leads to rearrangements in the subcellular polar localization of PIN proteins (Sauer et al. 2006). This auxin effect on PIN polarity is cell specific, does not depend on PIN transcription, and involves the Aux/IAA-ARF (indole-3-acetic acid-auxin response factor) signaling pathway (Sauer et al. 2006).

Auxin can also inhibit PIN endocytosis and promote its PM localization, which is specific to biologically active auxins and requires activity of the Calossin-like protein BIG (Paciorek et al. 2005). By inhibiting the internalization step of PIN constitutive cycling, auxin increases levels of PINs at the PM, providing a mechanism for the feedback regulation of PAT (Paciorek et al. 2005). Pan et al. reported that the $SCF^{TIR1/AFB}$-dependent processes are involved in auxin regulation of endocytosis, recycling, and PM accumulation of the auxin efflux transporter PIN2 in *Arabidopsis thaliana* (Pan et al. 2009). Another recent report (Robert et al. 2010) showed that auxin signaling mediated by the auxin receptor AUXIN-BINDING PROTEIN 1 (ABP1) inhibits the clathrin-mediated internalization of PIN proteins. ABP1 acts as a positive factor in clathrin recruitment to the PM, thereby promoting endocytosis (Robert et al. 2010). Auxin binding to ABP1 interferes with this action and leads to the inhibition of clathrin-mediated endocytosis (Robert et al. 2010). Thus, ABP1 mediates a non-transcriptional auxin signaling that regulates the evolutionarily conserved process of clathrin-mediated endocytosis and suggests that this signaling may be essential for the developmentally important feedback of auxin on its own transport (Robert et al. 2010).

3.2 Cytokinin-Mediated Regulation of PAT

It was reported that exogenous cytokinin (CK) exhibits a negative effect on the transcription of several PIN efflux carriers (Laplaze et al. 2007). Significantly, the B-type response regulators (ARRs) can directly activate the expression of *SHY2/IAA3*, a negative regulator of auxin responses, leading to downregulation of *PIN* transcription in the root meristem (Dello Ioio et al. 2008).

A new study has recently shown that cytokinin regulates endocytic recycling of PIN1 by redirecting it for lytic degradation in vacuoles (Marhavý et al. 2011). Stimulation of the lytic PIN1 degradation is a specific mechanism to rapidly modulate the auxin distribution in cytokinin-mediated developmental processes. In another recent study, multiple *arr* mutants, which have a reduced root apical meristem (RAM) phenotype, were found to have reduced PIN protein levels, but not decreased *PIN* transcript levels (Zhang et al. 2011). In contrast, disruption of type-A *Arabidopsis* ARRs, which are negative regulators of cytokinin signaling, alters the levels of PIN proteins and results in increased sensitivity to N-1-naphthylphthalamic acid (NPA), an inhibitor of PAT (Zhang et al. 2011). Cytokinin, acting through the type-A ARRs, alters the level of several PIN efflux carriers and thus regulates the distribution of auxin within the root tip.

3.3 Gibberellin-Mediated Regulation of PAT

A previous study in *Populus* wood showed that gibberellin (GA) stimulates PAT and has a common transcriptome with auxin (Björklund et al. 2007). By feeding isotope-labeled IAA, the experiment showed that GA indeed increases auxin levels in the stem by stimulating PAT (Björklund et al. 2007). A recent study in *Arabidopsis* showed that gibberellin regulates PIN abundance and is required for auxin transport-dependent growth and development. PAT is reduced in the inflorescences of *Arabidopsis* mutants defective in GA biosynthesis and signaling. This reduced PAT correlates with a reduction in the PIN abundance in GA-deficient plants and that PIN protein levels recovered to the wild-type levels following GA treatment. The regulation of PIN protein levels cannot be explained by a transcriptional regulation of the *PIN* genes but that GA deficiency promotes, at least in the case of PIN2, the targeting of PIN proteins for vacuolar degradation (Willige et al. 2011). Thus, GA-dependent modulation of PIN turnover may be causative for the differential growth responses.

3.4 Brassinosteroid-Mediated Regulation of PAT

Brassinosteroids (BRs) are important plant growth regulators in multiple developmental processes. Previous reports have shown that BR treatment enhances auxin-related responses (Bao et al. 2004; Nakamura et al. 2003). Recent study showed that BRs stimulate PAT capacities and modify the distribution of endogenous auxin (Li et al. 2005). In plants treated with BR or defective in BR biosynthesis or signaling, the transcription of *PIN* was differentially regulated (Li et al. 2005). In addition, BRs enhance plant tropistic responses by promoting the accumulation of the PIN2 protein from the root tip to the elongation zone and stimulating the expression and dispersed localization of ROP2 during tropistic responses (Li et al. 2005).

3.5 Strigolactone-Mediated Regulation of PAT

During the last century, two key hypotheses have been proposed to explain apical dominance in plants: auxin promotes the production of a second messenger that moves up into buds to repress their outgrowth and auxin saturation in the stem inhibits auxin transport from buds, thereby inhibiting bud outgrowth (Crawford et al. 2010). Strigolactones (SLs), or their derivatives, were recently demonstrated to act as endogenous shoot branching inhibitors: auxin moving down the main stem inhibits branch activity by preventing the establishment of auxin transport out of axillary branches and that SLs act by dampening PAT, thus enhancing competition between branches (Crawford et al. 2010).

3.6 Jasmonate-Mediated Regulation of PAT

Previously, we have shown that jasmonate (JA) upregulates auxin biosynthesis and represses polar auxin transport during *Arabidopsis* lateral root formation and gravitropic growth (Sun et al. 2009). JA displays two distinct aspects of CORONATINE INSENSITIVE 1 (COI1)- and AUXIN RESISTANT 1 (AXR1)-dependent effects on PIN2 subcellular distribution: at lower concentration, JA inhibits PIN2 endocytosis, whereas, at higher concentration, JA reduces PIN2 accumulation in the PM. Mutations of *ASA1* (*ANTHRANILATE SYNTHASE a1*) and the *TIR1/AFBs* auxin receptor genes impair the inhibitory effect of JA on PIN2 endocytosis, suggesting that a lower concentration of JA inhibits PIN2 endocytosis through interaction with the auxin pathway. In contrast, mutations of *ASA1* and the *TIR1/AFBs* auxin receptor genes enhance, rather than impair, the reduction effect of JA on the PM accumulation of PIN2, suggesting that this action of JA is independent of the auxin pathway. In addition to the JA effects on PIN2 endocytosis and PM residence, JA also alters lateral auxin redistribution in response to gravistimulation and therefore impairs the root gravitropic response (Sun et al. 2011). Our studies highlight the importance of JA–auxin interaction in the coordination of plant growth and the adaptation response.

3.7 ABA-Mediated Regulation of PAT

Lateral root (LR) formation is promoted by auxin and inhibited by cytokinin and abscisic acid (ABA). A recent study showed that mutation of *ABSCISIC ACID INSENSITIVE4* (*ABI4*), which encodes an ABA-regulated AP2 domain transcription factor, results in an increased number of LRs. Expression of the auxin-efflux carrier protein PIN1 is reduced in the *ABI4* overexpressors, enhanced in the *abi4* mutants, and is less sensitive to inhibition by CK and ABA in the *abi4* mutants than in the wild-type plants. Transport levels of exogenously applied auxin were elevated in the *abi4* mutants and reduced in the *ABI4* overexpressors. Therefore, ABI4 mediates ABA and CK inhibition of LR formation via reduction of PAT (Shkolnik-Inbar and Bar-Zvi 2010).

3.8 Ethylene-Mediated Regulation of PAT

Root growth is controlled by the coordinated action of several phytohormones, including auxin and ethylene. Recent studies showed that the effect of ethylene on root growth is largely mediated by the regulation of the auxin biosynthesis and transport-dependent local auxin distribution (Ruzicka et al. 2007; Stepanova et al. 2007; Swarup et al. 2007). Ethylene stimulates auxin biosynthesis and basipetal

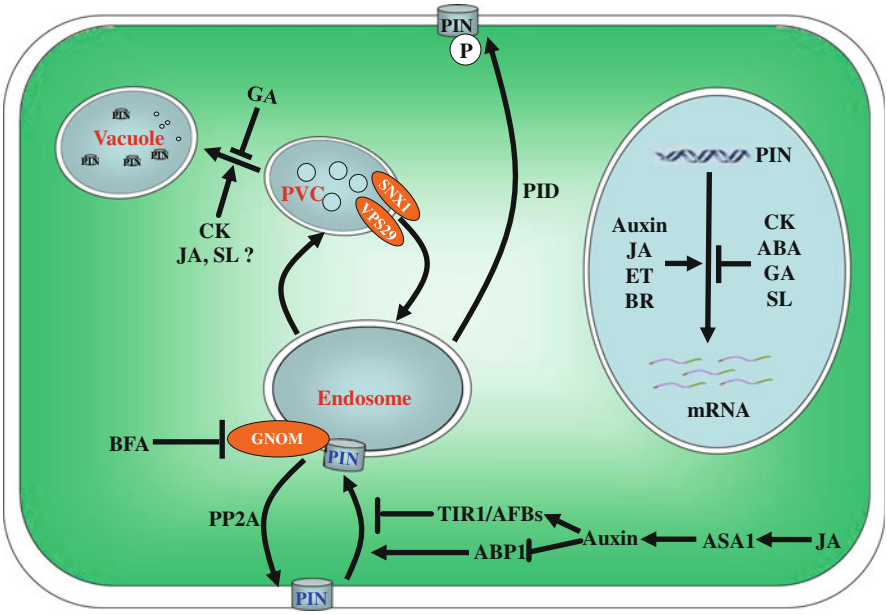

Fig. 1 Hormonal regulation of PIN proteins. Generally, hormones regulate PIN proteins at both transcriptional and posttranscriptional levels. Transcriptionally, auxin, JA, ET, and BR have been demonstrated to have positive effects on the expression of *PINs*, while CK, ABA, GA, and SL have negative effects. At the posttranscriptional level, PIN proteins cycle continuously between endosomal compartments and the plasma membrane. The endocytosis occurs in a clathrin-dependent manner, while the exocytotic step requires the activity of GNOM, an ADP-ribosylation factor GTPase guanine nucleotide exchange factor (ARF-GEF). The reversible phosphorylation of PIN proteins by the PINOID kinase (PID) and protein phosphatase 2A (PP2A) is important for the decision about apical or basal targeting of PIN proteins. Auxin inhibits PIN endocytosis through TIR1/AFBs auxin signaling pathways or affecting the ABP function in endocytosis. Lower concentrations of JA also inhibit endocytosis of PIN proteins through upregulation of ASA1-dependent auxin biosynthesis. CK and GA have contrasting effects on the PIN abundance. CK regulates endocytic recycling of PIN1 by redirecting it for lytic degradation in vacuoles. GA deficiency promotes, at least in the case of PIN2, the targeting of PIN proteins for vacuolar degradation. JA and SL reduce the PIN protein abundance at the plasma membrane posttranscriptionally through the unknown mechanisms. *JA* jasmonates, *ET* ethylene, *BRs* brassinosteroids, *CK* cytokinin, *ABA* abscisic acid, *GA* gibberellins, *SLs* strigolactones, *PVC* pre-vacuolar compartments

auxin transport towards the elongation zone, where it activates a local auxin response leading to inhibition of cell elongation. Consistently, in mutants affected in auxin biosynthesis (*wei2* and *wei7*) or basipetal auxin transport (*pin2* and *aux1*), ethylene cannot activate the auxin response nor regulate the root growth. In addition, ethylene modulates the transcription of several components of the auxin transport machinery (Ruzicka et al. 2007). Thus, ethylene achieves a local activation of the auxin signaling pathway and regulates root growth by both stimulating the auxin biosynthesis and modulating the auxin transport machinery.

4 Conclusions

The principal auxin transporters PIN can be regulated at multilevels, i.e., transcription, polarity, endocytosis, exocytosis, recycling, transcytosis, and degradation. The complexity and dynamic turnover of PIN provide multiple interaction nodes between auxin and other hormones, furnishing plants with great developmental flexibility and environmental plasticity. As discussed here, present studies have demonstrated that hormones can regulate PIN proteins at both transcriptional and posttranscriptional levels (Fig. 1). However, most of these studies are relatively limited to the alteration of PIN transcription or plasma membrane abundance, and the detailed cellular and molecular mechanisms underlying how hormones regulate PIN proteins remain largely unclear.

Acknowledgements This work was supported by grants from the Ministry of Science and Technology of China (2011CB915400) and the National Natural Science Foundation of China (31030006, 91117013).

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