Regulation of the Arabidopsis AGC kinase PINOID by PDK1 and the microtubule cytoskeleton

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PIN-DRIVEN POLAR AUXIN TRANSPORT IN PLANT DEVELOPMENTAL PLASTICITY: A KEY TARGET FOR ENVIRONMENTAL AND ENDOGENOUS SIGNALS

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Summary

Plants master the art of coping with environmental challenges in two ways: on the one hand, through their extensive defense systems, and on the other, by their developmental plasticity. The plant hormone auxin plays an important role in a plant's adaptations to its surroundings, as it specifies organ orientation and positioning by regulating cell growth and division in response to internal and external signals. Important in auxin action is the family of PIN-FORMED (PIN) auxin transport proteins that generate auxin maxima and minima by driving polar cell-to-cell transport of auxin through their asymmetric subcellular distribution. Here, we review how regulatory proteins, the cytoskeleton, and membrane trafficking affect PIN expression and localization. Transcriptional regulation of *PIN* genes alters protein abundance, provides tissue-specific expression, and enables feedback based on auxin concentrations and crosstalk with other hormones. Post-transcriptional modification, for example by PIN phosphorylation or ubiquitination, provides regulation through protein trafficking and degradation, changing the direction and quantity of the auxin flow. Several plant hormones affect PIN abundance, resulting in another means of crosstalk between auxin and these hormones. In conclusion, PIN proteins are instrumental in directing plant developmental responses to environmental and endogenous signals.

Introduction

Plant development is flexible and indeterminate in nature. This is in contrast to animal development, where at birth the young animal has acquired most, if not all, of the organs and limbs, and thus resembles the adult organism. During plant embryogenesis, only the basic body plan is laid down, and the shape of the adult plant differs considerably from that of the embryo. As sessile organisms, plants have acquired two important features that allow them to adapt and optimize their architecture to (changes in) their environment. The first comprises groups of stem cells organized in meristems in the root and the shoot apex that continuously produce new cell files and organs, respectively. The second is a plethora of signaling pathways that allow plants to accurately monitor their environment and to adapt their growth in response to external stimuli.
Based on observations on the bending of oat coleoptiles in response to directional light, Charles Darwin and his son concluded that something in the coleoptile tip was acted upon by light, resulting in bending of the coleoptile (Darwin & Darwin, 1880). These initial observations led to the identification of the responsible compound in this process, the plant hormone IAA, which was named auxin after the Greek word *auxein* for 'to grow' (Went, 1926; Kögl & Haagen-Smit, 1931). Intensive research on this plant hormone has revealed that auxin instructs plant development by regulating very basic processes such as cell division, growth, and differentiation in a concentration-dependent manner. This research has also unraveled a unique characteristic of auxin, its polar cell-to-cell transport, which acts in concert with auxin biosynthesis and metabolism to generate dynamic auxin maxima and minima that direct plant development and growth. The differential auxin concentrations are subsequently sensed and translated into a cellular response by complex signaling networks (Perrot-Rechenmann, 2010; Vernoux *et al.*, 2010; Ruiz Rosquete *et al.*, 2012). In this review, we will briefly summarize what is known about auxin signaling and transport, and then focus on the PIN-FORMED (PIN) proteins that mediate and direct polar auxin transport (PAT), and how endogenous and external signals act on transcriptional and post-transcriptional mechanisms to regulate their activity.

Auxin action

Auxin is sensed at different subcellular locations

The response of a cell to a hormone is determined, on the one hand, by its concentration, and on the other, by the sensitivity of the cell to the hormone. The initial search for auxin receptors identified three auxin binding sites: in the nucleus, the endoplasmatic reticulum (ER) and at the plasmamembrane (PM). Auxin binding in the ER and at the PM appeared to be mediated by the same protein, the AUXIN BINDING PROTEIN1 (ABP1; Hertel *et al.*, 1972; Ray, 1977; Feldwisch *et al.*, 1992; Jones & Herman, 1993). The PM localization suggested that ABP1 mediates rapid cellular responses to auxin (Rück *et al.*, 1993), such as the induction of cell division and cell expansion (Steffens *et al.*, 2001; David *et al.*, 2007; Braun *et al.*, 2008; Dahlke *et al.*, 2010). Despite its early identification, the function of ABP1 as auxin receptor has remained unclear for many years. Although it is likely that ABP1 activates multiple signaling pathways, the most well established effect of ABP1 is its stimulatory role in clathrin-mediated endocytosis (Robert *et al.*, 2010) via the Rho of Plants (ROP) family of GTPases (Xu *et al.*, 2010, 2014; Chen *et al.*, 2012). Disruption of the ABP1-ROP signaling pathway results in different developmental defects depending on the strength of the knockdown, ranging from arrest of embryo development (Chen *et al.*, 2001) to defects in pavement cell (PC) interdigitation (Xu *et al.*, 2010), leaf venation patterning, and gravitropic responses (Wang *et al.*, 2013).
A second receptor was initially identified through a mutation in the Arabidopsis *TRANSPORT INHIBITOR RESISTANT 1* (*TIR1*) gene (Ruegger *et al.*, 1998), but its function as auxin co-receptor acting in the nucleus to regulate auxin-responsive gene expression was uncovered much later (Dharmasiri *et al.*, 2005a; Kepinski & Leyser, 2005). TIR1 was found to act redundantly with five homologous AUXIN-RESPONSIVE F-BOX (AFB) proteins (Dharmasiri *et al.*, 2005b). Auxin-responsive gene expression is mediated by two classes of proteins: the DNA-binding auxin response factors (ARFs) that either activate or repress transcription, and the Aux/IAA family of transcriptional repressors (Fig. 1). In Arabidopsis, the ARFs comprise a family of 23 proteins, most of which have four conserved domains (Remington *et al.*, 2004; Okushima *et al.*, 2005). The DNA-binding domain at the N-terminus allows the ARFs to bind to the TGTCxC core sequence containing auxin response elements (AuxREs) in

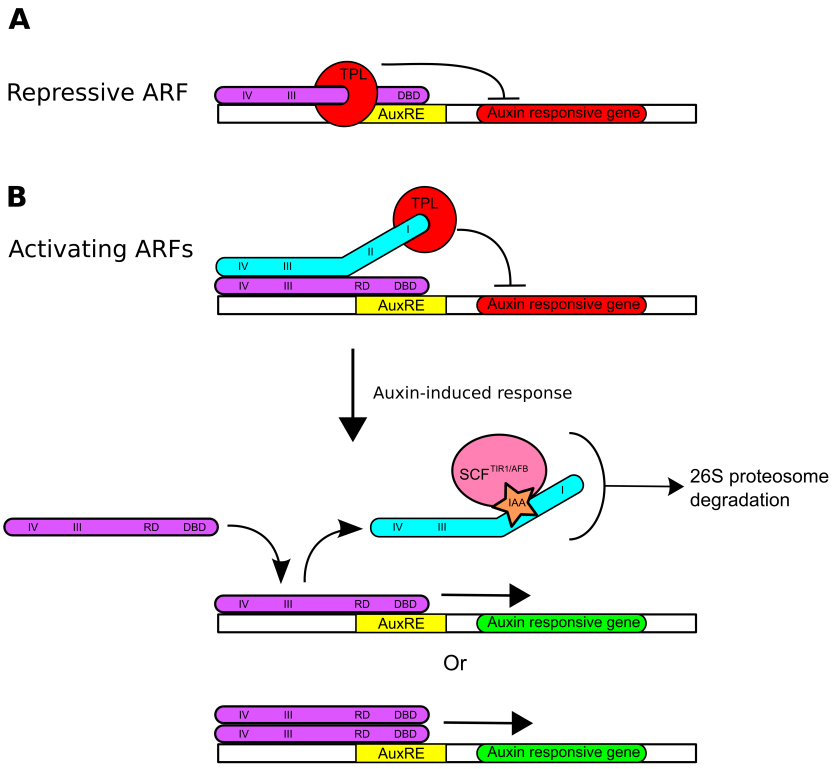

Figure 1: Model of the regulation of auxin responsive gene expression by the auxin response factor (ARF) transcription factors and Aux/IAA repressor proteins. Two types of ARFs exist: repressive (a) and activating (b). (a) Repressive ARFs are thought to block gene expression when bound to auxin-responsive elements (AuxREs) through their interaction with TOPLESS (TPL). (b) Activating ARFs block gene expression while forming a dimer with an Aux/IAA protein in complex with TPL. In the presence of auxin, the TRANSPORT INHIBITOR RESISTANT 1 (TIR1) receptor and the Aux/IAA coreceptor form a complex, leading to Aux/IAA ubiquitination and its targeting for degradation by the 26S proteasome. The ARFs remaining at the AuxRE can then promote auxin-responsive gene transcription as monomer or dimer. DBD, DNA-binding domain; RD, regulatory domain; SCF, SKP1-LIKE CULLIN1 AND F-BOX protein complex; AFB, AUXIN-RESPONSIVE F-BOX PROTEIN; IAA, auxin.

the promoters of auxin-responsive genes (Ulmasov *et al.*, 1995, 1997). The middle domain is involved in either activating or repressing gene expression, depending on the amino acid residues present (Ulmasov *et al.*, 1999a). At the C-terminus, the conserved domains III and IV are located, which are found in both ARFs and Aux/IAA proteins and are involved in dimerization with other ARFs or with Aux/IAA proteins (Ulmasov *et al.*, 1999b; Tiwari *et al.*, 2003). Aux/IAA proteins are encoded by a family of 29 genes in Arabidopsis (Liscum & Reed, 2002). Apart from

the conserved C-terminal domains III and IV involved in protein–protein dimerization, the N-terminal domain I in most Aux/IAA proteins contains an ERF-associated Amphiphilic Repression (EAR) motif (LxLxL) that is required for binding of the transcriptional corepressors TOPLESS or the four TOPLESS RELATED proteins (TPL/TPRs). Aux/IAAs need TPL binding for their repressing function (Tiwari *et al.*, 2004; Szemenyei *et al.*, 2008).
The middle domain II of Aux/IAA proteins is involved in protein stability and is the binding target for the TIR1/AFB F-box proteins. Together with an SKP1-like protein and CULLIN1, the TIR1/AFB proteins form the E3 ubiquitin protein ligase $SCF^{TIR1/AFB}$. Auxin promotes the interaction between TIR1/AFBs and domain II of the Aux/IAA coreceptors (Tan *et al.*, 2007; Calderón Villalobos *et al.*, 2012), thereby recruiting the Aux/IAA proteins for ubiquitination and subsequent degradation by the 26S proteasome (Dos Santos Maraschin *et al.*, 2009). After Aux/IAA degradation, the ARF remaining at the AuxRE in a promoter region can then activate the downstream gene either as a monomer or as a dimer with another ARF protein (Ulmasov *et al.*, 1999b; Tiwari *et al.*, 2003). Recently, TPL/TPR proteins were shown to interact with several repressive ARFs, suggesting that TPL/TPR proteins act in both Aux/IAA- and ARF-mediated transcriptional repression (Causier *et al.*, 2012).

Polar auxin transport-generated auxin maxima and minima

As described earlier, the response of a cell to auxin is, for the most part, determined by the concentration of the hormone in the cell, which, in addition to auxin biosynthesis and metabolism, is determined by polar cell-to-cell transport of auxin. PAT is a complex process that is mediated by at least three types of transporters. In line with the chemiosmotic hypothesis proposed for PAT (Rubery & Sheldrake, 1974; Raven, 1975), in the relatively acidic environment of the apoplast *c.* 15% of the auxin molecules are in the protonated state (IAAH), which allows auxin to pass the PM by diffusion. However, the majority of auxin is in the deprotonated form (IAA^-) and requires active uptake by the AUXIN1/LIKE-AUX1 (AUX/LAX) import carriers (Bennett *et al.*, 1996; Swarup & Péret, 2012). In the more alkaline cytosol, auxin molecules are deprotonated and the resulting anions can only pass the PM with the help of auxin efflux carriers. Polar placement of such carriers in the PM at the same side of a row of cells thus leads to polar cell-to-cell transport.

To date, two classes of auxin efflux carriers have been identified: the family of PIN proteins, consisting of eight members in Arabidopsis (Friml *et al.*, 2003); and the ABC-B/MULTI-DRUG RESISTANT/P-GLYCOPROTEIN (ABCB/MDR/PGP) transporters that belong to a subfamily of 20 proteins in Arabidopsis (Kaneda *et al.*, 2011).

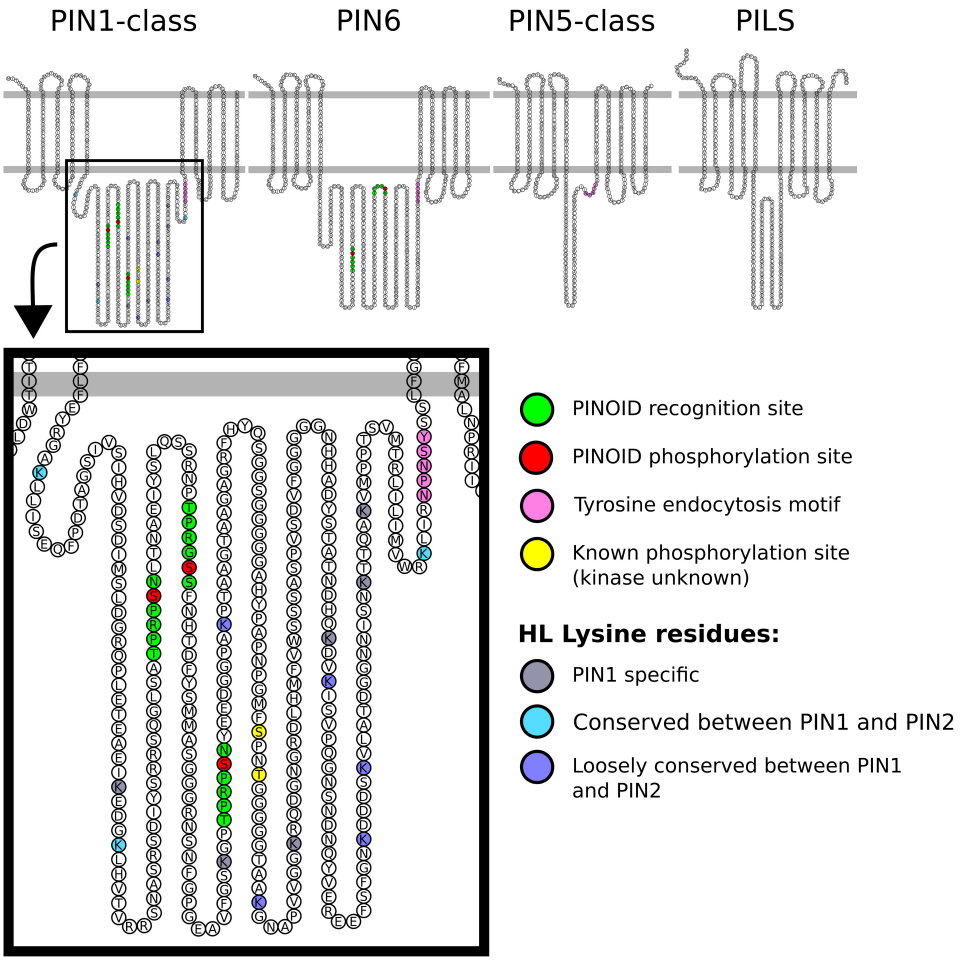

Figure 2: Schematic representation of the protein structures of PIN1 (representing the PIN1-class), PIN6, PIN5 (representing the PIN5-class), and PILS2 (representing the PILS family). Important amino acids in the PIN1 hydrophilic loop (HL) are color-coded, including the lysines and the five serine/threonine residues whose phosphorylation by PINOID, WAG1 or WAG2 (red) or other unknown kinases (yellow) has been shown to direct PIN polarity. HL, hydrophilic loop.

Arabidopsis ABCB family members were identified as auxin transporters because loss-of-function mutants showed auxin-related developmental

defects and reduced auxin transport, and the proteins were found to bind to the PAT inhibitor 1-naphthylphthalamic acid (NPA; Noh *et al.*, 2001; Geisler *et al.*, 2005). For ABCB1 and -19 the role of nonpolar auxin efflux carriers has now been well established, and they are thought to act in auxin transport channels to regulate the intracellular auxin available for the polar transport pathway (Petrášek *et al.*, 2006; Mravec *et al.*, 2008). More recent data suggest that ABCB14 and ABCB15 act as auxin efflux carriers in this pathway as well (Kaneda *et al.*, 2011), whereas ABCB4 seems to act as both an auxin influx carrier and an auxin efflux carrier, depending on the intracellular auxin concentrations (Kubeš *et al.*, 2012).
In contrast to the nonpolarly localized ABCB proteins, five of the Arabidopsis PIN proteins do show asymmetric localization at the PM (Gälweiler *et al.*, 1998; Müller *et al.*, 1998; Friml *et al.*, 2002a,b, 2003). Because the action of these PIN proteins appeared to be rate-limiting (Petrášek *et al.*, 2006), and their subcellular distribution at the PM correlated well with the direction of PAT (Benková *et al.*, 2003; Wiśniewska *et al.*, 2006), these PIN proteins are now considered to be the auxin efflux carriers proposed in the chemiosmotic model, driving and channeling polar cell-to-cell auxin transport. PIN proteins typically consist of two hydrophobic, transmembrane regions, interrupted by a short or long hydrophilic loop (HL, Fig. 2). All PM localized PINs have a long HL, and are referred to as PIN1-type or long PIN proteins (Viaene *et al.*, 2013). The importance of these long PIN proteins in their contribution to PAT is shown by loss-of-function mutants. Of the single mutant alleles, only those of the founding *PIN-FORMED/PIN1* gene show strong defects in development, with needle-like inflorescences that lack lateral organs (Gälweiler *et al.*, 1998), whereas mutations in *PIN2* and *PIN3* only reduce the ability of plants to respond to external signals, such as gravity and light (Luschnig *et al.*, 1998; Friml *et al.*, 2002b). By combining mutations in three to four *PIN* genes, very severe defects in early embryogenesis are obtained, on the one hand indicating strong functional redundancy between *PIN* genes and, on the other, corroborating the crucial role of PIN-mediated PAT in plant development (Friml *et al.*, 2003; Blilou *et al.*, 2005). The long PINs are often asymmetrically distributed over the PM (PIN1, PIN2, PIN4 and PIN7) or are able to polarize after external stimulation (PIN3; Tanaka *et al.*, 2006).

An ancient role for the endoplasmatic reticulum in controlling auxin action

The other three members of the PIN family in Arabidopsis, PIN5, PIN6 and PIN8, localize to the ER and, in some cell-types to the PM (Mravec *et al.*, 2009; Ganguly *et al.*, 2010, 2014; Dal Bosco *et al.*, 2012; Ding *et al.*, 2012; Sawchuk *et al.*, 2013). PIN5 and PIN8 are classified as short PINs, based on the length of their HLs (Viaene *et al.*, 2013), and their predominant ER localization suggests that PM localization of the long PINs is promoted by sequences in their long HL. A conserved tyrosine motif (NPXXY) present at the C-terminal end of the HL has been suggested as a possible interaction site for adapter proteins during clathrin-mediated endocytosis (Zažímalová *et al.*, 2007). That this motif is also conserved in the HL of PIN5 and PIN8 (Fig. 2), is in line with the recently observed clathin-mediated endocytosis of these short PINs in young root epidermis cells, where they localize to the PM (Ganguly *et al.*, 2014).

An *in silico* screen for proteins with homology and a similar topology to the PIN family members in Arabidopsis has identified seven ER-localized PIN-LIKES (PILS) proteins (Barbez *et al.*, 2012; Fig. 2). Despite the limited sequence similarity with PINs, PILS proteins contain the InterPro auxin carrier domain that is also present in PINs, and for PILS2 and PILS5, evidence of auxin transport activity has been obtained. The fact that they, and not the PIN proteins, occur in unicellular algae, suggests that PILS are evolutionarily older than PINs (Feraru *et al.*, 2012).

In contrast to the obvious function of the PM-localized PINs as drivers of PAT, the role of the ER-localized auxin transporters (PINs and PILS) is not yet clear. Several auxin-conjugating enzymes have been reported to localize in the ER (Bartel & Fink, 1995; Woodward & Bartel, 2005), and both phenotypic analysis and IAA metabolic profiling of lines overexpressing the ER-localized auxin transporters have indicated that they seem to act antagonistically (PIN6 and PIN8, efflux; PIN5 and PILS, influx) in controlling auxin homeostasis, and thus the amount of free auxin available in the cytosol for PAT, or in the nucleus for auxin response (Mravec *et al.*, 2009; Barbez *et al.*, 2012; Ding *et al.*, 2012; Sawchuk *et al.*, 2013). Two mechanisms have been proposed for a possible feedback on the action of ER-localized PINs in controlling auxin homeostasis and signaling. The first mechanism relates to the observation that the majority of the ABP1 protein pool is located in the ER and could potentially

regulate the activity or trafficking of the ER-localized PINs. However, at a pH of 7, ABP1 has been reported to bind auxin inefficiently, making it unlikely that auxin triggers ABP1 signaling in the ER (Ray, 1977; Jones & Herman, 1993; Tian *et al.*, 1995). Another possibility is that direct transport of auxin from the ER into the nucleus via PIN6/8 (Sawchuk *et al.*, 2013) could possibly provide feedback control on auxin homeostasis through TIR1/AFB signaling.

For the PILS in unicellular algae, the most obvious function would be regulation of auxin homeostasis. For multicellular systems, however, mathematical modeling of ER-localized auxin influx and efflux carriers, together with the feedback systems described earlier, has predicted that intracellular auxin retention in the ER, combined with controlled release in the cytosol/nucleus, could lead to canalization of auxin transport, giving rise to localized auxin maxima (Wabnik *et al.*, 2011). Interestingly, this model is supported by recent data suggesting that ER-localized PINs generate tissue-specific context and enhance PAT during vein patterning in leaves (Sawchuk *et al.*, 2013). Whether the observed partial PM localization of PIN5, PIN6 and PIN8 is important for their role in vein patterning, is currently unclear (Ganguly *et al.*, 2010, 2014).

PIN regulation by a complex network of feedback loops

Transcriptional regulation of PIN abundance: a matter of redundancy

Detailed expression studies have shown that each of the individual Arabidopsis *PIN* genes shows a specific expression pattern and that, in developmental processes such as embryogenesis or root growth, multiple PINs act in concert to generate and maintain dynamic auxin maxima that steer development and growth (reviewed in Tanaka *et al.*, 2006; Křeček *et al.*, 2009). For most *PIN* genes their expression pattern only partially correlates with the developmental defects observed in corresponding loss-of-function mutants (Gälweiler *et al.*, 1998; Friml *et al.*, 2003; Scarpella *et al.*, 2006).

In various single and multiple *pin* loss-of-function mutants, PIN proteins were found to be ectopically expressed, most likely because of the imbalance in auxin homeostasis (Blilou *et al.*, 2005; Vieten *et al.*, 2005; Rigas *et al.*, 2013). Pronounced embryo defects were only observed in quadruple *pin* mutant combinations that included *pin4* and *pin7* (Friml

et al., 2003; Blilou *et al.*, 2005). This shows that there is a molecular mechanism that uses the redundancy of the PIN proteins to overcome the effects of these mutations to some extent.

An important part of this redundancy is mediated by the auxin responsiveness of *PIN* expression. Vieten *et al.* (2005) used heat shock promoter-driven dominant *axr3/iaa17* or *solitary-root-1*(*slr-1*)/*iaa14* mutant genes to suppress auxin-responsive expression of the five 'long' *PIN* genes. This confirmed that these *PIN* genes are regulated through the Aux/IAA and ARF system (Vieten *et al.*, 2005). In addition, *PIN1* expression was found to be regulated by MONOPTEROS(MP)/ARF5 (Wenzel *et al.*, 2007), which interacts with and is repressed by BODENLOS (BDL)/IAA12 (Hamann *et al.*, 2002). A recently described dominant mutant allele of *MP autobahn*, of which the encoded protein (MP^{abn}) no longer interacts with BDL, suggests that the MP–BDL interaction not only restricts *PIN1* expression, but also determines PIN1 asymmetric localization to canalize PAT during vascular development (Garrett *et al.*, 2012). Although the authors do not rule out the possibility that PIN apolarity is a result of its enhanced expression, the proposed second regulatory role of the MP–BDL complex might correspond to the observed canalization of PAT by ARF-Aux/IAA-dependent feedback on PIN polarity (Sauer *et al.*, 2006).

We used known Arabidopsis *PIN* promoter sequences to detect putative AuxREs (Fig. 3). Surprisingly we did not find a clear correlation between the number of AuxREs in an upstream region and the reported auxin responsiveness of the corresponding gene. For example, *PIN1*, *PIN3*, and *PIN7* all react strongly to auxin application (Vieten *et al.*, 2005), whereas the *PIN3* and *PIN7* promoters contain much more known AuxREs compared with the *PIN1* promoter (Fig. 3). A possible explanation might lie in the presence of as yet uncharacterized AuxREs in the *PIN1* promoter, and also possibly in the recent finding that efficient DNA binding and dimerization of ARFs depend on the distance between two AuxREs (Boer *et al.*, 2014). Remarkably, in the shoot apical meristem of the *pin1* mutant, the expression of other *PIN* genes was not found to be elevated (Guenot *et al.*, 2012), suggesting that feedback regulation of auxin on *PIN* transcription does not work in every tissue.

Another group of transcription factors that is known to regulate *PIN* expression are the BABY BOOM (BBM)/PLETHORA (PLT) AP-2 domain transcription factors (Boutilier *et al.*, 2002; Blilou *et al.*, 2005;

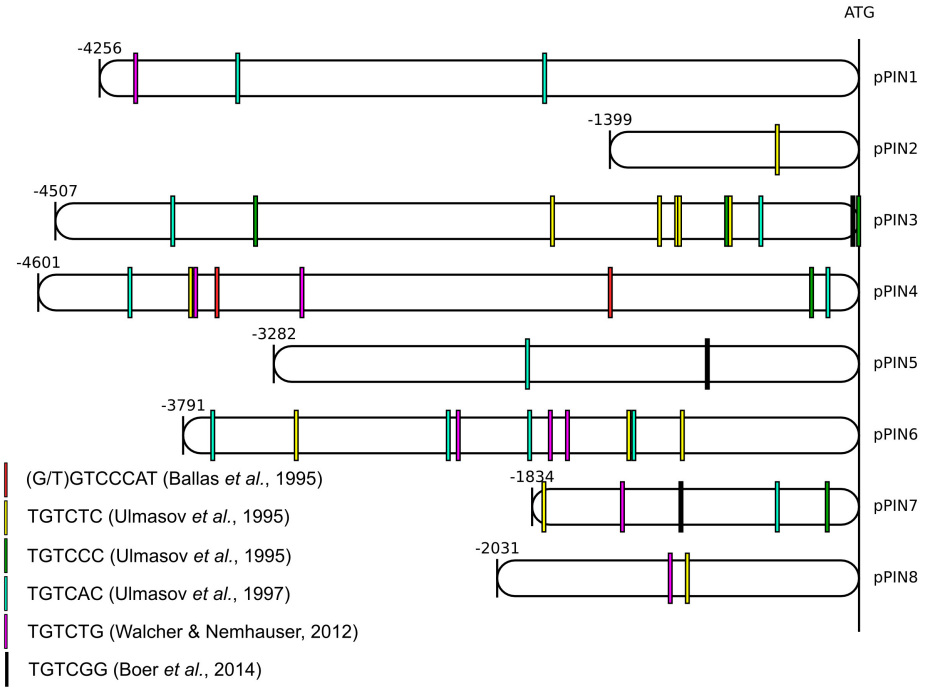

Figure 3: Schematic representation of the *Arabidopsis thaliana PIN* upstream regions, indicating the positions of putative auxin-responsive elements (AuxREs). The selected upstream regions are from the stop codon of the upstream gene until the *AtPIN* ATG start codon.

Galinha *et al.*, 2007). These transcription factors play an important role in maintaining the stem cell niche and in tissue patterning. In the embryo and the root meristem, PIN proteins restrict ARF-mediated *PLT* gene expression, and in turn PLTs act in concert with the SCARECROW (SCR) and SHORT ROOT (SHR) transcription factors to determine which *PIN* genes are expressed, thereby providing reciprocal regulatory loops between auxin and the PLTs (Blilou *et al.*, 2005; Xu *et al.*, 2006). Initial observations suggested that PLT3, PLT5, and PLT7 are involved in phyllotactic patterning in the shoot apical and inflorescence meristems by enhancing *PIN1* gene expression (Prasad *et al.*, 2011). More recently, evidence was obtained that PLTs are required for phyllotactic patterning by activating auxin biosynthesis in the center of the inflorescence meristem, suggesting that PLTs do not necessarily directly regulate *PIN* gene expression (Pinon *et al.*, 2013). It will be important to determine whether

BBM/PLTs directly bind the promoters of *PIN* genes.

Regulation of PIN abundance and polarity by membrane trafficking

After the *PIN* genes are transcribed, the newly synthesized short PINs (including PIN6) are retained in the ER, and the long PINs traffic via the trans-Golgi network/early endosomes (TGN/EE) to the PM in a nonpolar fashion. At this point, the PIN proteins start to undergo continuous endocytosis and recycling back to the PM, a process that can coincide with transcytosis, and which is required for the establishment and maintenance of PIN polarity (Geldner *et al.*, 2001; Dhonukshe *et al.*, 2008, 2010; Fig. 4). PIN endocytosis occurs via clathrin-coated vesicles, and disrupting the clathrin machinery reduces endocytosis, which causes changes in auxin distribution and leads to developmental defects (Dhonukshe *et al.*, 2007; Kitakura *et al.*, 2011). Auxin was shown to interfere with PIN endocytosis and, as a result, to stabilize PINs at the PM, thereby enhancing auxin efflux (Paciorek *et al.*, 2005). This was shown to be mediated by the apoplastic ABP1: ABP1 normally stimulates endocytosis, and binding of auxin inhibits this activity. In this way, ABP1 provides a positive feedback loop by which exported auxin induces local stabilization of PINs at the PM, thereby enhancing auxin efflux at that same position (Robert *et al.*, 2010; Čovanová *et al.*, 2013).

PIN endocytosis, transcytosis, and recycling require the actin cytoskeleton and the action of specific ADP-ribosylation factor-(ARF)-type GTPases and the corresponding ARF-GTP exchange factors (ARF-GEFs). In general, recycling of PIN proteins to the basal (rootward) PM in root cells is dependent on the ARF-GEF GNOM, which is sensitive to the fungal toxin brefeldin A (BFA; Geldner *et al.*, 2001, 2003; Kleine-Vehn *et al.*, 2008a). Exposure of roots to high BFA concentrations results in accumulation of PIN proteins in large intracellular structures called 'BFA compartments'. PIN-loaded BFA compartments are readily formed in cells that show basal PIN localization, whereas only limited PIN cargo accumulates in BFA compartments in cells where PINs show apical (shootward) localization. Moreover, long-term exposure to intermediate BFA concentrations leads to transcytosis of basal PIN proteins to the apical PM of root cells, suggesting that transcytosis and apical recycling are mediated by BFA-insensitive ARF-GEFs (Kleine-Vehn *et al.*, 2008a).

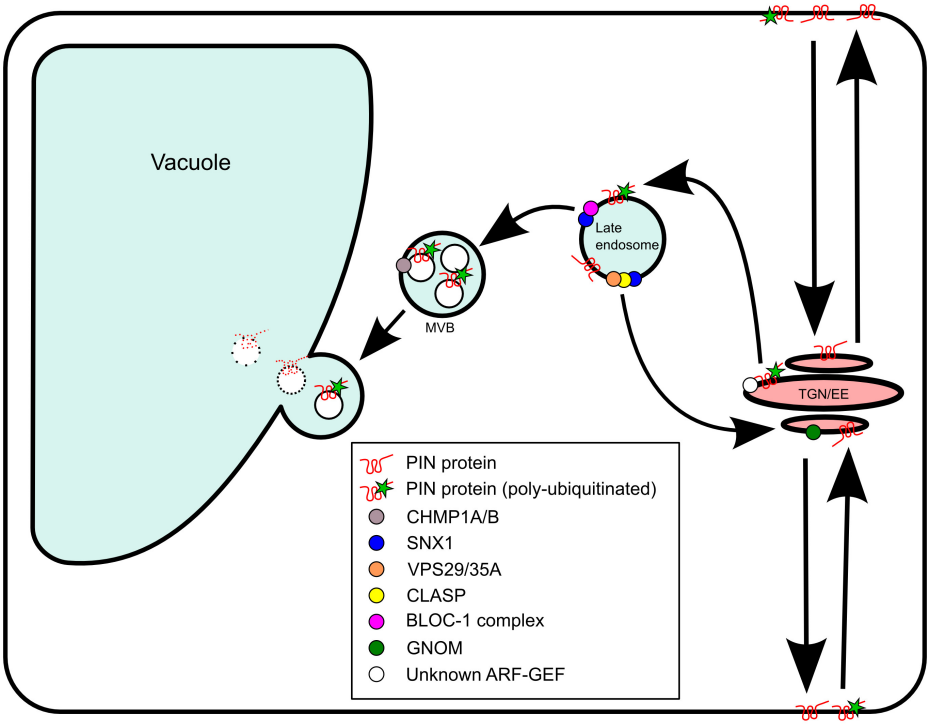

Figure 4: Regulation of PIN protein trafficking by phosphorylation and ubiquitination. Following their biosynthetic delivery via the trans-Golgi endosomes (TGN/EE) to the plasma membrane (PM), PIN proteins undergo continuous recycling between the PM and the TGN/EE. Unphosphorylated PINs, or those dephosphorylated by PP2A/PP6 phosphatase, are recycled to the PM by the brefeldin A (BFA)-sensitive ADP-ribosylation factor-guanine nucleotide exchange factors (ARF-GEF) GNOM, whereas phosphorylation of PIN proteins by PINOID (PID) results in their GNOM-independent recycling to the opposite PM. Monoubiquitination and subsequent polyubiquitination of PIN proteins induce their endocytosis, followed by trafficking from the TGN/EE to late endosomes, from where the SNX1/BLOC-1 complex mediates transfer to multivesicular bodies (MVBs) for vacuolar degradation. Alternatively, the SNX1/CLASP/VPS29/ retromer complex recruits PIN proteins from the late endosomes back to the TGN/EE. CHMP1A/B, CHARGED MULTIVESICULAR BODY PROTEIN/CHROMATIN MODIFYING PROTEIN 1A/B; SNX1, SORTING NEXIN 1; VPS29/35A, VACUOLAR PROTEIN SORTING 29/35A; CLASP, CLIP-ASSOCIATED PROTEIN; BLOC-1, BIOGENESIS OF LYSOSOME-RELATED ORGANELLES COMPLEX 1.

Reversible phosphorylation of PINs signals their polar subcellular distribution

Based on pharmacological experiments with suspension-cultured tobacco cells, it was concluded that protein phosphorylation is important

to sustain auxin efflux activity (Delbarre *et al.*, 1998). Support for this hypothesis was provided by the identification of the protein kinase PINOID (PID) as a positive regulator of PAT (Benjamins *et al.*, 2001). PID belongs to the plant-specific AGCVIII subfamily of the large family of AGC protein kinases (Christensen *et al.*, 2000; Benjamins *et al.*, 2001). The *PID* gene was named after the main phenotype of loss-of-function mutants, which develop pin-like inflorescences just like the *pin1* mutant. Other mutant phenotypes are seedlings with three instead of two cotyledons, defects in leaf venation, the altered floral phyllotaxis and the trumpet-shaped pistil in the few flowers that are formed (Christensen *et al.*, 2000; Benjamins *et al.*, 2001; Kleine-Vehn *et al.*, 2009). These defects were found to be caused by a shift in PIN1 polarity from the apical to the basal side of the cells. By contrast, *PID* overexpression resulted in a switch of basally localized PINs (PIN1, PIN2 and PIN4) to the apical PM of root cells, implying that PID activity is involved in apical PIN polarity establishment (Friml *et al.*, 2004). Serine residues in three conserved TPRXS(N/S) motives in the PIN hydrophilic loop have been identified as the targets for PID phosphorylation (Fig. 2), and expression of loss-of-phosphorylation or phosphomimic versions of PIN1-GFP or PIN2-VENUS in their respective mutant background demonstrated that PIN phosphorylation is essential and sufficient to direct PIN polarity (Dhonukshe *et al.*, 2010; Huang *et al.*, 2010).

Phylogenetic analysis of the kinase domains of the Arabidopsis AGCVIII kinases showed that PID clusters in the AGC3 clade together with three other protein kinases, these being WAVING AGRAVITROPIC ROOT1 (WAG1), WAG2, and an as yet uncharacterized kinase named AGC3-4 (Galván-Ampudia & Offringa, 2007). WAG1 and WAG2 were found to be involved in root waving (Santner & Watson, 2006) and to act redundantly with PID in apical polarity establishment of PIN2 in the root epidermis and lateral root cap to regulate (gravitropic) root growth, and of PIN1 in the protoderm of the embryo during cotyledon initiation. In line with their redundant action, WAG1 and WAG2 were found to phosphorylate the same serine residues in the PIN HL as PID (Dhonukshe *et al.*, 2010). While these three kinases show functional redundancy and have overlapping expression domains, they are also differentially expressed (Santner & Watson, 2006; Cheng *et al.*, 2008; Dhonukshe *et al.*, 2010) and a differential role for PID and WAG2 has been suggested in valve margin specification during Arabidopsis fruit development (Sorefan *et al.*, 2009).

Mass spectrometry analysis has identified several other amino acid residues in the PIN HL that are targets for phosphorylation (reviewed in Offringa & Huang, 2013), of which Ser337 and/or Thr340 in the PIN1 HL were shown to be important for PIN1 polarity establishment. As these residues are clearly not phosphorylated by PID, WAG1, or WAG2 (Dhonukshe *et al.*, 2010; Huang *et al.*, 2010; Zhang *et al.*, 2010), it is likely that they are targets of other kinases. A member of the Ca^{2+}/calmodulin-dependent kinase-related family CRK5 was able to phosphorylate the PIN2 HL, and the *crk5-1* mutant showed reduced PIN2 exocytosis, suggesting that phosphorylation of the CRK5 phosphorylation site enhances PIN2 exocytosis (Rigó *et al.*, 2013). Also the four D6 PROTEIN KINASES (D6PKs), which are members of the AGC1 subfamily of AGCVIII kinases, were found to phosphorylate the PIN HL *in vitro* (Galván-Ampudia & Offringa, 2007; Zourelidou *et al.*, 2009). Because the D6PKs do not affect PIN protein localization (Dhonukshe *et al.*, 2010), these kinases most likely target a different, possibly overlapping, set of serine/threonine residues than the AGC3 kinases. The fact that *d6pk* loss-of-function mutants show reduced auxin transport suggests that these kinases might be involved in regulating PIN auxin transport activity rather than polarity.

AGC3 kinases label PIN proteins following their nonpolar biosynthetic secretion to the PM, and this then leads to their asymmetric distribution through clathrin-dependent endocytosis, transcytosis, and recycling (Dhonukshe *et al.*, 2008, 2010). How the phosphorylation status of PIN cargo is perceived by the endomembrane trafficking system is currently unclear. The fact that D6 kinases are able to phosphorylate PIN proteins, most likely at different residues, but do not alter PIN polarity suggests that the PIN phosphorylation status is monitored by specific adaptor proteins that are able to distinguish which residues in cargo proteins are phosphorylated.

Apart from the AGC3 kinases, trimeric phosphatases were found to act antagonistically in determining the phosphorylation status of the PIN HL (Michniewicz *et al.*, 2007). Earlier research had shown that a mutation in a gene encoding a regulatory A subunit of a PP2A type phosphatase ROOTS CURL IN NAPHTHYLPHTHALAMIC ACID1 (RCN1/PP2A-A1) resulted in PAT-related root growth defects (Garbers *et al.*, 1996). Loss-of-function mutants in two of the three *PP2A-A* genes phenocopied some of the seedling phenotypes observed in *PID* overexpression lines and resulted in the same basal to apical shift of PIN polarity in the root (Michniewicz

et al., 2007). Initially, the PP2A-A subunits were shown not to be part of a typical PP2A holoenzyme, but rather to form a PP6-type heterotrimeric complex together with a PP6 catalytic (C) subunit (FyPP1 or FyPP3), and a SAPS domain-Like protein (SAL1-4) as a B subunit. Interestingly, yeast two-hybrid analysis suggested that SAL1 binding to the PIN HL was enhanced by phosphorylation (Dai *et al.*, 2012). More recent data, however, suggest that the PP2A-A subunits are promiscuous and that the PP2A holoenzyme might be specifically active during embryogenesis (Ballesteros *et al.*, 2013).

PIN trafficking regulated by environmental signals

AGC3 kinase and PIN polarity regulation by external signals

The amazing flexibility of plant development and growth is exemplified by the growth responses to external signals, such as light and gravity, through which a plant can optimize the position and orientation of its organs to its environment. AGC3 kinase-mediated PIN phosphorylation not only leads to apical targeting of PIN proteins for organ initiation in the embryo or in the inflorescence meristem, but is also required for proper root growth. *wag1 wag2* double mutant roots grow hyper-wavy on tilted agar plates, and *pid wag1 wag2* triple mutant roots are agravitropic (Santner & Watson, 2006; Dhonukshe *et al.*, 2010). The latter phenotype can be mimicked by expressing a nonphosphorylatable PIN2 S>A-YFP in the *pin2* loss-of-function mutant background, indicating that regulation of PIN2 polarity through phosphorylation by these kinases is important for gravitropic root growth. In addition, PID was also shown to play a role in phototropic response of the hypocotyl. In the dark, PIN3 was shown to be apolarly localized in the endodermis, and PHOT1-mediated signaling of unilateral blue light triggered a reduction in *PID* expression, resulting in a GNOM-dependent switch in PIN3 polarity to the inner-lateral PM, which initiates polar transport of auxin to the dark side (Ding *et al.*, 2011).
In the phototropism example, PID activity is regulated through its expression. Another way the activity of these kinases might be changed in response to internal and external signals is through their interacting regulatory proteins. For PID, several binding proteins have been identified, of which the calcium-regulated interaction with two calcium-binding proteins is very likely to link with signaling pathways that trigger calcium responses in the cell (Benjamins *et al.*, 2003). In addition, PID was found

to bind to, and to be phosphorylated by, the 3-phosphoinositide-dependent kinase 1 (PDK1), resulting in its hyperactivation (Zegzouti *et al.*, 2006a). PDK1 is an upstream regulator of several AGC kinases and involved in many developmental and stress-related processes (Bögre *et al.*, 2003; Zegzouti *et al.*, 2006b), making it difficult to deduce the *in vivo* significance of its interaction with PID.

Regulation of PIN PM abundance by gravity and light

When a seedling or plant is turned on its side, the shoot will bend up against the gravity vector (negative gravitropism), whereas the root will bend down with the gravity vector (positive gravitropism). In both cases, the growth response is the result of asymmetric auxin distribution, with elevated concentrations at the lower side of the tissue and reduced concentrations at the upper side. The mechanism behind gravity-induced asymmetric auxin distribution has been studied in most detail in roots. In a vertically oriented Arabidopsis root tip, apolar PIN3 and PIN7 redistribute auxin from the maximum in the collumella initials to the epidermis and lateral root cap, from where PIN2 drives the symmetric shootward-directed flow of auxin through the epidermis. Gravity stimulation of roots induces rapid polarization of PIN3 and PIN7 toward the lateral PM, resulting in enhanced auxin transport to the lower side of the root (Friml *et al.*, 2002b; Tanaka *et al.*, 2006; Kleine-Vehn *et al.*, 2010). This initial asymmetry in auxin distribution can already be observed a few minutes after gravity stimulation (Band *et al.*, 2012), and is significantly enhanced by strong post-translational regulation of PIN2 PM abundance. The reduced auxin concentrations destabilize PIN2 in the upper epidermis of the root, whereas the enhanced auxin concentrations in the lower epidermis cells stabilize PIN2 at the apical PM in an ABP1-dependent manner, resulting in canalization of auxin transport through the lower epidermis (Paciorek *et al.*, 2005; Abas *et al.*, 2006; Robert *et al.*, 2010). About 2h after gravity stimulation, when root bending has reached the 40° 'tipping point', the elevated auxin concentrations at the lower side now destabilize PIN2 in a $SCF^{TIR1/AFB}$-dependent way, thereby allowing auxin distribution to normalize again (Abas *et al.*, 2006; Band *et al.*, 2012; Baster *et al.*, 2013). It is well established that the turnover of PM proteins requires their ubiquitination, which triggers endocytosis and trafficking to the lytic vacuole for degradation (reviewed in Korbei & Luschnig, 2013). PIN2 is lysine-63-chain-ubiquitinated at multiple lysine residues in its hydrophilic

loop. Only when the majority of the lysines in the hydrophilic loop are substituted for arginines is PIN2 ubiquitination severely reduced, meaning that the mutant protein can no longer complement the *pin2* mutant, corroborating the idea that ubiquitination and vacuolar trafficking are relevant for PIN2 functionality. PIN2 alleles mimicking constitutive monoubiquitination were endocytosed, whereas vacuolar targeting was found to coincide with the formation of K63-linked polyubiquitin chains (Leitner *et al.*, 2012). When using Arabidopsis seedlings expressing a PIN2-GFP fusion, the turnover and vacuolar accumulation of this fusion protein can be nicely visualized by incubation in the dark, as the GFP moiety is stabilized in the vacuole under these conditions (Tamura *et al.*, 2003). At the same time, light stabilizes PIN2 at the PM, and by introducing the *PIN2:GFP* construct in different mutant backgrounds, it was shown that PIN2 turnover most likely involves the COP9 signalosome (CSN), the light-regulated COP1 ubiquitin E3 ligase and the basic helix–loop–helix transcription factor HY5. Dark-grown *PIN2:GFP* seedlings accumulate GFP in the vacuoles, and *cop9* mutants show reduced vacuolar GFP signal when grown in the dark, whereas *hy5* mutants show reduced PM-localized PIN2:GFP when grown in the light (Laxmi *et al.*, 2008). The involvement of the COP1 E3 ubiquitin ligase in PIN turnover was supported by the fact that *cop1* mutants show increased PIN1 and PIN2 PM localization and display a reduced gravitropic response (Sassi *et al.*, 2012).

The post-translational regulation of PIN2 is essential for the generation of a sufficiently strong asymmetric auxin distribution required for a full gravitropic growth response. This is demonstrated by the *pin2* mutant, where PIN1 is ectopically expressed in the root epidermis and cortex. Even though PIN1 in the *pin2* mutant is expressed in the PIN2 domain, where it shows the correct apical and basal polarity in the epidermis and cortex, respectively (Vieten *et al.*, 2005; Rigas *et al.*, 2013), it fails to restore the gravitropic root growth (Luschnig *et al.*, 1998). Moreover, ectopic PIN1 expression in the epidermis and cortex in *35S::PIN1* seedlings also leads to root agravitropic growth (Petrášek *et al.*, 2006). The reason that PIN2 is more sensitive to turnover than PIN1 could lie in the number of lysines in the HL (13 in PIN1 and 20 in PIN2) or in the entire protein (22 for PIN1 and 28 for PIN2). The fact that multiple lysine-to-arginine substitutions in PIN2 HL are necessary to obtain noncomplementing versions corroborates this hypothesis (Leitner *et al.*, 2012).

Recently, the small GOLVEN (GLV) peptides were identified to regulate PIN2 subcellular localization and influence root gravitropism (Whitford *et al.*, 2012). Arabidopsis contains 11 genes encoding GLV peptides, which are expressed in various domains throughout the plant (Fernandez *et al.*, 2013). After application of GLV peptides to roots, increased PIN2 PM membrane localization can be observed, while other GLV peptides cause PIN2 to accumulate in internal vesicles (Fernandez *et al.*, 2013). How these GLV peptides regulate PIN2 trafficking and what their function is in the gravitropic response remains to be shown. As for the GLV peptides, which are specifically expressed in the shoot, it would be interesting to see if these peptides could be linked to other external responses where auxin is involved, for example, phototropism.

PIN turnover: ubiquitination-driven sorting or anchoring

As described earlier, PIN ubiquitination has a dual role. Mono-ubiquitination triggers PIN endocytosis, and subsequent poly-ubiquitination labels PIN proteins for trafficking to and degradation in the lytic vacuole (Leitner *et al.*, 2012). Whether PINs labeled for degradation use the same endocytosis route as PINs that enter the recycling pathway is currently not clear.

For endocytosed PINs, the endosomal trafficking to the vacuole is at least partially separate from the normal recycling pathway (Jaillais *et al.*, 2007), and occurs GNOM-independently by another BFA-sensitive ARF-GEF (Kleine-Vehn *et al.*, 2008b) from the EEs via late endosomes (LEs) and multivesicular bodies (MVBs) to the vacuole (Fig. 4). LEs are labeled with the associated proteins SORTING NEXIN 1 (SNX1), VACUOLAR PROTEIN SORTING 29 (VPS29) and CLIP-ASSOCIATED PROTEIN (CLASP; Jaillais *et al.*, 2006, 2007; Ambrose *et al.*, 2013). VPS29 was found to interact with VPS35A and loss-of-function mutants show enhanced internal PIN accumulation, suggesting that VPS29 and VPS35A work in a complex in PIN vacuolar trafficking (Nodzyński *et al.*, 2013). Loss-of-function mutants in any of the corresponding genes show reduced PIN2 at the PM, indicating that SNX1, VPS29, and CLASP are part of the retromer that rescues PIN2 from degradation, thereby regulating its PM abundance (Kleine-Vehn *et al.*, 2008b; Ambrose *et al.*, 2013).

CLASP is a microtubule (MT)-associated protein involved in MT rescue and stabilization (Al-Bassam & Chang, 2011), but was also found to interact with SNX1 (Ambrose *et al.*, 2013). This suggests that the MT

cytoskeleton is important in preventing PIN degradation. PIN2-GFP seedlings treated with the MT-destabilizing drug oryzalin indeed show enhanced vacuolar GFP signal (Ambrose *et al.*, 2013), suggesting that CLASP and MT are important in retromer-mediated recycling of PIN proteins from the LEs via the TGN/EE back to the PM.

The mammalian BLOC-1 complex is involved in endosome trafficking from EE to lysosome-related organelles (Setty *et al.*, 2007). Two components of this complex, BLOS1 and BLOS2, were identified in Arabidopsis as interacting partners of SNX1. RNAi-mediated knockdown of BLOS1 resulted in increased PIN1 and PIN2 PM abundance (Cui *et al.*, 2010). These results suggest that the Arabidopsis BLOC-1 complex is involved in sorting the LEs to MVBs to enhance PIN degradation. At the same time, the results imply a dual function for SNX1, both in recycling PIN vesicles from the LE to the TGN/EE as part of the retromer complex, and in trafficking of PIN vesicles from the LE to the MVBs. Merging of MVBs with the vacuole exposes the PIN proteins to the lytic environment of the vacuole and causes their degradation (Fig. 4). However, if LEs were to merge directly with the vacuole, the PIN proteins would localize to the tonoplast instead of being degraded. This can be observed in double mutants in the *CHARGED MULTIVESICULAR BODY PROTEIN/CHROMATIN MODIFYING PROTEIN 1A* and *1B* (*CHMP1A* and *CHMP1B*) genes that fail to accumulate PIN LEs as lumenal vesicles of MVBs (Spitzer *et al.*, 2009).

Recent detailed analysis and modeling of PIN dynamics suggest that some PIN pools are in immobilized membrane fractions, and that PIN polarity is established by reducing diffusion and localizing endocytosis rather than through polar exocytosis (Kleine-Vehn *et al.*, 2011). One way in which PINs seem to be immobilized is by direct interaction with the cell wall, as genetic and pharmacological disruption of the cellulose matrix in the cell walls results in increased PIN diffusion and PIN polarity defects (Feraru *et al.*, 2011). In addition, some PIN-binding proteins have been identified that could reduce PIN turnover by enhancing PIN stability at the PM. For example, the interaction between ABCB19 and PIN1 (Blakeslee *et al.*, 2007; Titapiwatanakun *et al.*, 2009) was suggested to keep PIN1 in immobilized membrane fractions.

Other proteins that might keep PINs in nonmobile PM domains are the *MACCHI-BOU 4*/ENHANCER OF PINOID-Like (MEL)/NAKED PINS IN YUC MUTANTS (NPY) proteins. MEL/NPYs are typical scaffold

proteins that colocalize with the polarly localized PIN proteins in the epidermis of the embryo and root, and stabilize PIN polarity by reducing their internalization (Furutani *et al.*, 2011).

Regulation of PIN proteins by internal signals

Auxin-regulated PIN trafficking: the ABP1, AGC3 kinase, cytoskeleton module

Research into the influence of auxin on interdigitation of PCs yielded a pathway that involves ABP1, Rho GTPases and both the actin and microtubule cytoskeleton. Various mutants within known auxin-related genes show reduced interdigitation of PCs. External auxin application only rescues a subset of these mutants (Xu *et al.*, 2010).

After sensing auxin, the apoplastic ABP1 signals to the RhoGTP-ases ROP2 and ROP6 through its interaction with the PM-localized receptor-like transmembrane kinases (TMKs; Xu *et al.*, 2014). In leaf PCs, ROP2 and ROP6 activate ROP interactive CRIB motif-containing proteins RIC4 and RIC1, respectively (Xu *et al.*, 2010). ROP2/RIC4 stabilizes the actin cytoskeleton in the lobes (Fu *et al.*, 2002), reducing PIN1 endocytosis and thereby promoting PIN1 PM localization in the lobes (Nagawa *et al.*, 2012). ROP6 loads RIC1 onto the MT, causing it to promote MT ordering, and inhibiting exocytosis, thereby generating the indentations. By contrast, ROP2 removes RIC1 from the MT, possibly to enhance local outgrowth during lobe formation (Fu *et al.*, 2005).

With PIN1 being stabilized in the lobes, the exported auxin is sensed by ABP1, which again acts on ROP6 in the indentation of the opposite cell and back again on the ROP2 in the lobe. In roots, ROP6 seems to fulfill the role of ROP2, preventing PIN2 endocytosis by promoting actin stabilization (Chen *et al.*, 2012; Lin *et al.*, 2012). This is surprising, and suggests that the function of these ROPs can vary depending on the tissue, possibly by tissue-specific modulators of ROP function.

PIN regulation by ABP1, the ROPs, and the cytoskeleton during interdigitated patterning of PC seems to be integrated with the PIN polar targeting pathway of the AGC3 kinases and the PP2A phosphatases (Li *et al.*, 2011). In the PP2A phosphatase mutant *fypp1* and the *35S::PID* overexpression plants, PIN1 localization was shifted from the lobes to the indentations, resulting in PCs with a reduced number of lobes (Li *et al.*, 2011). This confirms that placement of PIN1 at the lobe tips is

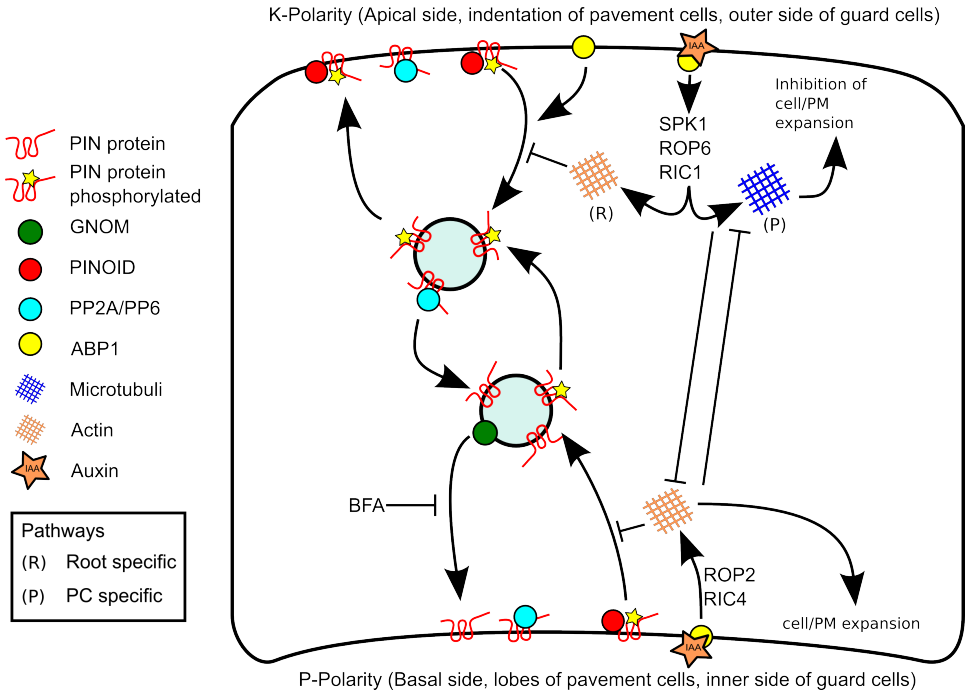

Figure 5: Combined model on the regulation of PIN trafficking by phosphorylation and the auxin binding protein 1/transmembrane kinase/Rho of Plants/ROP interactive CRIB-motif containing protein (ABP1/TMK/ROP/RIC) pathway. PIN proteins recycle continuously between the plasma membrane (PM) and trans-Golgi network/early endosomes (TGN/EE). Based on their phosphorylation status, which is determined by the antagonistic action of the PINOID kinase and PP2A/PP6 phosphatases, PIN proteins move either to the kinase (K)-polarity pole or the phosphatase (P)-polarity pole, respectively, through transcytosis and exocytosis. ABP1 acts on PIN endocytosis, dependent on the presence of auxin. Without auxin, ABP1 enhances PIN endocytosis. In the presence of auxin, ABP1 acts through TMK/ROP6/RIC1 or TMK/ROP2/RIC4 signaling to the actin cytoskeleton to inhibit PIN endocytosis. PP2A, PROTEIN PHOSPHATASE 2A; PP6, PROTEIN PHOSPHATASE 6; BFA, brefeldin A; PC, pavement cell.

important for proper indentation of PCs. Moreover, this suggests that there is a conserved mechanism where the AGC3 kinases and PP2A phosphatases regulate PIN polarity in all plant cells, but that the effect of PIN phosphorylation depends on the polarity field(s) in the cell (Fig. 5).

Regulation of PIN proteins by hormonal crosstalk

Apart from auxin, eight other plant hormones have been discovered, some of which are important in plant defense (salicylic acid and jasmonic acid), and others that have either a central (cytokinin

(CK), brassinosteroids (BRs)) or a more specific role (ABA, GA, strigolactones (SLs), ethylene) in directing plant development. Besides their well-established functions, a complex network of crosstalk has been uncovered between the signaling pathways of these hormones, and as part of this crosstalk, several hormones affect PIN action at either the transcriptional or the post-transcriptional level (Vanstraelen & Benková, 2012).

Strigolactones were initially identified as signaling molecules in symbiotic interaction between plants and arbuscular mycorrhizal fungi or parasitic weeds (Cook *et al.*, 1966; Akiyama *et al.*, 2005; Matusova *et al.*, 2005). Later, it was discovered that the same molecules are present in plants and that their amounts were reduced in the pea *ramosus* (*rms*), rice *dwarf* (*d*) and Arabidopsis *more axillary branching* (*max*) shoot branching mutants (Gomez-Roldan *et al.*, 2008; Umehara *et al.*, 2008). Over the years, two models emerged to explain the action of SLs. The first model proposes that a second messenger is produced in the main stem vasculature and transported upward into the bud, where it represses outgrowth. The second model involves the auxin canalization theory, where SLs reduce PIN abundance and basipetal PAT in the inflorescence stem, thereby inhibiting auxin efflux from the lateral buds (Bennett *et al.*, 2006). Various publications support the first model (Brewer *et al.*, 2009), including the discovery of an SL- and CK-responsive transcription factor that inhibits bud outgrowth (Braun *et al.*, 2012; Dun *et al.*, 2013). In favor of the second model, it was recently shown that SL application reduces PM levels of PIN1 by enhancing clathrin-mediated endocytosis (Crawford *et al.*, 2010; Shinohara *et al.*, 2013). This in turn would suppress the induction of canalized auxin transport from the buds, thereby maintaining their dormant state (Bennett *et al.*, 2006; Crawford *et al.*, 2010). In addition, it was shown that SLs promote root branching under phosphate-limiting conditions, by reducing PIN PM abundance in the root (Ruyter-Spira *et al.*, 2011).

Similar to SLs, CK application also resulted in a rapid reduction of PIN1:GFP abundance at the PM in lateral root primordia. In this case, an enhanced GFP signal could be observed in the vacuoles, when seedlings were incubated in the dark, suggesting that CK enhances PIN1 degradation. This regulation of PIN1 is mediated through the CK-responsive ARABIDOPSIS HISTIDINE KINASE 4 (AHK4), but not AHK2 and AHK3, and by B-type Arabidopsis response regulator (ARR)

components ARR2 and ARR12. Other PINs, such as PIN2 and PIN7, are not sensitive to CK, suggesting that this type of regulation is specific for PIN1 (Marhavý *et al.*, 2011). CK also represses *PIN* gene transcription. Upon CK detection, the AHK3 receptor relays the signal to ARR1 and ARR12, which activate SHY2/IAA3 and cause suppression of *PIN* expression (Dello Ioio *et al.*, 2008). Both *PIN1* and *PIN4* are down-regulated and *PIN7* is up-regulated by CK application (Růžička *et al.*, 2009). In line with these CK application experiments, genetic evidence was obtained by the analysis of the *auxin up-regulated f-box protein1* (*auf1*) mutant. The *AUF1* gene was found to be regulated by auxin, and AUF1 was found to act on ARR1, thereby forming a feedback loop between auxin and CK on PIN-mediated auxin transport (Zheng *et al.*, 2011). The analysis of the influence of CK is tricky, because ethylene is formed after CK application and ethylene is another hormone that influences *PIN* expression. An earlier publication reported that *PIN1*, *PIN2* and *PIN4* were found to be up-regulated by ethylene and that *PIN7* did not respond to the treatment (Růžička *et al.*, 2007). This is in strong contrast with the report of (Žádníková *et al.*, 2010) Žádníková *et al.* (2010), in which *PIN1* and *PIN4* were found to be down-regulated by ethylene and *PIN2* did not change expression. This discrepancy in observations could possibly be explained by the different tissues that were observed, in these cases being the root vs the apical hook.

Two other hormone families that show crosstalk with auxin by affecting PIN stability are GAs and BRs. Auxin is known to promote the GA-mediated degradation of DELLA proteins, thereby enhancing the cellular response to GA (Fu & Harberd, 2003), and in turn GA promotes the PM localization of PIN proteins. In various GA mutants, reduced amounts of PIN proteins are observed at the PM and the vacuolar targeting of PIN2:GFP is increased, whereas asymmetric GA distribution during root gravitropism is involved in decreasing PIN2 vacuolar targeting in the lower root epidermis (Willige *et al.*, 2011; Löfke *et al.*, 2013). BRs provide a delicate modulation to PIN abundance. Reduction of endogenous BRs by inhibiting BR synthesis increases *PIN2* and *PIN4* transcription, while supplying exogenous BRs causes a decrease in the expression of these *PIN* genes. In the BR receptor mutant *bri1*, however, a large reduction of both PIN2 and PIN4 can be observed, suggesting that BR signaling is required to prevent PIN turnover (Hacham *et al.*, 2012). This shows that BRs regulate PIN2 and PIN4 in the root at both the

transcriptional and post-transcriptional level.

Conclusions/future perspectives

As the initial identification of auxin is based on phototropic growth experiments of plant coleoptiles, it was clear from the start that this plant hormone plays an important role in adaptations of a plant's growth and development to environmental signals. In view of the central role of the polar transport-driven asymmetric distribution of auxin, it is not surprising that the PM-localized PIN auxin efflux carriers, and especially their post-translational regulation, are important targets for such signaling pathways. Several signaling pathways interfere with the post-translational modification of these PINs by phosphorylation or ubiquitination, thereby altering their PM abundance or polarity (Abas *et al.*, 2006; Michniewicz *et al.*, 2007; Dhonukshe *et al.*, 2008). Recently, ABP1-mediated PIN regulation through ROPs and the actin and microtubule cytoskeleton revealed another pathway that seems independent of PIN modification (Xu *et al.*, 2010; Chen *et al.*, 2012; Lin *et al.*, 2012). The fact that PID kinase activity can modulate the ABP1 pathway (Li *et al.*, 2011) suggests that the two pathways are likely to converge at some point. In the field of transcriptional regulation and hormonal crosstalk, a lot is still unknown. We know more or less when and where PIN proteins are expressed, but which factors exactly contribute to these expression patterns, and how their expression and subcellular distribution is regulated by environmental signals remain largely unknown. Several hormones (among which auxin itself) were not only shown to alter *PIN* transcription (Dello Ioio *et al.*, 2008; Hacham *et al.*, 2012), but also to influence the PIN abundance at the PM by modulating turnover of these auxin carriers (Crawford *et al.*, 2010; Willige *et al.*, 2011; Hacham *et al.*, 2012). A basic model starts to emerge on PIN turnover (Fig. 4) and over time this will be integrated into the model that describes the PIN endocytosis, polarity, and regulation by AGC3 kinases and ABP1/ROP/RIC (Fig. 5). Other regulators such as the GLV peptides, the MEL/NPYs and the AGC3 kinase binding proteins will most likely fit into a specific region of this model, as they are likely to function in specific developmental processes, or under specific stress conditions.

In this review, we have tried to cover the most important aspects of PIN regulation and to show the vast complexity of the regulatory

networks involved. These networks contain many feedback loops, and several mathematical models have been developed that describe PAT to help understand its complex regulation, and its function and dynamics in developmental processes such as vascular development, lateral root initiation, and phyllotaxis (van Berkel *et al.*, 2013). PIN-driven PAT is at the basis of plant developmental plasticity, and future models describing the control of these regulatory networks by different internal and external signals will allow the optimization of the development of crop plants to the growers' needs by tweaking their growth conditions.

Thesis outline

The review presented in this chapter provides the scientific basis for the other chapters in this thesis. Not only gives it the reader a solid background in understanding the experimental chapters, but it also shows that knowledge presented in this chapter is subject for new and somewhat controversial scientific insights. The best example for this can be found in **chapter 2**, where newly created *null abp1* lines show no embryo lethality, as observed in the original *abp1* mutant. We give an overview of the APB1 research until this finding and possible reasons for the observed discrepancies. **Chapter 3** describes that PDK1-mediated phosphorylation of PID causes its relocalization to the MT in protoplasts and that this effect can be copied or inhibited by creating mutant PID versions. These mutations can overcome to some degree the *pid wag1 wag2* embryo and adult phenotypes *in planta*, but we did not observe MT localization of the mutant proteins with confocal microscopy. **Chapter 4** shows a cellular mechanism that is responsible for the observed MT localization of PID after phosphorylation by PDK1. The family of BTB and TAZ domain scaffold (BT) proteins bind to PID and inhibit its phosphorylation function. Cotransfections of BT1 and PID result in a nuclear localized PID in protoplasts. The BT proteins also provide a bridge to the plant-specific At1 family of kinesins that add MT-binding capabilities to PID. **Chapter 5** provides an *in silico* phylogenetic analysis of the At1 kinesin family and investigates the conservation of the NPK1 binding and activation domain in the family members. T-DNA insert lines for the BT-interacting kinesins were obtained and examined for phenotypes in higher order mutant lines. The quadruple mutant did not give any strong phenotypes and RT-PCR showed that two of the four genes were not *null*

mutations, because residual expression could be detected. The expression domains of the kinesins and their response to external stimuli were tested with promoter-GUS constructs. In protoplasts the kinesins showed MT localization, however *in planta* the proteins were targetted for degradation by the 26S proteasome. Even after preventing this degradation, no MT localization could be observed.

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Bibliography

Abas, L., Benjamins, R., Malenica, N., Paciorek, T., Wiśniewska, J., Moulinier-Anzola, J. C., Sieberer, T., Friml, J. & Luschnig, C. (2006). Intracellular trafficking and proteolysis of the *Arabidopsis* auxin-efflux facilitator PIN2 are involved in root gravitropism. Nature Cell Biology **8**, 249–256.

Akiyama, K., Matsuzaki, K.-i. & Hayashi, H. (2005). Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature **435**, 824–827.

Al-Bassam, J. & Chang, F. (2011). Regulation of microtubule dynamics by TOG-domain proteins XMAP215/Dis1 and CLASP. Trends in Cell Biology **21**, 604–614.

Ambrose, C., Ruan, Y., Gardiner, J., Tamblyn, L. M., Catching, A., Kirik, V., Marc, J., Overall, R. & Wasteneys, G. O. (2013). CLASP interacts with Sorting Nexin 1 to link microtubules and auxin transport via PIN2 recycling in *Arabidopsis thaliana*. Developmental Cell **24**, 649–659.

Ballesteros, I., Domínguez, T., Sauer, M., Paredes, P., Duprat, A., Rojo, E., Sanmartín, M. & Sánchez-Serrano, J. J. (2013). Specialized functions of the PP2A subfamily II catalytic subunits PP2A-C3 and PP2A-C4 in the distribution of auxin fluxes and development in arabidopsis. Plant Journal **73**, 862–872.

Band, L. R., Wells, D. M., Larrieu, A., Sun, J., Middleton, A. M., French, A. P., Brunoud, G., Sato, E. M., Wilson, M. H., Péret, B., Oliva, M., Swarup, R., Sairanen, I., Parry, G., Ljung, K., Beeckman, T., Garibaldi, J. M., Estelle, M., Owen, M. R., Vissenberg, K., Hodgman, T. C., Pridmore, T. P., King, J. R., Vernoux, T. & Bennett, M. J. (2012). Root gravitropism is regulated by a transient lateral auxin gradient controlled by a tipping-point mechanism. Proceedings of the National Academy of Sciences of the United States of America **109**, 4668–4673.

Barbez, E., Kubeš, M., Rolčík, J., Béziat, C., Pěnčík, A., Wang, B., Ruiz Rosquete, M., Zhu, J., Dobrev, P. I., Lee, Y., Zažímalová, E., Petrášek, J., Geisler, M., Friml, J.

& Kleine-Vehn, J. (2012). A novel putative auxin carrier family regulates intracellular auxin homeostasis in plants. Nature **485**, 119–122.

Bartel, B. & Fink, G. R. (1995). ILR1, an amidohydrolase that releases active indole-3-acetic acid from conjugates. Science **268**, 1745–1748.

Baster, P., Robert, S., Kleine-Vehn, J., Vanneste, S., Kania, U., Grunewald, W., De Rybel, B., Beeckman, T. & Friml, J. (2013). SCFTIR1/AFB-auxin signalling regulates PIN vacuolar trafficking and auxin fluxes during root gravitropism. EMBO Journal **32**, 260–274.

Benjamins, R., Galván-Ampudia, C. S., Hooykaas, P. J. J. & Offringa, R. (2003). PINOID-mediated signaling involves calcium-binding proteins. Plant Physiology **132**, 1623–1630.

Benjamins, R., Quint, A., Weijers, D., Hooykaas, P. J. J. & Offringa, R. (2001). The PINOID protein kinase regulates organ development in *arabidopsis* by enhancing polar auxin transport. Development **128**, 4057–4067.

Benková, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertová, D., Jürgens, G. & Friml, J. (2003). Local, efflux-dependent auxin gradients as a common module for plant organ formation. Cell **115**, 591–602.

Bennett, M. J., Marchant, A., Green, H. G., May, S. T., Ward, S. P., Millner, P. A., Walker, A. R., Schulz, B. & Feldmann, K. A. (1996). *Arabidopsis AUX1* gene: A permease-like regulator of root gravitropism. Science **273**, 948–950.

Bennett, T., Sieberer, T., Willett, B., Booker, J., Luschnig, C. & Leyser, O. (2006). The *Arabidopsis* MAX pathway controls shoot branching by regulating auxin transport. Current Biology **16**, 553–563.

Blakeslee, J. J., Bandyopadhyay, A., Lee, O. R., Mravec, J., Titapiwatanakun, B., Sauer, M., Makam, S. N., Cheng, Y., Bouchard, R., Adamec, J., Geisler, M., Nagashima, A., Sakai, T., Martinoia, E., Friml, J., Peer, W. A. & Murphy, A. S.

(2007). Interactions among PIN-FORMED and P-Glycoprotein auxin transporters in *Arabidopsis*. Plant Cell **19**, 131–147.

Blilou, I., Xu, J., Wildwater, M., Willemsen, V., Paponov, I., Friml, J., Heidstra, R., Aida, M., Palme, K. & Scheres, B. (2005). The PIN auxin efflux facilitator network controls growth and patterning in *Arabidopsis* roots. Nature **433**, 39–44.

Boer, D. R., Freire-Rios, A., Van den Berg, W. A., Saaki, T., Manfield, I. W., Kepinski, S., López-Vidrieo, I., Franco-Zorrilla, J. M., De Vries, S. C., Solano, R., Weijers, D. & Coll, M. (2014). Structural basis for DNA binding specificity by the auxin-dependent ARF transcription factors. Cell **156**, 577–589.

Bögre, L., Ökrész, L., Henriques, R. & Anthony, R. G. (2003). Growth signalling pathways in *Arabidopsis* and the AGC protein kinases. Trends in Plant Science **8**, 424–431.

Boutilier, K., Offringa, R., Sharma, V. K., Kieft, H., Ouellet, T., Zhang, L., Hattori, J., Liu, C.-M., van Lammeren, A. A. M., Miki, B. L. A., Custers, J. B. M. & van Lookeren Campagne, M. M. (2002). Ectopic expression of BABY BOOM triggers a conversion from vegetative to embryonic growth. Plant Cell **14**, 1737–1749.

Braun, N., de Saint Germain, A., Pillot, J.-P., Boutet-Mercey, S., Dalmais, M., Antoniadi, I., Li, X., Maia-Grondard, A., Le Signor, C., Bouteiller, N., Luo, D., Bendahmane, A., Turnbull, C. & Rameau, C. (2012). The pea TCP transcription factor PsBRC1 acts downstream of strigolactones to control shoot branching. Plant Physiology **158**, 225–238.

Braun, N., Wyrzykowska, J., Muller, P., David, K., Couch, D., Perrot-Rechenmann, C. & Fleming, A. J. (2008). Conditional repression of AUXIN BINDING PROTEIN1 reveals that it coordinates cell division and cell expansion during postembryonic shoot development in *Arabidopsis* and tobacco. Plant Cell **20**, 2746–2762.

Brewer, P. B., Dun, E. A., Ferguson, B. J., Rameau, C. & Beveridge, C. A. (2009). Strigolactone acts downstream of auxin to

regulate bud outgrowth in pea and Arabidopsis. Plant Physiology **150**, 482–493.

Calderón Villalobos, L. I. A., Lee, S., De Oliveira, C., Ivetac, A., Brandt, W., Armitage, L., Sheard, L. B., Tan, X., Parry, G., Mao, H., Zheng, N., Napier, R., Kepinski, S. & Estelle, M. (2012). A combinatorial TIR1/AFB–Aux/IAA co-receptor system for differential sensing of auxin. Nature Chemical Biology **8**, 477–485.

Causier, B., Ashworth, M., Guo, W. & Davies, B. (2012). The TOPLESS interactome: A framework for gene repression in Arabidopsis. Plant Physiology **158**, 423–438.

Chen, J.-G., Ullah, H., Young, J. C., Sussman, M. R. & Jones, A. M. (2001). ABP1 is required for organized cell elongation and division in *Arabidopsis* embryogenesis. Genes & Development **15**, 902–911.

Chen, X., Naramoto, S., Robert, S., Tejos, R., Löfke, C., Lin, D., Yang, Z. & Friml, J. (2012). ABP1 and ROP6 GTPase signaling regulate clathrin-mediated endocytosis in *Arabidopsis* roots. Current Biology **22**, 1326–1332.

Cheng, Y., Qin, G., Dai, X. & Zhao, Y. (2008). NPY genes and AGC kinases define two key steps in auxin-mediated organogenesis in Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America **105**, 21017–21022.

Christensen, S. K., Dagenais, N., Chory, J. & Weigel, D. (2000). Regulation of auxin response by the protein kinase PINOID. Cell **100**, 469–478.

Cook, C. E., Whichard, L. P., Turner, B., Wall, M. E. & Egley, G. H. (1966). Germination of Witchweed (Striga lutea Lour.): isolation and properties of a potent stimulant. Science **154**, 1189–1190.

Čovanová, M., Sauer, M., Rychtář, J., Friml, J., Petrášek, J. & Zažímalová, E. (2013). Overexpression of the AUXIN BINDING PROTEIN1 modulates PIN-dependent auxin transport in tobacco cells. PLoS ONE **8**, e70050.

Crawford, S., Shinohara, N., Sieberer, T., Williamson, L., George, G., Hepworth, J., Müller, D., Domagalska, M. A. & Leyser, O. (2010). Strigolactones enhance competition between shoot branches by dampening auxin transport. Development **137**, 2905–2913.

Cui, Y., Li, X., Chen, Q., He, X., Yang, Q., Zhang, A., Yu, X., Chen, H., Liu, N., Xie, Q., Yang, W., Zuo, J., Palme, K. & Li, W. (2010). BLOS1, a putative BLOC-1 subunit, interacts with SNX1 and modulates root growth in *Arabidopsis*. Journal of Cell Science **123**, 3727–3733.

Dahlke, R. I., Luethen, H. & Steffens, B. (2010). ABP1: An auxin receptor for fast responses at the plasma membrane. Plant Signaling & Behavior **5**, 1–3.

Dai, M., Zhang, C., Kania, U., Chen, F., Xue, Q., Mccray, T., Li, G., Qin, G., Wakeley, M., Terzaghi, W., Wan, J., Zhao, Y., Xu, J., Friml, J., Deng, X. W. & Wang, H. (2012). A PP6-type phosphatase holoenzyme directly regulates PIN phosphorylation and auxin efflux in *Arabidopsis*. Plant Cell **24**, 2497–2514.

Dal Bosco, C., Dovzhenko, A., Liu, X., Woerner, N., Rensch, T., Eismann, M., Eimer, S., Hegermann, J., Paponov, I. A., Ruperti, B., Heberle-Bors, E., Touraev, A., Cohen, J. D. & Palme, K. (2012). The endoplasmic reticulum localized PIN8 is a pollen-specific auxin carrier involved in intracellular auxin homeostasis. Plant Journal **71**, 860–870.

Darwin, C. R. & Darwin, F. (1880). The power of movement in plants. John Murray, London, UK.

David, K. M., Couch, D., Braun, N., Brown, S., Grosclaude, J. & Perrot-Rechenmann, C. (2007). The auxin-binding protein 1 is essential for the control of cell cycle. Plant Journal **50**, 197–206.

Delbarre, A., Muller, P. & Guern, J. (1998). Short-lived and phosphorylated proteins contribute to carrier-mediated efflux, but not to influx, of auxin in suspension-cultured tobacco cells. Plant Physiology **116**, 833–844.

Dello Ioio, R., Nakamura, K., Moubayidin, L., Perilli, S., Taniguchi, M., Morita, M. T., Aoyama, T., Costantino, P. &

Sabatini, S. (2008). A genetic framework for the control of cell division and differentiation in the root meristem. Science **322**, 1380–1384.

Dharmasiri, N., Dharmasiri, S. & Estelle, M. (2005a). The F-box protein TIR1 is an auxin receptor. Nature **435**, 441–445.

Dharmasiri, N., Dharmasiri, S., Weijers, D., Lechner, E., Yamada, M., Hobbie, L., Ehrismann, J. S., Jürgens, G. & Estelle, M. (2005b). Plant development is regulated by a family of Auxin receptor F Box Proteins. Developmental Cell **9**, 109–119.

Dhonukshe, P., Aniento, F., Hwang, I., Robinson, D. G., Mravec, J., Stierhof, Y.-D. & Friml, J. (2007). Clathrin-mediated constitutive endocytosis of PIN auxin efflux carriers in *Arabidopsis*. Current Biology **17**, 520–527.

Dhonukshe, P., Huang, F., Galván-Ampudia, C. S., Mähönen, A. P., Kleine-Vehn, J., Xu, J., Quint, A., Prasad, K., Friml, J., Scheres, B. & Offringa, R. (2010). Plasma membrane-bound AGC3 kinases phosphorylate PIN auxin carriers at TPRXS(N/S) motifs to direct apical PIN recycling. Development **137**, 3245–3255.

Dhonukshe, P., Tanaka, H., Goh, T., Ebine, K., Mähönen, A. P., Prasad, K., Blilou, I., Geldner, N., Xu, J., Uemura, T., Chory, J., Ueda, T., Nakano, A., Scheres, B. & Friml, J. (2008). Generation of cell polarity in plants links endocytosis, auxin distribution and cell fate decisions. Nature **456**, 962–966.

Ding, Z., Galván-Ampudia, C. S., Demarsy, E., Łangowski, Ł., Kleine-Vehn, J., Fan, Y., Morita, M. T., Tasaka, M., Fankhauser, C., Offringa, R. & Friml, J. (2011). Light-mediated polarization of the PIN3 auxin transporter for the phototropic response in *Arabidopsis*. Nature Cell Biology **13**, 447–452.

Ding, Z., Wang, B., Moreno, I., Dupláková, N., Simon, S., Carraro, N., Reemmer, J., Pěnčík, A., Chen, X., Tejos, R., Skůpa, P., Pollmann, S., Mravec, J., Petrášek, J., Zažímalová, E., Honys, D., Rolčík, J., Murphy, A., Orellana, A., Geisler, M. & Friml, J. (2012). ER-localized auxin transporter PIN8 regulates auxin homeostasis and male gametophyte development in *Arabidopsis*. Nature Communications **3**, 941.

Dos Santos Maraschin, F., Memelink, J. & Offringa, R. (2009). Auxin-induced, SCFTIR1-mediated poly-ubiquitination marks Aux/IAA proteins for degradation. Plant Journal **59**, 100–109.

Dun, E. A., de Saint Germain, A., Rameau, C. & Beveridge, C. A. (2013). Dynamics of strigolactone function and shoot branching responses in *Pisum sativum*. Molecular Plant **6**, 128–140.

Feldwisch, J., Zettl, R., Hesse, F., Schell, J. & Palme, K. (1992). An auxin-binding protein is localized to the plasma membrane of maize coleoptile cells: identification by photoaffinity labeling and purification of a 23-kda polypeptide. Proceedings of the National Academy of Sciences of the United States of America **89**, 475–479.

Feraru, E., Feraru, M. I., Kleine-Vehn, J., Martinière, A., Mouille, G., Vanneste, S., Vernhettes, S., Runions, J. & Friml, J. (2011). PIN polarity maintenance by the cell wall in *Arabidopsis*. Current Biology **21**, 338–343.

Feraru, E., Vosolsobě, S., Feraru, M. I., Petrášek, J. & Kleine-Vehn, J. (2012). Evolution and structural diversification of PILS putative auxin carriers in plants. Frontiers in Plant Science **3**, 227.

Fernandez, A., Drozdzecki, A., Hoogewijs, K., Nguyen, A., Beeckman, T., Madder, A. & Hilson, P. (2013). Transcriptional and functional classification of the GOLVEN/ROOT GROWTH FACTOR/CLE-Like signaling peptides reveals their role in lateral root and hair formation. Plant Physiology **161**, 954–970.

Friml, J., Benková, E., Blilou, I., Wiśniewska, J., Hamann, T., Ljung, K., Woody, S., Sandberg, G., Scheres, B., Jürgens, G. & Palme, K. (2002a). AtPIN4 mediates sink-driven auxin gradients and root patterning in *Arabidopsis*. Cell **108**, 661–673.

Friml, J., Vieten, A., Sauer, M., Weijers, D., Schwarz, H., Hamann, T., Offringa, R. & Jürgens, G. (2003). Efflux-dependent auxin gradients establish the apical-basal axis of *Arabidopsis*. Nature **426**, 147–153.

Friml, J., Wiśniewska, J., Benková, E., Mendgen, K. & Palme, K. (2002b). Lateral relocation of auxin efflux regulator PIN3 mediates tropism in *Arabidopsis*. Nature **415**, 806–809.

Friml, J., Yang, X., Michniewicz, M., Weijers, D., Quint, A., Tietz, O., Benjamins, R., Ouwerkerk, P. B. F., Ljung, K., Sandberg, G., Hooykaas, P. J. J., Palme, K. & Offringa, R. (2004). A PINOID-dependent binary switch in apical-basal PIN polar targeting directs auxin efflux. Science **306**, 862–865.

Fu, X. & Harberd, N. P. (2003). Auxin promotes *Arabidopsis* root growth by modulating gibberellin response. Nature **421**, 740–743.

Fu, Y., Gu, Y., Zheng, Z., Wasteneys, G. & Yang, Z. (2005). Arabidopsis interdigitating cell growth requires two antagonistic pathways with opposing action on cell morphogenesis. Cell **120**, 687–700.

Fu, Y., Li, H. & Yang, Z. (2002). The ROP2 GTPase controls the formation of cortical fine F-Actin and the early phase of directional cell expansion during Arabidopsis organogenesis. Plant Cell **14**, 777–794.

Furutani, M., Sakamoto, N., Yoshida, S., Kajiwara, T., Robert, H. S., Friml, J. & Tasaka, M. (2011). Polar-localized NPH3-like proteins regulate polarity and endocytosis of PIN-FORMED auxin efflux carriers. Development **138**, 2069–2078.

Galinha, C., Hofhuis, H., Luijten, M., Willemsen, V., Blilou, I., Heidstra, R. & Scheres, B. (2007). PLETHORA proteins as dose-dependent master regulators of *Arabidopsis* root development. Nature **449**, 1053–1057.

Galván-Ampudia, C. S. & Offringa, R. (2007). Plant evolution: AGC kinases tell the auxin tale. Trends in Plant Science **12**, 541–547.

Gälweiler, L., Guan, C., Müller, A., Wisman, E., Mendgen, K., Yephremov, A. & Palme, K. (1998). Regulation of polar auxin transport by AtPIN1 in *Arabidopsis* vascular tissue. Science **282**, 2226–2230.

Ganguly, A., Lee, S. H., Cho, M., Lee, O. R., Yoo, H. & Cho, H.-T. (2010). Differential auxin-transporting activities of

PIN-FORMED proteins in Arabidopsis root hair cells. Plant Physiology **153**, 1046–1061.

Ganguly, A., Park, M., Kesawat, M. S. & Cho, H.-T. (2014). Functional analysis of the hydrophilic loop in intracellular trafficking of *Arabidopsis* PIN-FORMED proteins. Plant Cell **26**.

Garbers, C., DeLong, A., Deruére, J., Bernasconi, P. & Söll, D. (1996). A mutation in protein phosphatase 2A regulatory subunit A affects auxin transport in *Arabidopsis*. EMBO Journal **15**, 2115–2124.

Garrett, J., Meents, M., Blackshaw, M., Blackshaw, L., Hou, H., Styranko, D., Kohalmi, S. & Schultz, E. (2012). A novel, semi-dominant allele of *MONOPTEROS* provides insight into leaf initiation and vein pattern formation. Planta **236**, 297–312.

Geisler, M., Blakeslee, J. J., Bouchard, R., Lee, O. R., Vincenzetti, V., Bandyopadhyay, A., Titapiwatanakun, B., Peer, W. A., Bailly, A., Richards, E. L., Ejendal, K. F. K., Smith, A. P., Baroux, C., Grossniklaus, U., Müller, A., Hrycyna, C. A., Dudler, R., Murphy, A. S. & Martinoia, E. (2005). Cellular efflux of auxin catalyzed by the Arabidopsis MDR/PGP transporter AtPGP1. Plant Journal **44**, 179–194.

Geldner, N., Anders, N., Wolters, H., Keicher, J., Kornberger, W., Muller, P., Delbarre, A., Ueda, T., Nakano, A. & Jürgens, G. (2003). The *Arabidopsis* GNOM ARF-GEF mediates endosomal recycling, auxin transport, and auxin-dependent plant growth. Cell **112**, 219–230.

Geldner, N., Friml, J., Stierhof, Y.-D., Jürgens, G. & Palme, K. (2001). Auxin transport inhibitors block PIN1 cycling and vesicle trafficking. Nature **413**, 425–428.

Gomez-Roldan, V., Fermas, S., Brewer, P. B., Puech-Pagès, V., Dun, E. A., Pillot, J.-P., Letisse, F., Matusova, R., Danoun, S., Portais, J.-C., Bouwmeester, H., Becard, G., Beveridge, C. A., Rameau, C. & Rochange, S. F. (2008). Strigolactone inhibition of shoot branching. Nature **455**, 189–194.

Guenot, B., Bayer, E., Kierzkowski, D., Smith, R. S., Mandel, T., Žádníková, P., Benková, E. & Kuhlemeier, C. (2012).

PIN1-independent leaf initiation in Arabidopsis. Plant Physiology **159**, 1501–1510.

Hacham, Y., Sela, A., Friedlander, L. & Savaldi-Goldstein, S. (2012). BRI1 activity in the root meristem involves post-transcriptional regulation of PIN auxin efflux carriers. Plant Signaling & Behavior **7**, 68–70.

Hamann, T., Benková, E., Bäurle, I., Kientz, M. & Jürgens, G. (2002). The *Arabidopsis BODENLOS* gene encodes an auxin response protein inhibiting MONOPTEROS-mediated embryo patterning. Genes & Development **16**, 1610–1615.

Hertel, R., Thomson, K.-S. & Russo, V. E. A. (1972). *In-vitro* auxin binding to particulate cell fractions from corn coleoptiles. Planta **107**, 325–340.

Huang, F., Kemel Zago, M., Abas, L., van Marion, A., Galván-Ampudia, C. S. & Offringa, R. (2010). Phosphorylation of conserved PIN motifs directs *arabidopsis* PIN1 polarity and auxin transport. Plant Cell **22**, 1129–1142.

Jaillais, Y., Fobis-Loisy, I., Miège, C., Rollin, C. & Gaude, T. (2006). AtSNX1 defines an endosome for auxin-carrier trafficking in *Arabidopsis*. Nature **443**, 106–109.

Jaillais, Y., Santambrogio, M., Rozier, F., Fobis-Loisy, I., Miège, C. & Gaude, T. (2007). The retromer protein VPS29 links cell polarity and organ initiation in plants. Cell **130**, 1057–1070.

Jones, A. M. & Herman, E. M. (1993). KDEL-containing auxin-binding protein is secreted to the plasma membrane and cell wall. Plant Physiology **101**, 595–606.

Kaneda, M., Schuetz, M., Lin, B. S. P., Chanis, C., Hamberger, B., Western, T. L., Ehlting, J. & Samuels, A. L. (2011). ABC transporters coordinately expressed during lignification of *Arabidopsis* stems include a set of ABCBs associated with auxin transport. Journal of Experimental Botany **62**, 2063–2077.

Kepinski, S. & Leyser, O. (2005). The *Arabidopsis* F-box protein TIR1 is an auxin receptor. Nature **435**, 446–451.

Kitakura, S., Vanneste, S., Robert, S., Löfke, C., Teichmann, T., Tanaka, H. & Friml, J. (2011). Clathrin mediates endocytosis and polar distribution of PIN auxin transporters in *Arabidopsis*. Plant Cell **23**, 1920–1931.

Kleine-Vehn, J., Dhonukshe, P., Sauer, M., Brewer, P. B., Wiśniewska, J., Paciorek, T., Benková, E. & Friml, J. (2008a). ARF GEF-dependent transcytosis and polar delivery of PIN auxin carriers in *Arabidopsis*. Current Biology **18**, 526–531.

Kleine-Vehn, J., Ding, Z., Jones, A. R., Tasaka, M., Morita, M. T. & Friml, J. (2010). Gravity-induced PIN transcytosis for polarization of auxin fluxes in gravity-sensing root cells. Proceedings of the National Academy of Sciences of the United States of America **107**, 22344–22349.

Kleine-Vehn, J., Huang, F., Naramoto, S., Zhang, J., Michniewicz, M., Offringa, R. & Friml, J. (2009). PIN auxin efflux carrier polarity is regulated by PINOID kinase-mediated recruitment into GNOM-independent trafficking in *Arabidopsis*. Plant Cell **21**, 3839–3849.

Kleine-Vehn, J., Leitner, J., Zwiewka, M., Sauer, M., Abas, L., Luschnig, C. & Friml, J. (2008b). Differential degradation of PIN2 auxin efflux carrier by retromer-dependent vacuolar targeting. Proceedings of the National Academy of Sciences of the United States of America **105**, 17812–17817.

Kleine-Vehn, J., Wabnik, K., Martinière, A., Łangowski, Ł., Willig, K., Naramoto, S., Leitner, J., Tanaka, H., Jakobs, S., Robert, S., Luschnig, C., Govaerts, W., W Hell, S., Runions, J. & Friml, J. (2011). Recycling, clustering, and endocytosis jointly maintain PIN auxin carrier polarity at the plasma membrane. Molecular Systems Biology **7**, 540.

Kögl, F. & Haagen-Smit, A. J. (1931). Über die chemie des wuchsstoffs. Koninklijke Akademie van Wetenschappen Amsterdam Proceedings of the Section of Sciences **34**, 1411–1416.

Korbei, B. & Luschnig, C. (2013). Plasma membrane protein ubiquitylation and degradation as determinants of positional growth in plants. Journal of Integrative Plant Biology **55**, 809–823.

Kubeš, M., Yang, H., Richter, G. L., Cheng, Y., Młodzińska, E., Wang, X., Blakeslee, J. J., Carraro, N., Petrášek, J., Zažímalová, E., Hoyerová, K., Peer, W. A. & Murphy, A. S. (2012). The Arabidopsis concentration-dependent influx/efflux transporter ABCB4 regulates cellular auxin levels in the root epidermis. The Plant Journal **69**, 640–654.

Křeček, P., Skůpa, P., Libus, J., Naramoto, S., Tejos, R., Friml, J. & Zažímalová, E. (2009). The PIN-FORMED (PIN) protein family of auxin transporters. Genome Biology **10**, 249.

Laxmi, A., Pan, J., Morsy, M. & Chen, R. (2008). Light plays an essential role in intracellular distribution of auxin efflux carrier PIN2 in *Arabidopsis thaliana*. PLoS ONE **3**, e1510.

Leitner, J., Petrášek, J., Tomanov, K., Retzer, K., Pařezová, M., Korbei, B., Bachmair, A., Zažímalová, E. & Luschnig, C. (2012). Lysine63-linked ubiquitylation of PIN2 auxin carrier protein governs hormonally controlled adaptation of Arabidopsis root growth. Proceedings of the National Academy of Sciences of the United States of America **109**, 8322–8327.

Li, H., Lin, D., Dhonukshe, P., Nagawa, S., Chen, D., Friml, J., Scheres, B., Guo, H. & Yang, Z. (2011). Phosphorylation switch modulates the interdigitated pattern of PIN1 localization and cell expansion in *Arabidopsis* leaf epidermis. Cell Research **21**, 970–978.

Lin, D., Nagawa, S., Chen, J., Cao, L., Chen, X., Xu, T., Li, H., Dhonukshe, P., Yamamuro, C., Friml, J., Scheres, B., Fu, Y. & Yang, Z. (2012). A ROP GTPase-dependent auxin signaling pathway regulates the subcellular distribution of PIN2 in *Arabidopsis* roots. Current Biology **22**, 1319–1325.

Liscum, E. & Reed, J. W. (2002). Genetics of Aux/IAA and ARF action in plant growth and development. Plant Molecular Biology **49**, 387–400.

Löfke, C., Zwiewka, M., Heilmann, I., Van Montagu, M. C. E., Teichmann, T. & Friml, J. (2013). Asymmetric gibberellin signaling regulates vacuolar trafficking of PIN auxin transporters during root gravitropism. Proceedings of the National Academy of Sciences of the United States of America **110**, 3627–3632.

Luschnig, C., Gaxiola, R. A., Grisafi, P. & Fink, G. R. (1998). EIR1, a root-specific protein involved in auxin transport, is required for gravitropism in *Arabidopsis thaliana*. Genes & Development **12**, 2175–2187.

Marhavý, P., Bielach, A., Abas, L., Abuzeineh, A., Duclercq, J., Tanaka, H., Pařezová, M., Petrášek, J., Friml, J., Kleine-Vehn, J. & Benková, E. (2011). Cytokinin modulates endocytic trafficking of PIN1 auxin efflux carrier to control plant organogenesis. Developmental Cell **21**, 796–804.

Matusova, R., Rani, K., Verstappen, F. W. A., Franssen, M. C. R., Beale, M. H. & Bouwmeester, H. J. (2005). The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanche* spp. are derived from the carotenoid pathway. Plant Physiology **139**, 920–934.

Michniewicz, M., Zago, M. K., Abas, L., Weijers, D., Schweighofer, A., Meskiene, I., Heisler, M. G., Ohno, C., Zhang, J., Huang, F., Schwab, R., Weigel, D., Meyerowitz, E. M., Luschnig, C., Offringa, R. & Friml, J. (2007). Antagonistic regulation of PIN phosphorylation by PP2A and PINOID directs auxin flux. Cell **130**, 1044–1056.

Mravec, J., Kubeš, M., Bielach, A., Gaykova, V., Petrášek, J., Skůpa, P., Chand, S., Benková, E., Zažímalová, E. & Friml, J. (2008). Interaction of PIN and PGP transport mechanisms in auxin distribution-dependent development. Development **135**, 3345–3354.

Mravec, J., Skůpa, P., Bailly, A., Hoyerová, K., Křeček, P., Bielach, A., Petrášek, J., Zhang, J., Gaykova, V., Stierhof, Y.-D., Dobrev, P. I., Schwarzerova, K., Rolčík, J., Seifertova, D., Luschnig, C., Benková, E., Zažímalová, E., Geisler, M. & Friml, J. (2009). Subcellular homeostasis of phytohormone auxin

is mediated by the ER-localized PIN5 transporter. Nature **459**, 1136–1140.

Müller, A., Guan, C., Gälweiler, L., Tänzler, P., Huijser, P., Marchant, A., Parry, G., Bennett, M., Wisman, E. & Palme, K. (1998). *AtPIN2* defines a locus of *Arabidopsis* for root gravitropism control. EMBO Journal **17**, 6903–6911.

Nagawa, S., Xu, T., Lin, D., Dhonukshe, P., Zhang, X., Friml, J., Scheres, B., Fu, Y. & Yang, Z. (2012). ROP GTPase-dependent actin microfilaments promote PIN1 polarization by localized inhibition of clathrin-dependent endocytosis. PLoS Biology **10**, e1001299.

Nodzyński, T., Feraru, M. I., Hirsch, S., De Rycke, R., Niculaes, C., Boerjan, W., Van Leene, J., De Jaeger, G., Vanneste, S. & Friml, J. (2013). Retromer subunits VPS35A and VPS29 mediate prevacuolar compartment (PVC) function in *Arabidopsis*. Molecular Plant **6**, 1849–1862.

Noh, B., Murphy, A. S. & Spalding, E. P. (2001). *multidrug resistance*–like genes of arabidopsis required for auxin transport and auxin-mediated development. Plant Cell **13**, 2441–2454.

Offringa, R. & Huang, F. (2013). Phosphorylation-dependent trafficking of plasma membrane proteins in animal and plant cells. Journal of Integrative Plant Biology **55**, 789–808.

Okushima, Y., Overvoorde, P. J., Arima, K., Alonso, J. M., Chan, A., Chang, C., Ecker, J. R., Hughes, B., Lui, A., Nguyen, D., Onodera, C., Quach, H., Smith, A., Yu, G. & Theologis, A. (2005). Functional genomic analysis of the *AUXIN RESPONSE FACTOR* gene family members in *Arabidopsis thaliana*: unique and overlapping functions of *ARF7* and *ARF19*. Plant Cell **17**, 444–463.

Paciorek, T., Zažímalová, E., Ruthardt, N., Petrášek, J., Stierhof, Y.-D., Kleine-Vehn, J., Morris, D. A., Emans, N., Jürgens, G., Geldner, N. & Friml, J. (2005). Auxin inhibits endocytosis and promotes its own efflux from cells. Nature **435**, 1251–1256.

Perrot-Rechenmann, C. (2010). Cellular responses to auxin: division versus expansion. Cold Spring Harbor Perspectives in Biology **2**, a001446.

Petrášek, J., Mravec, J., Bouchard, R., Blakeslee, J. J., Abas, M., Seifertová, D., Wiśniewska, J., Tadele, Z., Kubeš, M., Čovanová, M., Dhonukshe, P., Skůpa, P., Benková, E., Perry, L., Křeček, P., Lee, O. R., Fink, G. R., Geisler, M., Murphy, A. S., Luschnig, C., Zažímalová, E. & Friml, J. (2006). PIN proteins perform a rate-limiting function in cellular auxin efflux. Science **312**, 914–918.

Pinon, V., Prasad, K., Grigg, S. P., Sanchez-Perez, G. F. & Scheres, B. (2013). Local auxin biosynthesis regulation by PLETHORA transcription factors controls phyllotaxis in Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America **110**, 1107–1112.

Prasad, K., Grigg, S. P., Barkoulas, M., Yadav, R. K., Sanchez-Perez, G. F., Pinon, V., Blilou, I., Hofhuis, H., Dhonukshe, P., Galinha, C., Mähönen, A. P., Muller, W. H., Raman, S., Verkleij, A. J., Snel, B., Reddy, G. V., Tsiantis, M. & Scheres, B. (2011). *Arabidopsis* plethora transcription factors control phyllotaxis. Current Biology **21**, 1123–1128.

Raven, J. A. (1975). Transport of indoleacetic acid in plant cells in relation to pH and electrical potential gradients, and its significance for polar IAA transport. New Phytologist **74**, 163–172.

Ray, P. M. (1977). Auxin-binding sites of Maize coleoptiles are localized on membranes of the endoplasmic reticulum. Plant Physiology **59**, 594–599.

Remington, D. L., Vision, T. J., Guilfoyle, T. J. & Reed, J. W. (2004). Contrasting modes of diversification in the *Aux/IAA* and *ARF* gene families. Plant Physiology **135**, 1738–1752.

Rigas, S., Ditengou, F. A., Ljung, K., Daras, G., Tietz, O., Palme, K. & Hatzopoulos, P. (2013). Root gravitropism and root hair development constitute coupled developmental responses regulated

by auxin homeostasis in the *Arabidopsis* root apex. New Phytologist **197**, 1130–1141.

Rigó, G., Ayaydin, F., Tietz, O., Zsigmond, L., Kovács, H., Páy, A., Salchert, K., Darula, Z., Medzihradszky, K. F., Szabados, L., Palme, K., Koncz, C. & Cséplő, Á. (2013). Inactivation of plasma membrane–localized CDPK-RELATED KINASE5 decelerates PIN2 exocytosis and root gravitropic response in *Arabidopsis*. Plant Cell **25**, 1592–1608.

Robert, S., Kleine-Vehn, J., Barbez, E., Sauer, M., Paciorek, T., Baster, P., Vanneste, S., Zhang, J., Simon, S., Čovanová, M., Hayashi, K., Dhonukshe, P., Yang, Z., Bednarek, S. Y., Jones, A. M., Luschnig, C., Aniento, F., Zažímalová, E. & Friml, J. (2010). ABP1 mediates auxin inhibition of clathrin-dependent endocytosis in *Arabidopsis*. Cell **143**, 111–121.

Rubery, P. H. & Sheldrake, A. R. (1974). Carrier-mediated auxin transport. Planta **118**, 101–121.

Rück, A., Palme, K., Venis, M. A., Napier, R. M. & Felle, H. H. (1993). Patch-clamp analysis establishes a role for an auxin binding protein in the auxin stimulation of plasma membrane current in *Zea mays* protoplasts. The Plant Journal **4**, 41–46.

Ruegger, M., Dewey, E., Gray, W. M., Hobbie, L., Turner, J. & Estelle, M. (1998). The TIR1 protein of *Arabidopsis* functions in auxin response and is related to human SKP2 and yeast Grr1p. Genes & Development **12**, 198–207.

Ruiz Rosquete, M., Barbez, E. & Kleine-Vehn, J. (2012). Cellular auxin homeostasis: gatekeeping is housekeeping. Molecular Plant **5**, 772–786.

Ruyter-Spira, C., Kohlen, W., Charnikhova, T., van Zeijl, A., van Bezouwen, L., de Ruijter, N., Cardoso, C., Lopez-Raez, J. A., Matusova, R., Bours, R., Verstappen, F. & Bouwmeester, H. (2011). Physiological effects of the synthetic strigolactone analog GR24 on root system architecture in Arabidopsis: another belowground role for strigolactones? Plant Physiology **155**, 721–734.

Růžička, K., Ljung, K., Vanneste, S., Podhorská, R., Beeckman, T., Friml, J. & Benková, E. (2007). Ethylene regulates root growth through effects on auxin biosynthesis and transport-dependent auxin distribution. Plant Cell **19**, 2197–2212.

Růžička, K., Šimášková, M., Duclercq, J., Petrášek, J., Zažímalová, E., Simon, S., Friml, J., Van Montagu, M. C. E. & Benková, E. (2009). Cytokinin regulates root meristem activity via modulation of the polar auxin transport. Proceedings of the National Academy of Sciences of the United States of America **106**, 4284–4289.

Santner, A. A. & Watson, J. C. (2006). The WAG1 and WAG2 protein kinases negatively regulate root waving in Arabidopsis. Plant Journal **45**, 752–764.

Sassi, M., Lu, Y., Zhang, Y., Wang, J., Dhonukshe, P., Blilou, I., Dai, M., Li, J., Gong, X., Jaillais, Y., Yu, X., Traas, J., Ruberti, I., Wang, H., Scheres, B., Vernoux, T. & Xu, J. (2012). COP1 mediates the coordination of root and shoot growth by light through modulation of PIN1- and PIN2-dependent auxin transport in *Arabidopsis*. Development **139**, 3402–3412.

Sauer, M., Balla, J., Luschnig, C., Wiśniewska, J., Reinöhl, V., Friml, J. & Benková, E. (2006). Canalization of auxin flow by Aux/IAA-ARF-dependent feedback regulation of PIN polarity. Genes & Development **20**, 2902–2911.

Sawchuk, M. G., Edgar, A. & Scarpella, E. (2013). Patterning of leaf vein networks by convergent auxin transport pathways. PLoS Genetics **9**, e1003294.

Scarpella, E., Marcos, D., Friml, J. & Berleth, T. (2006). Control of leaf vascular patterning by polar auxin transport. Genes & Development **20**, 1015–1027.

Setty, S. R. G., Tenza, D., Truschel, S. T., Chou, E., Sviderskaya, E. V., Theos, A. C., Lamoreux, M. L., Di Pietro, S. M., Starcevic, M., Bennett, D. C., Dell'Angelica, E. C., Raposo, G. & Marks, M. S. (2007). BLOC-1 is required for cargo-specific sorting from vacuolar early endosomes toward lysosome-related organelles. Molecular Biology of the Cell **18**, 768–780.

Shinohara, N., Taylor, C. & Leyser, O. (2013). Strigolactone can promote or inhibit shoot branching by triggering rapid depletion of the auxin efflux protein PIN1 from the plasma membrane. PLoS Biology **11**, e1001474.

Sorefan, K., Girin, T., Liljegren, S. J., Ljung, K., Robles, P., Galván-Ampudia, C. S., Offringa, R., Friml, J., Yanofsky, M. F. & Ostergaard, L. (2009). A regulated auxin minimum is required for seed dispersal in *Arabidopsis*. Nature **459**, 583–586.

Spitzer, C., Reyes, F. C., Buono, R., Sliwinski, M. K., Haas, T. J. & Otegui, M. S. (2009). The ESCRT-related CHMP1A and B proteins mediate multivesicular body sorting of auxin carriers in *Arabidopsis* and are required for plant development. Plant Cell **21**, 749–766.

Steffens, B., Feckler, C., Palme, K., Christian, M., Böttger, M. & Lüthen, H. (2001). The auxin signal for protoplast swelling is perceived by extracellular ABP1. Plant Journal **27**, 591–599.

Swarup, R. & Péret, B. (2012). AUX/LAX family of auxin influx carriers-an overview. Frontiers in Plant Science **3**, 225.

Szemenyei, H., Hannon, M. & Long, J. A. (2008). TOPLESS mediates auxin-dependent transcriptional repression during *Arabidopsis* embryogenesis. Science **319**, 1384–1386.

Tamura, K., Shimada, T., Ono, E., Tanaka, Y., Nagatani, A., Higashi, S.-i., Watanabe, M., Nishimura, M. & Hara-Nishimura, I. (2003). Why green fluorescent fusion proteins have not been observed in the vacuoles of higher plants. Plant Journal **35**, 545–555.

Tan, X., Calderon-Villalobos, L. I. A., Sharon, M., Zheng, C., Robinson, C. V., Estelle, M. & Zheng, N. (2007). Mechanism of auxin perception by the TIR1 ubiquitin ligase. Nature **446**, 640–645.

Tanaka, H., Dhonukshe, P., Brewer, P. B. & Friml, J. (2006). Spatiotemporal asymmetric auxin distribution: a means to coordinate plant development. Cellular and Molecular Life Sciences **63**, 2738–2754.

Tian, H., Klämbt, D. & Jones, A. M. (1995). Auxin-binding protein 1 does not bind auxin within the endoplasmic reticulum despite this

being the predominant subcellular location for this hormone receptor. Journal of Biological Chemistry **270**, 26962–26969.

Titapiwatanakun, B., Blakeslee, J. J., Bandyopadhyay, A., Yang, H., Mravec, J., Sauer, M., Cheng, Y., Adamec, J., Nagashima, A., Geisler, M., Sakai, T., Friml, J., Peer, W. A. & Murphy, A. S. (2009). ABCB19/PGP19 stabilises PIN1 in membrane microdomains in Arabidopsis. Plant Journal **57**, 27–44.

Tiwari, S. B., Hagen, G. & Guilfoyle, T. (2003). The roles of Auxin Response Factor domains in auxin-responsive transcription. Plant Cell **15**, 533–543.

Tiwari, S. B., Hagen, G. & Guilfoyle, T. J. (2004). Aux/IAA Proteins contain a potent transcriptional repression domain. Plant Cell **16**, 533–543.

Ulmasov, T., Hagen, G. & Guilfoyle, T. J. (1997). ARF1, a transcription factor that binds to auxin response elements. Science **276**, 1865–1868.

Ulmasov, T., Hagen, G. & Guilfoyle, T. J. (1999a). Activation and repression of transcription by auxin-response factors. Proceedings of the National Academy of Sciences of the United States of America **96**, 5844–5849.

Ulmasov, T., Hagen, G. & Guilfoyle, T. J. (1999b). Dimerization and DNA binding of auxin response factors. Plant Journal **19**, 309–319.

Ulmasov, T., Liu, Z. B., Hagen, G. & Guilfoyle, T. J. (1995). Composite structure of auxin response elements. Plant Cell **7**, 1611–1623.

Umehara, M., Hanada, A., Yoshida, S., Akiyama, K., Arite, T., Takeda-Kamiya, N., Magome, H., Kamiya, Y., Shirasu, K., Yoneyama, K., Kyozuka, J. & Yamaguchi, S. (2008). Inhibition of shoot branching by new terpenoid plant hormones. Nature **455**, 195–200.

van Berkel, K., de Boer, R. J., Scheres, B. & ten Tusscher, K. (2013). Polar auxin transport: models and mechanisms. Development **140**, 2253–2268.

Vanstraelen, M. & Benková, E. (2012). Hormonal interactions in the regulation of plant development. Annual Review of Cell and Developmental Biology **28**, 463–487.

Vernoux, T., Besnard, F. & Traas, J. (2010). Auxin at the shoot apical meristem. Cold Spring Harbor Perspectives in Biology **2**, a001487.

Viaene, T., Delwiche, C. F., Rensing, S. A. & Friml, J. (2013). Origin and evolution of PIN auxin transporters in the green lineage. Trends in Plant Science **18**, 5–10.

Vieten, A., Vanneste, S., Wiśniewska, J., Benková, E., Benjamins, R., Beeckman, T., Luschnig, C. & Friml, J. (2005). Functional redundancy of PIN proteins is accompanied by auxin-dependent cross-regulation of PIN expression. Development **132**, 4521–4531.

Wabnik, K., Kleine-Vehn, J., Govaerts, W. & Friml, J. (2011). Prototype cell-to-cell auxin transport mechanism by intracellular auxin compartmentalization. Trends in Plant Science **16**, 468–475.

Wang, C., Yan, X., Chen, Q., Jiang, N., Fu, W., Ma, B., Liu, J., Li, C., Bednarek, S. Y. & Pan, J. (2013). Clathrin light chains regulate clathrin-mediated trafficking, auxin signaling, and development in *Arabidopsis*. Plant Cell **25**, 499–516.

Went, F. W. (1926). On growth-accelerating substances in the coleoptile of Avena sativa. Proceedings of the Koninklijke Akademie van Wetenschappen **30**, 10–19.

Wenzel, C. L., Schuetz, M., Yu, Q. & Mattsson, J. (2007). Dynamics of *MONOPTEROS* and PIN-FORMED1 expression during leaf vein pattern formation in *Arabidopsis thaliana*. Plant Journal **49**, 387–398.

Whitford, R., Fernandez, A., Tejos, R., Pérez, A. C., Kleine-Vehn, J., Vanneste, S., Drozdzecki, A., Leitner, J., Abas, L., Aerts, M., Hoogewijs, K., Baster, P., De Groodt, R., Lin, Y.-C., Storme, V., Van de Peer, Y., Beeckman, T., Madder, A., Devreese, B., Luschnig, C., Friml, J. & Hilson,

P. (2012). GOLVEN secretory peptides regulate auxin carrier turnover during plant gravitropic responses. Developmental Cell **22**, 678–685.

Willige, B. C., Isono, E., Richter, R., Zourelidou, M. & Schwechheimer, C. (2011). Gibberellin regulates PIN-FORMED abundance and is required for Auxin transport–dependent growth and development in *Arabidopsis thaliana*. Plant Cell **23**, 2184–2195.

Wiśniewska, J., Xu, J., Seifertová, D., Brewer, P. B., Růžička, K., Blilou, I., Rouquié, D., Benková, E., Scheres, B. & Friml, J. (2006). Polar PIN localization directs auxin flow in plants. Science **312**, 883.

Woodward, A. W. & Bartel, B. (2005). Auxin: regulation, action, and interaction. Annals of Botany **95**, 707–735.

Xu, J., Hofhuis, H., Heidstra, R., Sauer, M., Friml, J. & Scheres, B. (2006). A molecular framework for plant regeneration. Science **311**, 385–388.

Xu, T., Dai, N., Chen, J., Nagawa, S., Cao, M., Li, H., Zhou, Z., Chen, X., De Rycke, R., Rakusová, H., Wang, W., Jones, A. M., Friml, J., Patterson, S. E., Bleecker, A. B. & Yang, Z. (2014). Cell surface ABP1-TMK auxin-sensing complex activates ROP GTPase signaling. Science **343**, 1025–1028.

Xu, T., Wen, M., Nagawa, S., Fu, Y., Chen, J.-G., Wu, M.-J., Perrot-Rechenmann, C., Friml, J., Jones, A. M. & Yang, Z. (2010). Cell surface- and Rho GTPase-based auxin signaling controls cellular interdigitation in *Arabidopsis*. Cell **143**, 99–110.

Žádníková, P., Petrášek, J., Marhavý, P., Raz, V., Vandenbussche, F., Ding, Z., Schwarzerová, K., Morita, M. T., Tasaka, M., Hejátko, J., Van Der Straeten, D., Friml, J. & Benková, E. (2010). Role of PIN-mediated auxin efflux in apical hook development of *Arabidopsis thaliana*. Development **137**, 607–617.

Zažímalová, E., Křeček, P., Skůpa, P., Hoyerová, K. & Petrášek, J. (2007). Polar transport of the plant hormone auxin – the role of PIN-FORMED (PIN) proteins. Cellular and Molecular Life Sciences **64**, 1621–1637.

Zegzouti, H., Anthony, R. G., Jahchan, N., Bögre, L. & Christensen, S. K. (2006a). Phosphorylation and activation of PINOID by the phospholipid signaling kinase 3-phosphoinositide-dependent protein kinase 1 (PDK1) in arabidopsis. Proceedings of the National Academy of Sciences of the United States of America **103**, 6404–6409.

Zegzouti, H., Li, W., Lorenz, T. C., Xie, M., Payne, C. T., Smith, K., Glenny, S., Payne, G. S. & Christensen, S. K. (2006b). Structural and functional insights into the regulation of arabidopsis AGC VIIIa kinases. Journal of Biological Chemistry **281**, 35520–35530.

Zhang, J., Nodzyński, T., Pěnčík, A., Rolčík, J. & Friml, J. (2010). PIN phosphorylation is sufficient to mediate PIN polarity and direct auxin transport. Proceedings of the National Academy of Sciences of the United States of America **107**, 918–922.

Zheng, X., Miller, N. D., Lewis, D. R., Christians, M. J., Lee, K.-H., Muday, G. K., Spalding, E. P. & Vierstra, R. D. (2011). AUXIN UP-REGULATED F-BOX PROTEIN1 regulates the cross talk between auxin transport and cytokinin signaling during plant root growth. Plant Physiology **156**, 1878–1893.

Zourelidou, M., Müller, I., Willige, B. C., Nill, C., Jikumaru, Y., Li, H. & Schwechheimer, C. (2009). The polarly localized D6 PROTEIN KINASE is required for efficient auxin transport in *Arabidopsis thaliana*. Development **136**, 627–636.