# Chapter 8
# Auxin on the Road Navigated by Cellular PIN Polarity

**Pawel Baster and Jiří Friml**

**Abstract** The generation of asymmetry, at both cellular and tissue level, is one of the most essential capabilities of all eukaryotic organisms. It mediates basically all multicellular development ranging from embryogenesis and de novo organ formation till responses to various environmental stimuli. In plants, the awe-inspiring number of such processes is regulated by phytohormone auxin and its directional, cell-to-cell transport. The mediators of this transport, PIN auxin transporters, are asymmetrically localized at the plasma membrane, and this polar localization determines the directionality of intercellular auxin flow. Thus, auxin transport contributes crucially to the generation of local auxin gradients or maxima, which instruct given cell to change its developmental program. Here, we introduce and discuss the molecular components and cellular mechanisms regulating the generation and maintenance of cellular PIN polarity, as the general hallmarks of cell polarity in plants.

## 1 Introduction

The emergence of multicellularity during the evolution of species had its inevitable repercussions. Efficient intercellular communication was one of such. In other words, to achieve a mutual goal, neighboring cells (single elements of the system) needed to perceive and transduce externally or internally generated signals. As a result, the multicellular organism, as a whole, should be able to translate these

P. Baster • J. Friml (✉)
Department of Plant Systems Biology, VIB, 9052 Gent, Belgium

Department of Plant Biotechnology and Genetics, Ghent University, 9052 Gent, Belgium

Institute of Science and Technology Austria (IST Austria), 3400 Klosterneuburg, Austria
e-mail: Jiri.FRIML@ist.ac.at

E. Zažímalová et al. (eds.), *Auxin and Its Role in Plant Development*,
DOI 10.1007/978-3-7091-1526-8_8, 

signals into a developmental response. This basic necessity for efficient internal communication underlies the origin of the small signaling molecules, termed hormones, present in both plants and animals (Alberts et al. 2007). While the essential role of hormones in cell-to-cell communication is evident in both cases, the response of the organism into which the hormonal signal is translated, diverge between two kingdoms. In animal species, solutions based on a modulation of behavior were primarily promoted (Davies 2004). In contrast, plants, due to the sessile nature of their lifestyle, developed a remarkable repertoire of mechanisms which allow them, through fine-tuning of metabolism or body shaping, to adjust and survive in ever changing and often adverse environments (Tanaka et al. 2006). These mechanisms are facilitated not only by intercellular communication but also by tightly regulated cell division, morphogenesis, and differentiation. Importantly, most, if not all of them, are based on cell polarization and repolarization which guide tissue and organ patterning and thus underpin basic shape and functionality of an organism. The phenomenon of cell polarity can be reflected in various aspects like disproportional growth or asymmetrical distribution of the cellular components. The term itself, however, is much broader and in largest sense applies to the generation of any asymmetry in relation to an axis (Sauer and Friml 2004; Geldner 2009).

Despite its complexity, plant development, with its remarkable polarization-based flexibility, is coordinated most prominently by a single phytohormone—auxin—which serves itself as a polarizing cue (Chap. 14; Berleth and Sachs 2001; Sauer et al. 2006; Leyser 2011). Moreover, generation of local auxin accumulations (gradients) plays an essential role in plethora of crucial events during plant development, like embryogenesis (Chap. 9; Friml et al. 2003; Schlereth et al. 2010), organogenesis (Chaps. 10 and 11; Benková et al. 2003), phyllotaxis (Reinhardt et al. 2003), root meristem organization (Sabatini et al. 1999; Friml et al. 2002a), root stem cell differentiation (Ding and Friml 2010), or vascular tissue patterning (Scarpella et al. 2006). Although essentially all plant tissues possess the capacity for auxin biosynthesis (Mano and Nemoto 2012; Ljung 2013) and metabolism (Ludwig-Müller 2011; Ruiz Rosquete et al. 2012), it is the directional auxin transport that most significantly contributes for establishing of auxin gradients across plant tissues (Tanaka et al. 2006). The existence of such a cell-to-cell transport of auxin (see Chap. 5), which is the unique feature among other phytohormones, was predicted in the mid-1970s by so-called chemiosmotic model (Rubery and Sheldrake 1974; Raven 1975). This model postulated an auxin efflux from the cell, facilitated by polarly localized exporters, as a critical step during intercellular auxin translocation. These predictions were spectacularly confirmed by characterization of the broad spectrum of developmental phenotypes caused by mutations in the *PIN-FORMED* (*PIN*) genes (Gälweiler et al. 1998; Luschnig et al. 1998; Okada et al. 1991). Basically all different phenotypes found in various *pin* mutants could be mimicked by treatments with auxin transport inhibitors (Vieten et al. 2007). Transport assays from both plant and heterologous systems, provided later, shown that PIN proteins indeed mediate auxin export from the cells (Petrášek et al. 2006). The intercellular auxin transport, beside of PINs, rely on the

coordinated activity of two other transporter families. These are AUXIN RESISTANT1/LIKE AUX1 (AUX1/LAX) and MULTIDRUG RESISTANCE/PHOSPHOGLYCOPROTEIN/ATP-BINDING CASSETTE OF B-TYPE (MDR/PGP/ABCB) proteins, facilitating influx and efflux of auxin from the cell, respectively (Bennett et al. 1996; Noh et al. 2001; Kramer 2004; Yang et al. 2006; Mravec et al. 2008; Swarup et al. 2008; Verrier et al. 2008). Despite the fact that multiple components are involved, a critical control of the directionality of auxin flux is attributed to the efflux activity of the PIN transporters at their highly defined, polar, subcellular domains (Wiśniewska et al. 2006; Blakeslee et al. 2007; Titapiwatanakun et al. 2009). PIN family consist of eight members, most of which (PIN1, 2, 3, 4, 6, and 7) dependent on the tissue or developmental context exhibit plasma membrane (PM) localization restricted mainly to the apical (shootward; shoot-apex-facing) or basal (rootward; root-apex-facing) side of the cell (Zažímalová et al. 2007). Notably, also AUX/LAXs and ABCBs, which serve as an additional source of auxin for PIN-mediated transport (Geisler et al. 2005; Mravec et al. 2008; Christie et al. 2011; Kubeš et al. 2012), in some cases display asymmetric distribution (Swarup et al. 2001; Panikashvili et al. 2007; McFarlane et al. 2010).

Although some similarities can be found (Geldner 2009), in general, the mechanisms underlying cell polarity in plants differ from those characterized in animals (Tepass et al. 2001; Humbert et al. 2006; Wells et al. 2006; Chen et al. 2010). It seems that in both animal and plant systems the delivery of protein to the place of action by subcellular trafficking is equally important (Dudu et al. 2004; Altschuler et al. 2008; Geldner 2009; Shivas et al. 2010). On the other hand, the most prominent trafficking-based determinants of polarity found in animals, like CRUMBS, SCRIBBLE, and PAR complexes, are missing in plant genomes (Geldner 2009). Additionally, counterparts of so-called tight junctions which in animals serve as diffusion barriers, dividing PM of epithelial cells into apical and baso-lateral, polar domains (Giepmans and van Ijzendoorn 2009), are missing in majority of plant cell types. A similar structure is present in plants in form of 'Casparian Strip', belts of specialized cell wall material generating an extracellular diffusion barrier, found exclusively in endodermis (Roppolo et al. 2011). Polar trafficking pathways described in plants cells appear to be more complex than those found in animals. Besides apical and basal PM domains, characteristic for animal epidermal cells, also outer- and inner-lateral domains, with corresponding polar cargos, can be found in similar cell types in plants (Miwa et al. 2007; Langowski et al. 2010; Takano et al. 2010). What is more, the differences are reflected not only on the cellular but also on the tissue level. Plants in contrast to animals cannot use the mechanism of invasive, cell-migration-based tissue patterning due to the presence of the rigid extracellular matrix, cell wall, encapsulating plant cells and making them immobile (Dettmer and Friml 2011).

Considering the aforementioned differences it is not surprising that alternative, to animal, solutions were promoted by evolution in plant kingdom. In this chapter, these plant-specific mechanisms for cellular polarization are discussed. The polarity of cellular components is mainly considered in context of PIN proteins, due to their

essential role in auxin-mediated plant development. Moreover, in case of PIN family enough molecular components and polarity generating/maintaining signals are described for drawing a comprehensive and interesting overview. First, subcellular trafficking machinery relevant for PIN polarity is characterized. Next, cell structure and cargo-related determinants for PIN targeting and maintenance at the polar domain are presented. Finally, the feedback mechanisms for PIN polarization are discussed. It should be taken into account that such a categorization, due to frequently redundant nature of the biological processes, is largely subjective and serves mainly for presentation purposes.

## 2 Long Journey with Unsure Destination: Trafficking for PIN Polarization

Polar localization of PIN auxin transporters, restricted to the specific side of the cell relies, among other mechanisms, on the function of complicated network, encompassing multiple bypassing and interconnected pathways collectively referred to as 'intracellular trafficking' (Paul and Frigerio 2007; Bassham et al. 2008; Robinson et al. 2008; Irani and Russinova 2009; Žárský et al. 2009). Auxin transporters, subjected to intracellular trafficking, originate from de novo synthesis and are delivered to the PM through so-called anterograde route. This mode of intracellular transport generally involves sequential steps including protein folding in endoplasmic reticulum (ER), translocation through the cis- and trans-cisternae of the Golgi Apparatus (GA) with final arrival to the cell surface (Vitale and Denecke 1999; Matheson et al. 2006).

### *2.1 Constitutive PIN Cycling for Rapid Repolarizations*

Contrary to the usually presented rather static snapshot pictures, the polar localization of PIN proteins is in reality very dynamic. Once delivered to the cell surface, PINs undergo continuous shuttling between PM and intracellular compartments by rounds of internalization (endocytosis) and polar recycling (exocytosis). These processes are jointly referred to as 'constitutive endocytic cycling' (Geldner et al. 2001; Dhonukshe et al. 2007; Kleine-Vehn et al. 2011). It is not entirely clear to which extent the initial secretion of de novo synthesized PINs to the PM occurs in polar fashion (Langowski and Friml, unpublished) or whether the endocytic cycling is generating the polar distribution following apolar secretion (Dhonukshe et al. 2008a).

Nevertheless, the first station reached by internalized PINs on their endocytic trafficking route from the PM is early endosome (EE). This subcellular compartment is critical as it is the intersection between secretory and endocytic routes and,

in plants, originates from and associates with trans-Golgi network (TGN) (Dettmer et al. 2006; Lam et al. 2007; Viotti et al. 2010). Thus, sorting of vesicular cargo for various subcellular destinations occurs at EE/TGN (Liu et al. 2002; Dettmer et al. 2006). Once destined for recycling, PINs are translocated from EE/TGN to the hypothetical compartment called recycling endosome (RE), where they fall under the control of ADP-Ribosylation Factor GTPase (ARF-GTPase) machinery. ARF-GTPases, by recruitment of vesicle coat proteins and organizing cytoskeleton at membrane surfaces, control vesicle trafficking. Their spatiotemporal activity is determined by the antagonistic activity of ARF guanine nucleotide exchange factors (ARF-GEFs) and ARF-GTPase activating proteins (ARF-GAPs), which are activating and deactivating ARF-GTPase complex, respectively (D'Souza-Schorey and Chavrier 2006). GNOM, a protein belonging to the golgi-associated, brefeldin-A resistant guanine nucleotide exchange factor (GBF) class of ARF-GEFs, most prominently controls the polar recycling of PINs to the PM (Fig. 8.1; Geldner et al. 2003). This protein, and more specifically its Sec7 domain, is a target of fungal toxin brefeldin A (BFA), which by inhibiting GNOM-mediated exocytosis, causes reversible intracellular accumulation of constitutively endocytosed proteins and aggregation of TGN into so-called BFA compartments or BFA bodies. This effect serves as a tool to visualize the constitutive cycling of PM proteins (Geldner et al. 2001). Interestingly, GNOM controls preferentially PIN recycling to the basal side of the cell, whereas pathway by which PINs are targeted to the apical domain is, most likely, additionally controlled by uncharacterized, BFA-insensitive ARF-GEF (Kleine-Vehn et al. 2008a, b).

Besides ARF-GTPases, also RabA1B, a member of small Rab-GTPase family and INTERACTOR OF CONSTITUTIVE ACTIVE ROP1 (ICR1), an effector of RHO OF PLANTS1 (ROP1) RAC-GTPase, were recently associated with defective PIN recycling (Fig. 8.1; Hazak et al. 2010; Feraru et al. 2012). Importantly, genetic interference with ICR1 results with severely disturbed polarity of PIN1 and PIN2, as well as various developmental defects (Hazak et al. 2010). Moreover, ICR1 was shown to interact with Sec3A (Lavy et al. 2007), one of the exocyst complex (EC) components. EC is known to participate in the extensive fusion of exocytic vesicles at specific sites of PM during so-called polarized exocytosis. Polarized exocytosis is controlled, among others, by Rho GTPases (Žárský et al. 2009). Interestingly, another subunit of the EC, Exo70, influences polar auxin transport through the regulation PIN1 and PIN2 recycling (Drdová et al. 2013).

The fundamental role of the cellular scaffolding and cytoskeleton for intracellular PIN trafficking should be also underlined here. PIN constitutive cycling seems to depend mainly on actin filaments since pharmacological interference with the integrity of this component abolishes internalization and recycling of PINs. On the other hand, microtubules are essential for both PIN trafficking in dividing cells as well as for maintenance of the general polarity of the cell (Geldner et al. 2001; Friml et al. 2002b; Petrášek and Schwarzerová 2009; Dhonukshe et al. 2008b; Boutté et al. 2006; Kleine-Vehn et al. 2006, 2008b, c; Kleine-Vehn and Friml 2008). The components of cytoskeleton not only serve as an orientation cues,

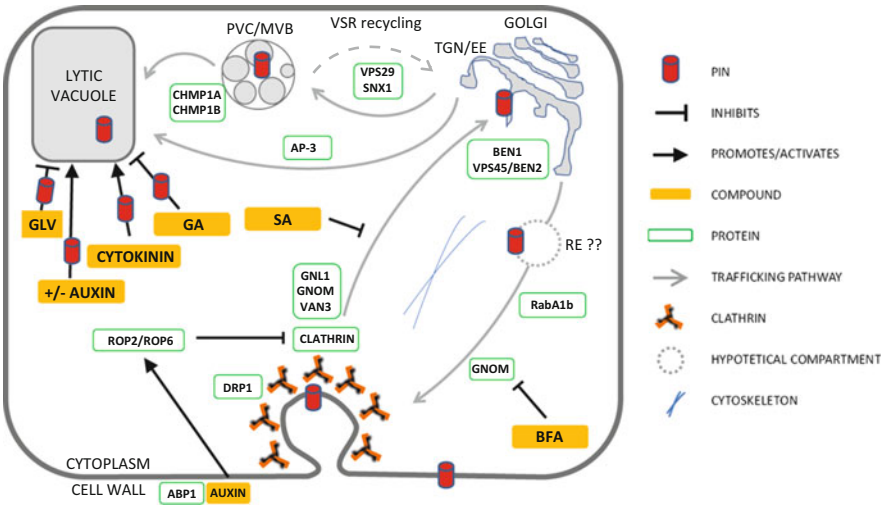


**Fig. 8.1** Intracellular trafficking for polarization of PINs. Auxin, when extracellularly bound to ABP1, rapidly inhibits clathrin-mediated PIN endocytosis through mutually exclusive ROP2/ROP6 signaling. Formation of the PIN-containing, clathrin-coated vesicles, in case of cell plate formation, requires the function of DRP1. Internalization of cargo vesicles from the plasma membrane is mediated by Rab5 GTPase ARA7, ARF-GEFs GNOM, GNL1, and ARF-GAP VAN3, inhibited by SA and proceeds along cytoskeletal cell scaffold. Early endocytic trafficking of PINs requires function of VPS45 BEN2 and ARF-GEF BEN1. PIN recycling depends on RabA1b GTPase and GNOM, a target of BFA-mediated inhibition of exocytosis. PIN vacuolar targeting route passing through PVC/MVB includes the regulation by retromer complex components VPS29 and SNX1, as well as ESCRT components CHMP1A and CHMP1B. PVC/MVB-bypassing route for regulating vacuolar function is mediated by AP-3 complex. Cytokinin and long-term, above- or below-optimal auxin levels reduce the membrane abundance of PINs by promoting their turnover. In contrast, vacuolar targeting of PINs is inhibited by GA and GLV peptides

according to which PINs polarize (Heisler et al. 2010), but also provide guidance for vesicle trafficking (Voigt et al. 2005) (Fig. 8.1).

The functional significance of an energy-demanding process such as constitutive cycling still remains unclear. Evidently, this process provides the means for fast and de novo synthesis-independent repositioning of PIN auxin transporters, in response to internal and external cues. It, thus, allows developmental flexibility conditioned by rapid redirection of auxin fluxes within tissues (Michniewicz et al. 2007a; Vanneste and Friml 2009). Another, attractive scenario highlights a possible analogy between auxin efflux and neurotransmitter release. In this scenario, PIN transporters localized at the surface of constitutively cycling intracellular vesicles would mediate uptake of auxin from the cytosol into these vesicles and after their arrival and fusion with the PM, auxin would be released from the cell, similarly to the synaptic release of neurotransmitters (Friml and Palme 2002; Baluška et al. 2003). Such a hypothesis was to some extent supported by experiments based on auxin immunolocalization and manipulation of secretory pathway using phospholipase

Dζ2 mutant (Schlicht et al. 2006; Mancuso et al. 2007). The validation of the 'neurotransmitter' hypothesis would provide a connection between the effect of established inhibitors of auxin transport on both vesicle trafficking and on PIN-dependent auxin transport (Geldner et al. 2001; Dhonukshe et al. 2008b). Finally, auxin transporters were proposed to have an additional function, similar to known, dual function receptor/transporters (Hertel 1983; Foti et al. 2004; Holler and Dikic 2004). In this scenario, endocytic cycling, analogously to the situation in animals, would serve as a way to transduce the signal and to regenerate receptors during ligand-dependent endocytosis. It is important to note that all the interpretations for functionality of constitutive cycling in the process of auxin transport are not mutually exclusive, but the latter two ('neurotransmitter' and transporter/receptor) remain rather speculative.

On the other hand, changes in PIN polarity have been observed in courses of many developmental processed including embryogenesis (Friml et al. 2003), organogenesis (Benková et al. 2003; Reinhardt et al. 2003), fruit development (Sorefan et al. 2009), vascular tissue formation and regeneration (Scarpella et al. 2006; Balla et al. 2011), as well as in response to light (Ding et al. 2011) or gravity (Kleine-Vehn et al. 2010; Rakusova et al. 2011). Such a dynamic translocation of polar cargos from one cell side to another via recycling endosomes is called transcytosis (Tuma and Hubbard 2003; Kleine-Vehn et al. 2008a, b) and it appears that plants evolved to utilize this mechanism to adjust their development in response to different cues via redirecting of PIN-dependent auxin fluxes.

## *2.2 Early Endocytic Processes for PIN Polarization*

The first step of endocytic recycling is the internalization from the PM. The most prominent route of PIN internalization occurs through the creation of the membrane curvature from which upon scission the intracellular vesicle is formed, during clathrin-mediated endocytosis (CME) (Fig. 8.1). Clathrin is a self-assembling protein, recruited to the membranes where it contributes to membrane deformation and serves as a vesicular coat constituent (Chen et al. 2011). The fundamental role of CME in PIN trafficking and thus its essential contribution to generation of intrinsic polar localization of PINs is well characterized. It was demonstrated by pharmacological and genetic interference with the clathrin function (Dhonukshe et al. 2007; Kitakura et al. 2011). The notion that auxin efflux carriers from the PIN family are cargos of CME was further supported by identification of DYNAMIN-RELATED PROTEIN 1 (DRP1) as associated with PIN1 and important for its endocytosis (Fig. 8.1; Mravec et al. 2011). In plants, the precise function of the dynamin superfamily, represented among others by DRP1, is not well characterized. It is speculated that these proteins might regulate membrane dynamics by modulation of its scission and tubulation (Praefcke and McMahon 2004; Heymann and Hinshaw 2009). Importantly, proteins belonging to this family were previously implicated in plant CME (Konopka et al. 2008; Fujimoto et al. 2010) and

interference with DRP1 function results in altered distribution of PIN1 in dividing cells which eventually leads to a range of developmental phenotypes related to defective auxin transport (Mravec et al. 2011).

Interestingly, ARF-GEF GNOM, function of which is typically associated with recycling to the PM, was also implicated in the regulation of endocytosis, based on its partial localization at the PM and PIN endocytosis defects observed in *gnom* knock-down mutants (Fig. 8.1; Naramoto et al. 2010). In addition, another GBF subfamily member GNOM LIKE1 (GNL1) and ARF-GAP—VASCULAR NETWORK DEFECTIVE 3 (VAN3)—have been functionally associated with PM and endocytic processes, corroborating the notion that ARF GTPase machinery is involved in endocytosis (Fig. 8.1; Teh and Moore 2007; Naramoto et al. 2010).

Following internalization from the PM, the early endocytic trafficking of PINs has been shown to rely on the BFA-VISUALIZED ENDOCYTIC TRAFFICKING DEFECTIVE1/HOPM INTERACTOR7 (BEN1/MIN7/BIG5) ARF-GEF, belonging to BFA-inhibited guanine nucleotide exchange protein (BIG) subfamily (Fig. 8.1; Tanaka et al. 2009). PIN internalization is regulated also by Rab5/ARA7, a member of Rab GTPase family (Fig. 8.1; Dhonukshe et al. 2008a) and an universal component of membrane fusion in eukaryotes—VACUOLAR PROTEIN SORTING45 (VPS45/BEN2) (Fig. 8.1; Tanaka et al. 2013). Genetic interference with all aforementioned components regulating endocytosis or early endocytic trafficking leads, besides perturbations in PIN trafficking and polarity, to significant developmental aberrations like embryogenesis and organogenesis defects, reduced growth and apical dominance, leaf venation pattern discontinuity, and root meristem disorganization. These observation links early endocytic processes to PIN polarity and auxin-mediated development.

## *2.3 Late Endocytic Trafficking for PIN Abundance at the Cell Surface*

Certain proportion of internalized PINs, based on signals which are not fully understood, is targeted for the late endocytic pathway. This mode of transport originates generally at EE/TGN, where proteins are sorted, proceeds through late endosomes (LE), prevacuolar compartments/multivesicular bodies (PVC/MVB) and terminates at the final destination of membrane proteins—the lytic vacuole. This subcellular route eventually results with protein degradation. Vacuolar targeting defines additional mechanism, by which polar localization of PINs and specifically the aspect of their membrane abundance can be controlled (Müller et al. 2007; Scheuring et al. 2011). As mentioned above, plant trafficking machinery encompasses multiple bypassing, often unidirectional transport routes. Accordingly, PINs can be retrieved from late endocytic pathway and thus avoid degradation. This is accomplished by the Retromer Complex (RC) capacity for retrieval of certain trafficking components like VACUOLAR SORTING RECEPTORS (VSR)

from PVC to EE/TGN (Arighi et al. 2004; Seaman 2005). Both the core component of this complex—VACUOLAR PROTEIN SORTING29 (VPS29) and its prominent interactor—SORTIN NEXIN1 (SNX1) were shown to control the rate of PINs progression toward the vacuole (Fig. 8.1; Kleine-Vehn et al. 2008c; Nodzyński et al. 2013). Such a mechanism is in agreement with widely accepted and evolutionary conserved function of RC (Arighi et al. 2004; Seaman 2005; Shimada et al. 2006). Notably, a more unorthodox function, related more directly to PIN polarity, has been also proposed for RC at the level of early endocytic recycling (Jaillais et al. 2006, 2007).

The significance of the final steps during late endocytic trafficking of PIN proteins for their polar localization and abundance is not to be underestimated. The endosomal-sorting complexes required for transport (ESCRT) machinery, controlling the formation of internal vesicles within PVC/MVB, which upon fusion are released into vacuolar lumen, appears to play important role for PIN polarization (Winter and Hauser 2006; Wollert et al. 2009; Scheuring et al. 2011). Interfering with the function of CHARGED MULTIVESICULAR BODY PROTEIN/CHROMATIN MODIFYING PROTEIN 1A and 1B (CHMP1A/CHMP1B), components of ESCRT machinery, leads to severe developmental defects including seedling lethality. These phenotypes were associated with inaccurately generated auxin distribution correlated with the ectopic (PVC/MVB and vacuolar membranes) PIN localization (Fig. 8.1; Spitzer et al. 2009). Also an alternative, PVC-bypassing, late endocytic pathway, dependent on the ADAPTOR PROTEIN (AP) Complex 3 subunits β and δ, generally regulates vacuolar function and thus PIN degradation rate, although it does not affect PIN polarity or abundance at the PM (Fig. 8.1; Feraru et al. 2010; Zwiewka et al. 2011).

## 3 Where to Go and Why to Stay? Cues and Cellular Requirements for Pin Polarity

### *3.1 Cargo-Based Determinants for Polar PIN Localization*

One of the most important initial findings concerning the determination of PIN polarity was derived from the ectopic PIN expression in particular cell types. The PIN2 promoter-driven expression of PIN1 targeted this protein predominantly to the basal side of root epidermal cells contrasting to the native apical PIN2 localization in the same cells. Consecutive introduction of the fluorescent tag into certain place within central hydrophilic loop of PIN1 was sufficient to cause basal-to-apical switch in PIN1 localization. This was a clear indication that some determinants of polar PIN localization are encoded intrinsically in PIN amino acid sequence (Wiśniewska et al. 2006). This sequence-based instruction turned out to be the phosphorylation status of specific serine residues located within PIN central hydrophilic loop (Huang et al. 2010; Zhang et al. 2010). Current model postulates that

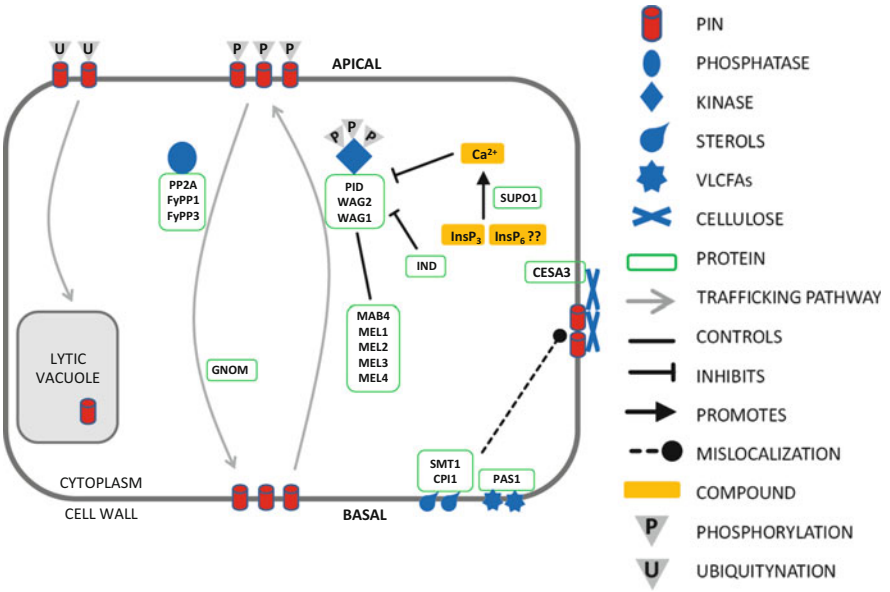


**Fig. 8.2** Posttranslational modifications and cellular requirements for polarization of PINs. PINs dephosphorylated by PP2A, FyPP1, or FyPP3 phosphatases are preferentially recruited to the basal, GNOM-dependent, BFA-sensitive pathway, whereas phosphorylation by PID, WAG1, or WAG2 kinases targets PINs into apical cell site independently of GNOM function. The transcription of PID kinase is controlled by IND transcription factor. The activity of the kinases is influenced also by MAB4, its homologs MELs and by $InsP_3$ (or alternatively $InsP_6$) that regulate cellular $Ca^{2+}$ levels with the assistance of SUPO1. The polar localization of PINs depends on the sterol and VLCFA composition of the membrane controlled by SMT1, CPI1, and PAS1, respectively. Membrane abundance of PINs, regulated by their vacuolar targeting, depends on the ubiquitination status of the protein. Cellulose content of the cell wall regulated by CESA3 contributes to the maintenance of PIN polarity

dephosphorylated PINs are preferentially recruited to the basal, GNOM-dependent and BFA-sensitive pathway, whereas phosphorylation targets PINs into the apical cell side, independently of GNOM function (Fig. 8.2; Friml et al. 2004; Michniewicz et al. 2007b; Kleine-Vehn et al. 2009).

Readjustments of PIN phosphorylation status rely on the antagonistic activity of protein kinases and phosphatases. AGC3 protein kinases PINOID (PID) and its homologs WAVY ROOT GROWTH1/2 (WAG1/WAG2) (Benjamins et al. 2001; Friml et al. 2004; Santner and Watson 2006; Dhonukshe et al. 2010) phosphorylate, while PROTEIN PHOSPHATASE2A (PP2A) (Muday and DeLong 2001; Michniewicz et al. 2007b; Ballesteros et al. 2013) dephosphorylate PINs (Fig. 8.2). Along with PID and WAG proteins, also other kinases such as D6 protein kinase (Zourelidou et al. 2009) or CDPK-RELATED KINASE5 (Rigó et al. 2013) can phosphorylate PIN proteins and regulate their function, but their exact role is less clear. On the other hand, phosphatase subunit PP2AA interacts with another Ser/Thr protein phosphatase, FyPP1, to form functional holoenzyme. FyPP1 and its

close homolog FyPP3 were reported to interact with and directly dephosphorylate PINs (Fig. 8.2; Dai et al. 2012). Importantly, fluctuations of PIN phosphorylation status both above and below certain native threshold lead eventually to severe developmental aberrations like defective embryogenesis and patterning of shoot apical meristem and root (Christensen et al. 2000; Benjamins et al. 2001; Friml et al. 2004; Michniewicz et al. 2007b; Dhonukshe et al. 2010; Li et al. 2011; Dai et al. 2012).

Given the crucial role of PID-mediated PIN phosphorylation in PIN polarity, it is important to highlight that the regulation of PID activity, on various levels, has also impact on polar PIN localization and thus on auxin fluxes. Calcium ($Ca^{2+}$), which is one of the most ubiquitous secondary messengers in eukaryotes, appears to be the prominent part of such a system for the regulation of PID kinase activity (Fig. 8.2). Early experiments in animal and yeast systems have associated the appearance of cytosolic $Ca^{2+}$ with the phospholipase C (PLC)-generated inositol trisphosphate ($InsP_3$). PLC signaling is known to be important for various biological processes including cell division and differentiation (Michell 2008). The plant field has followed this paradigm upon an observation that $InsP_3$ is able to trigger the release of $Ca^{2+}$ from the cellular storage compartments (Blatt et al. 1990; Gilroy et al. 1990; Krinke et al. 2007; Tang et al. 2007). Controversially, up to date no unambiguous $InsP_3$-activated $Ca^{2+}$ channel could be identified in plants (Testerink and Munnik 2011). Interestingly, there are indications that $InsP_6$ can function as a signaling molecule, triggering $Ca^{2+}$ release with a much higher potency than $InsP_3$ (Lemtiri-Chlieh et al. 2003). Moreover, $InsP_3$ when microinjected into plant can be rapidly converted into $InsP_6$ (Munnik and Testerink 2009), explaining the earlier observations of $InsP_3$ being able to release $Ca^{2+}$. PID kinase was shown to be regulated by both phospholipid and $Ca^{2+}$ signaling. Some PID interactors bind $Ca^{2+}$ (Benjamins et al. 2003; Zegzouti et al. 2006). Moreover, *suppressor of PIN1 overexpression*-1 (*supo-1*) mutant encoding ALTERED EXPRESSION OF APX2 8/FIERY1/HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES2/ROTUNDA1 (ALX8/FRY1/HOS2/RON1/SAL1) inositol polyphosphate 1-phosphatase also with 3′(2′),5′-bisphosphate nucleotidase activity exhibits aberrant PIN polarization presumably as a result of changed PID activity. It was proposed that this effect is a result of altered content of cytosolic $Ca^{2+}$ in a mutant due to disturbed $InsP_3$ metabolism (Fig. 8.2; Zhang et al. 2011a). Although in light of recent findings, an alternative explanation, in which $InsP_6$ would be the signal activating $Ca^{2+}$ cascade, seems more plausible (Munnik and Nielsen 2011).

Another mode of PID activity regulation applies to its transcription. INDEHISCENT (IND), a basic helix–loop–helix transcription factor through negative regulation of PID and WAG2 expression influences polar localization of PIN1 and PIN3 proteins (Fig. 8.2). Interestingly, in this case local auxin minimum, which is required for valve margin formation in *Arabidopsis* fruit, is not properly established. Consequently, abnormal fruits which fail to open and thus do not disperse seeds are observed in *ind* mutant (Sorefan et al. 2009). Another regulator of PID activity is ENHANCER OF PINOID/MACCHI-BOU4 (ENP/MAB4) (Treml et al. 2005; Furutani et al. 2007) and its close homologs MAB4/ENP/

NPY1-LIKEs (MEL1, MEL2, MEL3, and MEL4) that are known to influence polar PIN localization (Fig. 8.2; Furutani et al. 2011). MAB4 encodes NON-PHOTOTROPIC HYPOCOTYL3-like (NPH3)-like protein, which associates with light activated kinase PHOTOTROPIN1 (PHOT1), a blue-light receptor (Motchoulski and Liscum 1999). NPH3 has been shown to modulate PIN2 trafficking in root phototropism (Wan et al. 2012).

Regulation of PID activity provides also an entry point for various external signals, such as light or gravity, to which plants are able to dynamically respond by modulation of their growth. For example, during hypocotyl gravitropic response, PIN3 and PIN7 polarize to the bottom side of gravity-sensing endodermal cells and mediate the differential auxin accumulation at the lower side of hypocotyl for asymmetric bending and growth (Rakusova et al. 2011). Accordingly, blue-light-dependent signaling cascade causes polarization of PIN3 away from the light during phototropic response in hypocotyl endodermal cells. This coincides with an establishment of auxin maximum at the shaded side of an organ (Ding et al. 2011). Importantly, in both cases PIN repolarization is initiated by differential recruitment into GNOM-mediated trafficking pathway and depends on the PID-mediated phosphorylation status of the PIN protein (Ding et al. 2011; Rakusova et al. 2011).

Beside PIN phosphorylation, another posttranslational protein modification appears to be instructive for PIN localization. The destabilization of PINs from the PM and their sorting for vacuolar delivery was associated with the linking of the polyubiquitin chains to specific lysine residues within PIN2 central hydrophilic loop (Fig. 8.2; Leitner et al. 2012). Moreover, PIN2 degradation was shown to be dependent on the 26S proteasome, the universal proteolysis complex of eukaryotic organisms, which targets ubiquitinated, typically soluble proteins (Sieberer et al. 2000; Abas et al. 2006). Although recent data clearly show that ubiquitination is a crucial part of PIN abundance control (Abas et al. 2006; Leitner et al. 2012), it remains unclear how proteasome activity can contribute to the degradation of PM proteins such as PINs, which were shown to be targeted to the lytic vacuoles (Kleine-Vehn et al. 2008c; Laxmi et al. 2008; Shirakawa et al. 2009; Marhavý et al. 2011; Baster et al. 2013).

## *3.2 Cell Structural Determinants of PIN Polarity Maintenance*

The cues and mechanisms described in the previous paragraph dealt mainly with targeting of PINs to their respective polar domains but not with their maintenance there. As mentioned before, crucial polarity trafficking components as well as ‘tight junctions’, which in animals limit migration of the surface proteins between polar domains of epithelial cells, are absent in the plant kingdom. On the other hand, plant cells are surrounded by cell wall, a structure absent in animal cells. It appears that in

order to preserve abundance and asymmetry of proteins within fluid PM, in comparison to animals, plants developed both overlapping and alternative mechanisms. The migration of transmembrane proteins within the lipid bilayer can serve as an example of polarity regulation mechanism which is common for plants and animals. Interestingly, certain fraction of membrane localized PINs, as reported previously, appears to be surprisingly stagnant (Dhonukshe et al. 2008a; Men et al. 2008; Kleine-Vehn et al. 2011). This phenomenon was proposed to be linked with processes that actually immobilize this fraction of PINs within specific structures at the PM, called clusters, which have been detected by super-resolution microscopy approaches (Kleine-Vehn et al. 2011). The nature of these highly immobile structures is not entirely clear; however, their appearance might be related to specific sterol and lipid composition of the PM (Kleine-Vehn et al. 2011; Men et al. 2008; Roudier et al. 2010; Carland et al. 2010; Martinière et al. 2012). In fact, the *sterol methyl transferase 1* (*smt1*) mutant, function of which is required for appropriate synthesis and composition of major membrane sterols (Diener et al. 2000), is characterized by defective polar auxin transport correlating with mislocalization of PIN1 and PIN3 proteins (Fig. 8.2; Willemsen et al. 2003). Similarly, the improper reestablishment of PIN2 polarity following cytokinesis as a consequence of defective PIN2 endocytosis was reported for sterol biosynthesis, *cyclopropylsterol isomerase1-1 (cpi1-1)* mutant (Fig. 8.2; Men et al. 2008). The involvement of sterols in polar distribution of PIN proteins is additionally supported by the fact that the internalized PIN2 co-localizes with the sterol marker filipin and a prolonged disruption of membrane sterols by filipin treatments reduces the heterogeneity and polar localization of PIN2 in the PM (Grebe et al. 2003; Kleine-Vehn et al. 2006, 2011).

Apart from sterols, some other molecular components that physically scaffold structure of the membrane appear very important for maintaining PIN polarity. Sphingolipids, membrane constituents and signaling molecules (Dickson et al. 2006), were shown to influence cell polarity (Hoekstra et al. 2003; Nyasae et al. 2003). The immunophilin-like protein PASSTICINO1 (PAS1) (Bach et al. 2008; Roudier et al. 2010) is involved in the biosynthesis and metabolism of very-long-chain fatty acids (VLCFAs), one of the composites of sphingolipids. In case of *pas1* mutant, patterning defects at the cellular level were attributed to altered auxin distribution during key events in plant life. Disturbed formation of auxin gradients was associated with abnormal PM distribution of PIN1 protein due to defective VLCFA synthesis (Fig. 8.2; Roudier et al. 2010). Additionally, PIN1 abundance in its polar domain was shown to be stabilized by interaction with PGP1/PGP19 ABCB transporters (Titapiwatanakun et al. 2009). Notably, the efflux activity of these ABCB transporters at the PM is positively regulated by PID, most likely through direct phosphorylation. In this case, another immunophilin—TWISTED DWARF (TWD) by interaction with PID appears to decrease ABCB activity at the cell surface (Bouchard et al. 2006; Henrichs et al. 2012; Wang et al. 2012, 2013).

Finally, recent reports suggest that not only structure of the PM but also the integrity of the cell wall is required for maintenance of PIN polarity. Such a notion

was suggested upon characterization of *regulator of PIN polarity3 (repp3)* mutant (Feraru et al. 2011), exhibiting defects in localization of ectopically expressed PIN1. The mutation responsible for *repp* mutant phenotype was found in the gene coding for CELLULOSE SYNTHASE CATALYTIC SUBUNIT3/CONSTITUTIVE EXPRESSION OF VSP1/ISOXABEN RESISTANT1/ECTOPIC LIGNIN1 (CESA3/CEV1/IXR1/ELI1). CESA3 is a part of the enzymatic complex required for synthesis of 1,4 glucans, molecules which are able to associate to form cellulose microfibrils and thus scaffold cell wall (Fig. 8.2; Richmond and Somerville 2000; Ellis and Turner 2001; Scheible et al. 2001; Caño-Delgado et al. 2003; Desprez et al. 2007). Additionally, pharmacologically induced cell wall degradation or inhibition of the cell wall biosynthesis resulted in similar phenotypes as in case of *repp3* mutant. Interestingly, plasmolysis-based experiments forcing detachment of the polar domain from the cell wall suggested that the mechanisms immobilizing PIN-containing PM clusters might relate to cellulose-based connections between the polar domain and the cell wall (Feraru et al. 2011; Martinière et al. 2012).

## 4 Transport in Loops: Hormonal Feedback Regulations of PIN Polarity

### *4.1 Auxin Feedback on PIN-Dependent Auxin Transport*

Polar distribution of PIN auxin transporters can be regulated through plethora of controlling mechanisms with various molecular players involved. Intriguingly, another layer of complexity for auxin-driven plant development emerges from the self-organizing abilities of auxin transport. Already early experiments implied that auxin-induced changes could instruct capacity and directionality of auxin flow and thus auxin would have the ability to shape its own transport (Sachs 1981, 1991). Indeed, the effect of auxin on PIN amounts and PIN localization and thus existence of multiple feedback mechanisms at various levels has been validated experimentally. One of such mechanisms is a well-characterized nucleus-based $SCF^{TIR1/AFB}$-dependent auxin signaling (Dharmasiri and Estelle 2004; Dharmasiri et al. 2005a, b; Kepinski and Leyser 2005; Chapman and Estelle 2009), involved in the regulation of PIN transcription (Peer et al. 2004; Vieten et al. 2005; Heisler et al. 2005; Scarpella et al. 2006). $SCF^{TIR1/AFB}$-dependent auxin signaling appears to have a double role in transport feedback, controlling both PIN transcription and abundance at the PM by promoting, upon prolonged auxin exposure, PIN vacuolar targeting for degradation (Baster et al. 2013). The gravitropic response of the roots serve as an example of the process facilitated by such a dual mechanism in which fluctuations of auxin above or below certain physiological threshold through ($SCF^{TIR1/AFB}$)-dependent signaling mediate PIN degradation (Fig. 8.1; Abas et al. 2006; Kleine-Vehn et al. 2008c; Baster et al. 2013). In addition, this transcriptional signaling is

also required to feedback on PIN polarity and thus directionality of auxin transport in both root- and shoot-based model systems (Sauer et al. 2006; Balla et al. 2011).

The auxin feedback loop which has recently drawn considerable amount of attention, due to its proposed contribution to the polarization of auxin transporters, is the non-transcriptional auxin effect on PIN endocytosis. Indeed auxin, rapidly upon application, inhibits PIN internalization and promotes the retention of PINs at the PM correlating with increased auxin efflux capacity (Paciorek et al. 2005). Although the mechanism is still largely elusive, it was proposed that auxin, when extracellulary bound to AUXIN-BINDING PROTEIN1 (ABP1), executes this inhibitory function through dynamic activation of mutually exclusive RHO OF PLANTS2 and 6 (ROP2/ROP6) pathways downstream of ABP1 (Fig. 8.1). This mechanism is functionally important in developmental processes like patterning of the leaf epidermis or root gravitropism (Robert et al. 2010; Xu et al. 2010; Nagawa et al. 2012; Chen et al. 2012; Lin et al. 2012).

Additional complexity of the system, which depends on auxin-mediated feedback and regulates plant development, emerges from the fact that some of its elements are interconnected. For example CME, activity of which was shown to be nonuniform throughout the root meristem, directly influences auxin-responsive gene expression. Such a mechanism is based on the positive autoregulatory feedback mediated by BREVIS RADIX (BRX). This plant-specific transcription factor, through auxin-regulated PM-to-nucleus transfer and subsequent transcriptional activation of certain auxin response factor targets, controls the cell elongation and proliferation in the root tip. Therefore, the differential pattern of endocytosis splits the transcriptional auxin signaling within the root meristem and might thus provide additional positional information to interpret auxin gradients (Mouchel et al. 2004; Santuari et al. 2011).

Notably, the experimental approaches aiming to explain the principles of auxin feedback-mediated plant development are more and more prominently supported by computational models. For example, recently proposed ‘extracellular receptor-based polarization’ (ERP) model integrates transcription-based intracellular feedback mechanisms with a competitive utilization of auxin receptors in the cell exterior for the spatial regulation of PIN internalization (Wabnik et al. 2010, 2011). Given the fact that during various developmental processes, PINs can behave differentially, polarizing either toward or away from the auxin source (Grieneisen et al. 2007; Blilou et al. 2005; Benjamins and Scheres 2008; Kleine-Vehn et al. 2008b), it is worth mentioning that ERP model proposes mechanistic principles explaining these contrasting self-organizing properties of auxin transport (Wabnik et al. 2010, 2011). However, it remains to be seen whether this largely theoretical model corresponds to the biological reality. This question will be solved only after the molecular mechanism underlying the polarization of PINs and auxin transport will be elucidated.

### 4.2 Other Hormonal Regulations of PIN-Dependent Auxin Transport

It seems that not only auxin can shape the capacity and directionality of its transport. Other hormones, by influencing the PM stability of PIN auxin transporters, can be also integrated into the PIN-dependent auxin distribution network. Most of the plant hormones have been shown to regulate transcription of numerous genes downstream of their corresponding signaling pathways, thus many of them directly or indirectly influence also the transcription of *PIN* genes. Such an effect is well characterized in case of cytokinin and ethylene, both of which have also multiple developmental functions (Swarup et al. 2007; Růžička et al. 2007, 2009; Dello Ioio et al. 2008; Zhang et al. 2011b; Bishopp et al. 2011; Liu et al. 2013).

Other hormones prominently modulate PIN activity by posttranscriptional regulation. For example, a stabilization of PINs at the PM by interference with their endocytosis was observed when plants were subjected to pharmacologically or genetically induced accumulation of the plant hormone salicylic acid (SA) (Fig. 8.1; Du et al. 2013). In contrast, gibberellic acid (GA) deficiency, observed in GA biosynthesis mutants, promotes degradation of PIN proteins, whereas treatment with GA increases PIN protein stability by inhibiting PIN vacuolar trafficking. This mechanism appears to be important for correct gravitropic response of the root (Fig. 8.1; Willige et al. 2011; Löfke et al. 2013). The stabilization of PIN2 at the membrane resulting in the perturbations in root gravitropism could be also observed in both *Arabidopsis* plants overexpressing *GOLVEN* (*GLV*) genes encoding for small secretory peptides of ROOT GROWTH FACTOR (RGF) family as well as upon exogenous applications of such compounds (Fig. 8.1; Matsuzaki et al. 2010; Whitford et al. 2012). In contrast to these stabilizing effects, cytokinin promotes vacuolar trafficking of PINs thus destabilizing them from the PM. Functionality of this mechanism was demonstrated during lateral root organogenesis (Fig. 8.1; Marhavý et al. 2011). These frequent observations on the effects of various signaling pathways converging at the regulation of PIN-dependent auxin distribution network are in line with the model that PIN-mediated asymmetric auxin distribution functions as a versatile mechanism integrating multiple internal and external signals (Vanneste and Friml 2009).

## 5 Conclusions and Perspectives

In recent years, there has been a significant advance achieved in our understanding of the basic rules governing generation, maintenance, and refining of the cellular polarity in plants. It becomes clear how polar protein localization at the subcellular scale can underpin basic functionality of an organism and instruct its development. In particular, the studying of asymmetric distribution displayed by PIN auxin transporters allowed us to deepen the knowledge about polarity. At the same

time, it provided excellent means to comprehend auxin transport machinery and its contribution, by establishment of asymmetric auxin distribution, to the regulation of various developmental processes. An important open question remains: to which extent cellular mechanisms and molecular components of the PIN polar targeting machinery can be translated into proteins localized at different polar domains in plants. Another important aspect is the evolution of auxin transport machinery and thus mechanisms governing polarity generation in more ancient plant species. For this, the examination of evolutionary older than Angiosperms plant species like moss or algae, which is rapidly gaining popularity in the plant field, will hopefully significantly contribute to our understanding of the polarity phenomenon in the close future.

## References

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

Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P (2007) Molecular biology of the cell, 5th edn. Garland, New York

Altschuler SJ, Angenent SB, Wang Y, Wu LF (2008) On the spontaneous emergence of cell polarity. Nature 454:886–889

Arighi CN, Hartnell LM, Aguilar RC, Haft CR, Bonifacino JS (2004) Role of the mammalian retromer in sorting of the cation-independent mannose 6-phosphate receptor. J Cell Biol 165:123–133

Bach L, Michaelson LV, Haslam R, Bellec Y, Gissot L, Marion J, Da Costa M, Boutin JP, Miquel M, Tellier F, Domergue F, Markham JE, Beaudoin F, Napier JA, Faure JD (2008) The plant very long chain hydroxy fatty Acyl-CoA dehydratase PASTICCINO2 is essential and limiting for plant development. Proc Natl Acad Sci U S A 105:14727–14731

Balla J, Kalousek P, Reinöhl V, Friml J, Procházka S (2011) Competitive canalization of PIN-dependent auxin flow from axillary buds controls pea bud outgrowth. Plant J 65:571–577

Ballesteros I, Domínguez T, Sauer M, Paredes P, Duprat A, Rojo E, Sanmartín M, Sánchez-Serrano JJ (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 J 73:862–872

Baluška F, Samaj J, Menzel D (2003) Polar transport of auxin: carrier-mediated flux across the plasma membrane or neurotransmitter-like secretion? Trends Cell Biol 13:282–285

Bassham DC, Brandizzi F, Otegui MS, Sanderfoot AA (2008) The secretory system of *Arabidopsis*. Arabidopsis Book 6:e0116

Baster P, Robert S, Kleine-Vehn J, Vanneste S, Kania U, Grunewald W, De Rybel B, Beeckman T, Friml J (2013) SCF(TIR1/AFB)-auxin signaling regulates PIN vacuolar trafficking and auxin fluxes during root gravitropism. EMBO J 32:260–274

Benjamins R, Scheres B (2008) Auxin: the looping star in plant development. Annu Rev Plant Biol 59:443–465

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

Benjamins R, Ampudia CSG, Hooykaas PJJ, Offringa R (2003) PINOID-mediated signaling involves calcium-binding proteins. Plant Physiol 132:1623–1630

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 MJ, Marchant A, Green HG, May ST, Ward SP, Millner PA, Walker AR, Schulz B, Feldmann KA (1996) Arabidopsis AUX1 gene: a permease-like regulator of root gravitropism. Science 273:948–950

Berleth T, Sachs T (2001) Plant morphogenesis: long-distance coordination and local patterning. Curr Opin Plant Biol 4:57–62

Bishopp A, Help H, El-Showk S, Weijers D, Scheres B, Friml J, Benková E, Mähönen AP, Helariutta Y (2011) A mutually inhibitory interaction between auxin and cytokinin specifies vascular pattern in roots. Curr Biol 21:917–926

Blakeslee JJ, Bandyopadhyay A, Lee OR, Mravec J, Titapiwatanakun B, Sauer M, Makam SN, Cheng Y, Bouchard R, Adamec J, Geisler M, Nagashima A, Sakai T, Martinoia E, Friml J, Peer WA, Murphy AS (2007) Interactions among PIN-FORMED and P-glycoprotein auxin transporters in Arabidopsis. Plant Cell 19:131–147

Blatt MR, Thiel G, Trentham DR (1990) Reversible inactivation of K+ channels of Vicia stomatal guard cells following the photolysis of caged inositol 1,4,5-trisphosphate. Nature 346:766–769

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

Bouchard R, Bailly A, Blakeslee JJ, Oehring SC, Vincenzetti V, Lee OR, Paponov I, Palme K, Mancuso S, Murphy AS, Schulz B, Geisler M (2006) Immunophilin-like TWISTED DWARF1 modulates auxin efflux activities of *Arabidopsis* P-glycoproteins. J Biol Chem 281:30603–30612

Boutté Y, Crosnier MT, Carraro N, Traas J, Satiat-Jeunemaitre B (2006) The plasma membrane recycling pathway and cell polarity in plants: studies on PIN proteins. J Cell Sci 119:1255–1265

Caño-Delgado A, Penfield S, Smith C, Catley M, Bevan M (2003) Reduced cellulose synthesis invokes lignification and defense responses in *Arabidopsis thaliana*. Plant J 34:351–362

Carland F, Fujioka S, Nelson T (2010) The sterol methyltransferases SMT1, SMT2, and SMT3 influence *Arabidopsis* development through nonbrassinosteroid products. Plant Physiol 153:741–756

Chapman EJ, Estelle M (2009) Mechanism of auxin-regulated gene expression in plants. Annu Rev Genet 43:265–285

Chen CL, Gajewski KM, Hamaratoglu F, Bossuyt W, Sansores-Garcia L, Tao C, Halder G (2010) The apical-basal cell polarity determinant Crumbs regulates Hippo signaling in *Drosophila*. Proc Natl Acad Sci U S A 107:15810–15815

Chen X, Irani NG, Friml J (2011) Clathrin-mediated endocytosis: the gateway into plant cells. Curr Opin Plant Biol 14:674–682

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. Curr Biol 22:1326–1332

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

Christie JM, Yang H, Richter GL, Sullivan S, Thomson CE, Lin J, Titapiwatanakun B, Ennis M, Kaiserli E, Lee OR, Adamec J, Peer WA, Murphy AS (2011) phot1 inhibition of ABCB19 primes lateral auxin fluxes in the shoot apex required for phototropism. PLoS Biol 9(6): e1001076

D'Souza-Schorey C, Chavrier P (2006) ARF proteins: roles in membrane traffic and beyond. Nat Rev Mol Cell Biol 7:347–358

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 XW, Wang H (2012) A PP6-type phosphatase holoenzyme

directly regulates PIN phosphorylation and auxin efflux in Arabidopsis. Plant Cell 24:2497–2514

Davies PJ (2004) Plant hormones: biosynthesis, signal transduction, action! Kluwer Academic, London

Dello Ioio R, Nakamura K, Moubayidin L, Perilli S, Taniguchi M, Morita MT, 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

Desprez T, Juraniec M, Crowell EF, Jouy H, Pochylova Z, Parcy F, Höfte H, Gonneau M, Vernhettes S (2007) Organization of cellulose synthase complexes involved in primary cell wall synthesis in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 104:15572–15577

Dettmer J, Friml J (2011) Cell polarity in plants: when two do the same, it is not the same. Curr Opin Cell Biol 23:686–696

Dettmer J, Hong-Hermesdorf A, Stierhof Y-D, Schumacher K (2006) Vacuolar H+-ATPase activity is required for endocytic and secretory trafficking in *Arabidopsis*. Plant Cell 18:715–730

Dharmasiri N, Estelle M (2004) Auxin signaling and regulated protein degradation. Trends Plant Sci 9:302–308

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 JS, Jurgens G, Estelle M (2005b) Plant development is regulated by a family of auxin receptor F box proteins. Dev Cell 9:109–119

Dhonukshe P, Aniento F, Hwang I, Robinson DG, Mravec J, Stierhof YD, Friml J (2007) Clathrin-mediated constitutive endocytosis of PIN auxin efflux carriers in *Arabidopsis*. Curr Biol 17:520–527

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

Dhonukshe P, Grigoriev I, Fischer R, Tominaga M, Robinson DG, Hasek J, Paciorek T, Petrásek J, Seifertová D, Tejos R, Meisel LA, Zazímalová E, Gadella TW Jr, Stierhof YD, Ueda T, Oiwa K, Akhmanova A, Brock R, Spang A, Friml J (2008b) Auxin transport inhibitors impair vesicle motility and actin cytoskeleton dynamics in diverse eukaryotes. Proc Natl Acad Sci U S A 105:4489–4494

Dhonukshe P, Huang F, Galvan-Ampudia CS, Mähönen AP, 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

Dickson RC, Sumanasekera C, Lester RL (2006) Functions and metabolism of sphingolipids in *Saccharomyces cerevisiae*. Prog Lipid Res 45:447–465

Diener AC, Li H, Zhou W, Whoriskey WJ, Nes WD, Fink GR (2000) Sterol methyltransferase 1 controls the level of cholesterol in plants. Plant Cell 12:853–870

Ding Z, Friml J (2010) Auxin regulates distal stem cell differentiation in Arabidopsis roots. Proc Natl Acad Sci U S A 107:12046–12051

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

Drdová EJ, Synek L, Pečenková T, Hála M, Kulich I, Fowler JE, Murphy AS, Zárský V (2013) The exocyst complex contributes to PIN auxin efflux carrier recycling and polar auxin transport in *Arabidopsis*. Plant J 73:709–719

Du Y, Tejos R, Beck M, Himschoot E, Li H, Robatzek S, Vanneste S, Friml J (2013) Salicylic acid interferes with clathrin-mediated endocytic protein trafficking. Proc Natl Acad Sci U S A 110 (19):7946–7951

Dudu V, Pantazis P, Gonzalez-Gaitan M (2004) Membrane traffic during embryonic development: epithelial formation, cell fate decisions and differentiation. Curr Opin Cell Biol 16:407–414

Ellis C, Turner JG (2001) The *Arabidopsis* mutant cev1 has constitutively active jasmonate and ethylene signal pathways and enhanced resistance to pathogens. Plant Cell 13:1025–1033

Feraru E, Paciorek T, Feraru MI, Zwiewka M, De Groodt R, De Rycke R, Kleine-Vehn J, Friml J (2010) The AP-3 B adaptin mediates the biogenesis and function of lytic vacuoles in *Arabidopsis*. Plant Cell 22:2812–2824

Feraru E, Feraru MI, 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*. Curr Biol 21:338–343

Feraru E, Feraru MI, Asaoka R, Paciorek T, De Rycke R, Tanaka H, Nakano A, Friml J (2012) BEX5/RabA1b regulates trans-Golgi network-to-plasma membrane protein trafficking in Arabidopsis. Plant Cell 24:3074–3086

Foti M, Moukil MA, Dudognon P, Carpentier JL (2004) Insulin and IGF-1 receptor trafficking and signaling. Novartis Found Symp 262:125–141

Friml J, Palme K (2002) Polar auxin transport – old questions and new concepts? Plant Mol Biol 49:273–284

Friml J, Benková E, Blilou I, Wisniewska 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, 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, 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, Yang X, Michniewicz M, Weijers D, Quint A, Tietz O, Benjamins R, Ouwerkerk PB, Ljung K, Sandberg G, Hooykaas PJ, Palme K, Offringa R (2004) APINOID-dependent binary switch in apical-basal PIN polar targeting directs auxin efflux. Science 306:862–865

Fujimoto M, Arimura S, Ueda T, Takanashi H, Hayashi Y, Nakano A, Tsutsumi N (2010) *Arabidopsis* dynamin-related proteins DRP2B and DRP1A participate together in clathrin-coated vesicle formation during endocytosis. Proc Natl Acad Sci U S A 107:6094–6099

Furutani M, Kajiwara T, Kato T, Treml BS, Stockum C, Torres-Ruiz RA, Tasaka M (2007) The gene MACCHI-BOU 4/ENHANCER OF PINOID encodes a NPH3-like protein and reveals similarities between organogenesis and phototropism at the molecular level. Development 134:3849–3859

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

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

Geisler M, Blakeslee JJ, Bouchard R, Lee OR, Vincenzetti V, Bandyopadhyay A, Titapiwatanakun B, Peer WA, Bailly A, Richards EL, Ejendal KF, Smith AP, Baroux C, Grossniklaus U, Müller A, Hrycyna CA, Dudler R, Murphy AS, Martinoia E (2005) Cellular efflux of auxin catalyzed by the Arabidopsis MDR/PGP transporter AtPGP1. Plant J 44:179–194

Geldner N (2009) Cell polarity in plants: a PARspective on PINs. Curr Opin Plant Biol 12:42–48

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

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

Giepmans BN, van Ijzendoorn SC (2009) Epithelial cell-cell junctions and plasma membrane domains. Biochim Biophys Acta 1788:820–831

Gilroy S, Read ND, Trewavas AJ (1990) Elevation of cytoplasmic calcium by caged calcium or caged inositol triphosphate initiates stomatal closure. Nature 346:769–771

Grebe M, Xu J, Mobius W, Ueda T, Nakano A, Geuze HJ, Rook MB, Scheres B (2003) *Arabidopsis* sterol endocytosis involves actin-mediated trafficking via ARA6-positive early endosomes. Curr Biol 13:1378–1387

Grieneisen VA, Xu J, Marée AF, Hogeweg P, Scheres B (2007) Auxin transport is sufficient to generate a maximum and gradient guiding root growth. Nature 449:1008–1013

Hazak O, Bloch D, Poraty L, Sternberg H, Zhang J, Friml J, Yalovsky S (2010) A rho scaffold integrates the secretory system with feedback mechanisms in regulation of auxin distribution. PLoS Biol 8(1):e1000282

Heisler MG, Ohno C, Das P, Sieber P, Reddy GV, Long JA, Meyerowitz EM (2005) Patterns of auxin transport and gene expression during primordium development revealed by live imaging of the *Arabidopsis* inflorescence meristem. Curr Biol 15:1899–1911

Heisler MG, Hamant O, Krupinski P, Uyttewaal M, Ohno C, Jönsson H, Traas J, Meyerowitz EM (2010) Alignment between PIN1 polarity and microtubule orientation in the shoot apical meristem reveals a tight coupling between morphogenesis and auxin transport. PLoS Biol 8 (10):e1000516

Henrichs S, Wang B, Fukao Y, Zhu J, Charrier L, Bailly A, Oehring SC, Linnert M, Weiwad M, Endler A, Nanni P, Pollmann S, Mancuso S, Schulz A, Geisler M (2012) Regulation of ABCB1/PGP1-catalysed auxin transport by linker phosphorylation. EMBO J 31:2965–2980

Hertel R (1983) The mechanism of auxin transport as a model for auxin action. Z Pflanzenphysiol 112:53–67

Heymann JAW, Hinshaw JE (2009) Dynamins at a glance. J Cell Sci 122:3427–3431

Hoekstra D, Maier O, van der Wouden JM, Slimane TA, van IJzendoorn SC (2003) Membrane dynamics and cell polarity: the role of sphingolipids. J Lipid Res 44:869–877

Holler D, Dikic I (2004) Receptor endocytosis via ubiquitin-dependent and -independent pathways. Biochem Pharmacol 67:1013–1017

Huang F, Zago MK, Abas L, van Marion A, Galván-Ampudia CS, Offringa R (2010) Phosphorylation of conserved PIN motifs directs *Arabidopsis* PIN1 polarity and auxin transport. Plant Cell 22:1129–1142

Humbert PO, Dow LE, Russell SM (2006) The Scribble and Par complexes in polarity and migration: friends or foes? Trends Cell Biol 16:622–630

Irani NG, Russinova E (2009) Receptor endocytosis and signaling in plants. Curr Opin Plant Biol 12:653–659

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

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, Friml J (2008) Polar targeting and endocytic recycling in auxin-dependent plant development. Annu Rev Cell Dev Biol 24:447–473

Kleine-Vehn J, Dhonukshe P, Swarup R, Bennett M, Friml J (2006) Subcellular trafficking of the *Arabidopsis* auxin influx carrier AUX1 uses a novel pathway distinct from PIN1. Plant Cell 18:3171–3181

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

Kleine-Vehn J, Łangowski Ł, Wisniewska J, Dhonukshe P, Brewer PB, Friml J (2008b) Cellular and molecular requirements for polar PIN targeting and transcytosis in plants. Mol Plant 1:1056–1066

Kleine-Vehn J, Leitner J, Zwiewka M, Sauer M, Abas L, Luschnig C, Friml J (2008c) Differential degradation of PIN2 auxin efflux carrier by retromer-dependent vacuolar targeting. Proc Natl Acad Sci U S A 105:17812–17817

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, Ding Z, Jones AR, Tasaka M, Morita MT, Friml J (2010) Gravity-induced PIN transcytosis for polarization of auxin fluxes in gravity-sensing root cells. Proc Natl Acad Sci U S A 107:22344–22349

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, Hell SW, Runions J, Friml J (2011) Recycling, clustering, and endocytosis jointly maintain PIN auxin carrier polarity at the plasma membrane. Mol Syst Biol 25:540

Konopka CA, Backues SK, Bednarek SY (2008) Dynamics of *Arabidopsis* dynamin-related protein 1C and a clathrin light chain at the plasma membrane. Plant Cell 20:1363–1380

Kramer EM (2004) PIN and AUX/LAX proteins: their role in auxin accumulation. Trends Plant Sci 9:578–582

Krinke O, Novotná Z, Valentová O, Martinec J (2007) Inositol trisphosphate receptor in higher plants: is it real? J Exp Bot 58:361–376

Kubeš M, Yang H, Richter GL, Cheng Y, Młodzińska E, Wang X, Blakeslee JJ, Carraro N, Petrášek J, Zažímalová E, Hoyerová K, Peer WA, Murphy AS (2012) The Arabidopsis concentration-dependent influx/efflux transporter ABCB4 regulates cellular auxin levels in the root epidermis. Plant J 69:640–654

Lam SK, Siu CL, Hillmer S, Jang S, An G, Robinson DG, Jiang L (2007) Rice SCAMP1 defines clathrin-coated, trans-golgi-located tubular-vesicular structures as an early endosome in tobacco BY-2 cells. Plant Cell 19:296–319

Langowski L, Růzicka K, Naramoto S, Kleine-Vehn J, Friml J (2010) Trafficking to the outer polar domain defines the root-soil interface. Curr Biol 20:904–908

Lavy M, Bloch D, Hazak O, Gutman I, Poraty L, Sorek N, Sternberg H, Yalovsky S (2007) A Novel ROP/RAC effector links cell polarity, root-meristem maintenance, and vesicle trafficking. Curr Biol 17:947–952

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(1):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. Proc Natl Acad Sci U S A 109:8322–8327

Lemtiri-Chlieh F, MacRobbie EA, Webb AA, Manison NF, Brownlee C, Skepper JN, Chen J, Prestwich GD, Brearley CA (2003) Inositol hexakisphosphate mobilizes an endomembrane store of calcium in guard cells. Proc Natl Acad Sci U S A 100:10091–10095

Leyser O (2011) Auxin, self-organisation, and the colonial nature of plants. Curr Biol 21:R331–R337

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 Res 21:970–978

Lin D, Shingo N, Chen J, Chen X, Cao L, Xu T, Li H, Dhonukshe P, Friml J, Scheres B, Fu Y, Yang Z (2012) A ROP GTPase-dependent auxin signaling pathway inhibits PIN2 endocytosis in *Arabidopsis* roots. Curr Biol 22:1319–1325

Liu T, Mirschberger C, Chooback L, Arana Q, Dal Sacco Z, MacWilliams H, Clarke M (2002) Altered expression of the 100 kDa subunit of the *Dictyostelium* vacuolar proton pump impairs enzyme assembly, endocytic function and cytosolic pH regulation. J Cell Sci 115:1907–1918

Liu J, Mehdi S, Topping J, Friml J, Lindsey K (2013) Interaction of PLS and PIN and hormonal crosstalk in Arabidopsis root development. Front Plant Sci 4:75

Ljung K (2013) Auxin metabolism and homeostasis during plant development. Development 140:943–950

Löfke C, Zwiewka M, Heilmann I, Van Montagu MC, Teichmann T, Friml J (2013) Asymmetric gibberellin signaling regulates vacuolar trafficking of PIN auxin transporters during root gravitropism. Proc Natl Acad Sci U S A 110:3627–3632

Ludwig-Müller J (2011) Auxin conjugates: their role for plant development and in the evolution of land plants. J Exp Bot 62:1757–1773

Luschnig C, Gaxiola RA, Grisafi P, Fink GR (1998) EIR1, a root-specific protein involved in auxin transport, is required for gravitropism in *Arabidopsis thaliana*. Genes Dev 12:2175–2218

Mancuso S, Marras AM, Mugnai S, Schlicht M, Zársky V, Li G, Song L, Xue HW, Baluska F (2007) Phospholipase Dζ2 drives vesicular secretion of auxin for its polar cell-cell transport in the transition zone of the root apex. Plant Signal Behav 2:240–244

Mano Y, Nemoto K (2012) The pathway of auxin biosynthesis in plants. J Exp Bot 63:2853–2872

Marhavý P, Bielach A, Abas L, Abuzeineh A, Duclercq J, Tanaka H, Parezová M, Petrásek J, 917 Friml J, Kleine-Vehn J, Benková E (2011) Cytokinin modulates endocytic trafficking of PIN1 918 auxin efflux carrier to control plant organogenesis. Dev Cell 21:796–804

Martinière A, Lavagi I, Nageswaran G, Rolfe DJ, Maneta-Peyret L, Luu DT, Botchway SW, Webb SEW, Mongrand S, Maurel C, Martin-Fernandez ML, Kleine-Vehn J, Friml J, Moreau P, Runions J (2012) The cell wall constrains lateral diffusion of plant plasma-membrane proteins. Proc Natl Acad Sci U S A 109:12805–12810

Matheson LA, Hanton SL, Brandizzi F (2006) Traffic between the plant endoplasmic reticulum and Golgi apparatus: to the Golgi and beyond. Curr Opin Plant Biol 9:601–609

Matsuzaki Y, Ogawa-Ohnishi M, Mori A, Matsubayashi Y (2010) Secreted peptide signals required for maintenance of root stem cell niche in *Arabidopsis*. Science 329:1065–1067

McFarlane HE, Shin JJ, Bird DA, Samuels AL (2010) *Arabidopsis* ABCG transporters, which are required for export of diverse cuticular lipids, dimerize in different combinations. Plant Cell 22:3066–3075

Men S, Boutté Y, Ikeda Y, Li X, Palme K, Stierhof Y-D, Hartmann M-A, Moritz T, Grebe M (2008) Sterol-dependent endocytosis mediates post-cytokinetic acquisition of PIN2 auxin efflux carrier polarity. Nat Cell Biol 10:237–244

Michell RH (2008) Inositol derivatives: evolution and functions. Nat Rev Mol Cell Biol 9:151–161

Michniewicz M, Brewer PB, Friml J (2007a) Polar auxin transport and asymmetric auxin distribution. Arabidopsis Book 5:e0108

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

Miwa K, Takano J, Omori H, Seki M, Shinozaki K, Fujiwara T (2007) Plants tolerant of high boron levels. Science 318:1417

Motchoulski A, Liscum E (1999) *Arabidopsis* NPH3: a NPH1 photoreceptor-interacting protein essential for phototropism. Science 286:961–964

Mouchel CF, Briggs GC, Hardtke CS (2004) Natural genetic variation in *Arabidopsis* identifies BREVIS RADIX, a novel regulator of cell proliferation and elongation in the root. Genes Dev 18:700–714

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

Mravec J, Petrášek J, Li N, Boeren S, Karlova R, Kitakura S, Pařezová M, Naramoto S, Nodzyński T, Dhonukshe P, Bednarek SY, Zažímalová E, de Vries S, Friml J (2011) Cell plate restricted association of DRP1A and PIN proteins is required for cell polarity establishment in *Arabidopsis*. Curr Biol 21:1055–1060

Muday GK, DeLong A (2001) Polar auxin transport: controlling where and how much. Trends Plant Sci 6:535–542

Müller J, Mettbach U, Menzel D, Šamaj J (2007) Molecular dissection of endosomal compartments in plants. Plant Physiol 145:293–304

Munnik T, Nielsen E (2011) Green light for polyphosphoinositide signals in plants. Curr Opin Plant Biol 14:489–497

Munnik T, Testerink C (2009) Plant phospholipid signaling: 'in a nutshell'. J Lipid Res 50:260–265

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 Biol 10(4):e1001299

Naramoto S, Kleine-Vehn J, Robert S, Fujimoto M, Dainobu T, Paciorek T, Ueda T, Nakano A, Van Montagu MCE, Fukuda H, Friml J (2010) ADP-ribosylation factor machinery mediates endocytosis in plant cells. Proc Natl Acad Sci U S A 107:21890–21895

Nodzyński T, Feraru MI, Hirscha S, De Rycke R, Niculaesa C, Boerjana W, Van Leene J, De Jaeger G, Vanneste S, Friml J (2013) Retromer subunits VPS35A and VPS29 mediate prevacuolar compartment (PVC) function in Arabidopsis. Mol Plant 6:1849–1862

Noh B, Murphy AS, Spalding EP (2001) Multidrug resistance-like genes of *Arabidopsis* required for auxin transport and auxin-mediated development. Plant Cell 13:2441–2454

Nyasae LK, Hubbard AL, Tuma PL (2003) Transcytotic efflux from early endosomes is dependent on cholesterol and glycosphingolipids in polarized hepatic cells. Mol Biol Cell 14:2689–2705

Okada K, Ueda J, Komaki MK, Bell CJ, Shimura Y (1991) Requirement of the auxin polar transport system in early stages of *Arabidopsis* floral bud formation. Plant Cell 3:677–684

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

Panikashvili D, Savaldi-Goldstein S, Mandel T, Yifhar T, Franke RB, Höfer R, Schreiber L, Chory J, Aharoni A (2007) The *Arabidopsis* DESPERADO/AtWBC11 transporter is required for cutin and wax secretion. Plant Physiol 145:1345–1360

Paul MJ, Frigerio L (2007) Coated vesicles in plant cells. Semin Cell Dev Biol 18:471–478

Peer WA, Bandyopadhyay A, Blakeslee JJ, Makam SN, Chen RJ, Masson PH, Murphy AS (2004) Variation in expression and protein localization of the PIN family of auxin efflux facilitator proteins in flavonoid mutants with altered auxin transport in Arabidopsis thaliana. Plant Cell 16:1898–1911

Petrášek J, Schwarzerová K (2009) Actin and microtubule cytoskeleton interactions. Curr Opin Plant Biol 12:728–734

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

Praefcke GJK, McMahon HT (2004) The dynamin superfamily: universal membrane tubulation and fission molecules? Nat Rev Mol Cell Biol 5:133–147

Rakusova H, Gallego-Bartolomé J, Vanstraelen M, Robert HS, Alabadí D, Blázquez MA, Benková E, Friml J (2011) Polarization of PIN3-dependent auxin transport for hypocotyl gravitropic response in *Arabidopsis thaliana*. Plant J 67:817–826

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

Reinhardt D, Pesce ER, Stieger P, Mandel T, Baltensperger K, Bennett M, Traas J, Friml J, Kuhlemeier C (2003) Regulation of phyllotaxis by polar auxin transport. Nature 426:255–260

Richmond TA, Somerville CR (2000) The cellulose synthase superfamily. Plant Physiol 124:495–498

Rigó G, Ayaydin F, Tietz O, Zsigmond L, Kovács H, Páy A, Salchert K, Darula Z, Medzihradszky KF, Szabados L, Palme K, Koncz C, Cséplo A (2013) Inactivation of plasma membrane-localized CDPK-RELATED KINASE5 decelerates PIN2 exocytosis and root gravitropic response in Arabidopsis. Plant Cell 25(5):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 SY, Jones AM, Luschnig C, Aniento F, Zažímolová E, Friml J (2010) ABP1 mediates auxin inhibition of clathrin-dependent endocytosis in *Arabidopsis*. Cell 143:111–121

Robinson DG, Jiang L, Schumacher K (2008) The endosomal system of plants: charting new and familiar territories. Plant Physiol 147:1482–1492

Roppolo D, De Rybel B, Tendon VD, Pfister A, Alassimone J, Vermeer JE, Yamazaki M, Stierhof YD, Beeckman T, Geldner N (2011) A novel protein family mediates Casparian strip formation in the endodermis. Nature 473:380–383

Roudier F, Gissot L, Beaudoin F, Haslam R, Michaelson L, Marion J, Molino D, Lima A, Bach L, Morin H, Tellier F, Palauqui JC, Bellec Y, Renne C, Miquel M, Dacosta M, Vignard J, Rochat C, Markham JE, Moreau P, Napier J, Faure JD (2010) Very-long-chain fatty acids are involved in polar auxin transport and developmental patterning in Arabidopsis. Plant Cell 22:364–375

Rubery PH, Sheldrake AR (1974) Carrier-mediated auxin transport. Planta 118:101–121

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

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 MC, Benková E (2009) Cytokinin regulates root meristem activity via modulation of the polar auxin transport. Proc Natl Acad Sci U S A 106:4284–4289

Sabatini S, Beis D, Wolkenfelt H, Murfett J, Guilfoyle T, Malamy J, Benfey P, Leyser O, Bechtold N, Weisbeek P, Scheres B (1999) An auxin-dependent distal organizer of pattern and polarity in the Arabidopsis root. Cell 99:463–472

Sachs T (1981) The control of the patterned differentiation of vascular tissues. Adv Bot Res 9:151–262

Sachs T (1991) Cell polarity and tissue patterning in plants. Development 113:83–93

Santner AA, Watson JC (2006) The WAG1 and WAG2 protein kinases negatively regulate root waving in *Arabidopsis*. Plant J 45:752–764

Santuari L, Scacchi E, Rodriguez-Villalon A, Salinas P, Dohmann EM, Brunoud G, Vernoux T, Smith RS, Hardtke CS (2011) Positional information by differential endocytosis splits auxin response to drive Arabidopsis root meristem growth. Curr Biol 21:1918–1923

Sauer M, Friml J (2004) The matryoshka dolls of plant polarity. Development 131:5774–5775

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

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

Scheible WR, Eshed R, Richmond T, Delmer D, Somerville C (2001) Modifications of cellulose synthase confer resistance to isoxaben and thiazolidinone herbicides in *Arabidopsis* Ixr1 mutants. Proc Natl Acad Sci U S A 98:10079–10084

Scheuring D, Viotti C, Krüger F, Künzl F, Sturm S, Bubeck J, Hillmer S, Frigerio L, Robinson DG, Pimpl P, Schumacher K (2011) Multivesicular bodies mature from the trans-Golgi network/early endosome in *Arabidopsis*. Plant Cell 23:3463–3481

Schlereth A, Möller B, Liu W, Kientz M, Flipse J, Rademacher EH, Schmid M, Jürgens G, Weijers D (2010) MONOPTEROS controls embryonic root initiation by regulating a mobile transcription factor. Nature 464:913–916

Schlicht M, Strnad M, Scanlon MJ, Mancuso S, Hochholdinger F, Palme K, Volkmann D, Menzel D, Baluska F (2006) Auxin immunolocalization implicates vesicular neurotransmitter-like mode of polar auxin transport in root apices. Plant Signal Behav 1:122–133

Seaman MNJ (2005) Recycle your receptors with retromer. Trends Cell Biol 15:68–75

Shimada T, Koumoto Y, Li L, Yamazaki M, Kondo M, Nishimura M, Hara-Nishimura I (2006) AtVPS29, a putative component of a retromer complex, is required for the efficient sorting of seed storage proteins. Plant Cell Physiol 47:1187–1194

Shirakawa M, Ueda H, Shimada T, Nishiyama C, Hara-Nishimura I (2009) Vacuolar SNAREs function in the formation of the leaf vascular network by regulating auxin distribution. Plant Cell Physiol 50:1319–1328

Shivas JM, Morrison HA, Bilder D, Skop AR (2010) Polarity and endocytosis: reciprocal regulation. Trends Cell Biol 20:445–452

Sieberer T, Seifert GJ, Hauser MT, Grisafi P, Fink GR, Luschnig C (2000) Post-transcriptional control of the *Arabidopsis* auxin efflux carrier EIR1 requires AXR1. Curr Biol 10:1595–1598

Sorefan K, Girin T, Liljegren SJ, Ljung K, Robles P, Galván-Ampudia CS, Offringa R, Friml J, Yanofsky MF, Østergaard L (2009) A regulated auxin minimum is required for seed dispersal in *Arabidopsis*. Nature 459:583–586

Spitzer C, Reyes FC, Buono R, Sliwinski MK, Haas TJ, Otegui MS (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

Swarup R, Friml J, Marchant A, Ljung K, Sandberg G, Palme K, Bennett M (2001) Localization of the auxin permease AUX1 suggests two functionally distinct hormone transport pathways operate in the *Arabidopsis* root apex. Genes Dev 15:2648–2653

Swarup R, Perry P, Hagenbeek D, Van Der Straeten D, Beemster GT, Sandberg G, Bhalerao R, Ljung K, Bennett MJ (2007) Ethylene upregulates auxin biosynthesis in *Arabidopsis* seedlings to enhance inhibition of root cell elongation. Plant Cell 19:2186–2196

Swarup K, Benkova E, Swarup R, Casimiro I, Peret B, Yang Y, Parry G, Nielsen E, De Smet I, Vanneste S, Levesque MP, Carrier D, James N, Calvo V, Ljung K, Kramer E, Roberts R, Graham N, Marillonnet S, Patel K (2008) The auxin influx carrier LAX3 promotes lateral root emergence. Nat Cell Biol 10:946–954

Takano J, Tanaka M, Toyoda A, Miwa K, Kasai K, Fuji K, Onouchi H, Naito S, Fujiwara T (2010) Polar localization and degradation of Arabidopsis boron transporters through distinct trafficking pathways. Proc Natl Acad Sci U S A 107:5220–5225

Tanaka H, Dhonukshe P, Brewer PB, Friml J (2006) Spatiotemporal asymmetric auxin distribution: a means to coordinate plant development. Cell Mol Life Sci 63:2738–2754

Tanaka H, Kitakura S, De Rycke R, De Groodt R, Friml J (2009) Fluorescence imaging-based screen identifies ARF GEF component of early endosomal trafficking. Curr Biol 19:391–397

Tanaka H, Kitakura S, Rakusová R, Uemura T, Feraru MI, De Rycke R, Robert S, Kakimoto T, Friml J (2013) Cell polarity and patterning by PIN trafficking through early endosomal compartments in *Arabidopsis*. PLoS Genet 9(5):e1003540

Tang RH, Han S, Zheng H, Cook CW, Choi CS, Woerner TE, Jackson RB, Pei ZM (2007) Coupling diurnal cytosolic Ca2+ oscillations to the CAS-IP3 pathway in *Arabidopsis*. Science 315:1423–1426

Teh OK, Moore I (2007) An ARF-GEF acting at the golgi and in selective endocytosis in polarized plant cells. Nature 448:493–496

Tepass U, Tanentzapf G, Ward R, Fehon R (2001) Epithelial cell polarity and cell junctions in *Drosophila*. Annu Rev Genet 35:747–784

Testerink C, Munnik T (2011) Molecular, cellular and physiological responses to phosphatidic acid formation in plants. J Exp Bot 62:2349–2361

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

Treml BS, Winderl S, Radykewicz R, Herz M, Schweizer G, Hutzler P, Glawischnig E, Ruiz RA (2005) The gene ENHANCER OF PINOID controls cotyledon development in the *Arabidopsis* embryo. Development 132:4063–4074

Tuma PL, Hubbard AL (2003) Transcytosis: crossing cellular barriers. Physiol Rev 83:871–932

Vanneste S, Friml J (2009) Auxin: a trigger for change in plant development. Cell 136:1005–1016

Verrier PJ, Bird D, Burla B, Dassa E, Forestier C, Geisler M, Klein M, Kolukisaoglu Ü, Lee Y, Martinoia E, Murphy A, Rea PA, Samuels L, Schulz B, Spalding EP, Yazaki K, Theodoulou FL (2008) Plant ABC proteins—a unified nomenclature and updated inventory. Trends Plant Sci 13:151–159

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

Vieten A, Sauer M, Brewer PB, Friml J (2007) Molecular and cellular aspects of auxin transport-mediated development. Trends Plant Sci 12:160–168

Viotti C, Bubeck J, Stierhof YD, Krebs M, Langhans M, van den Berg W, van Dongen W, Richter S, Geldner N, Takano J, Jürgens G, de Vries SC, Robinson DG, Schumacher K (2010) Endocytic and secretory traffic in *Arabidopsis* merge in the trans-Golgi network/early endosome, an independent and highly dynamic organelle. Plant Cell 22:1344–1357

Vitale A, Denecke J (1999) The endoplasmic reticulum – gateway of the secretory pathway. Plant Cell 11:615–628

Voigt B, Timmers AC, Šamaj J, Müller J, Baluška F, Menzel D (2005) GFP-FABD2 fusion construct allows in vivo visualization of the dynamic actin cytoskeleton in all cells of *Arabidopsis* seedlings. Eur J Cell Biol 84:595–608

Wabnik K, Kleine-Vehn J, Balla J, Sauer M, Naramoto S, Reinöhl V, Merks RMH, Govaerts W, Friml J (2010) Emergence of tissue polarization from synergy of intracellular and extracellular auxin signaling. Mol Syst Biol 6:447

Wabnik K, Govaerts W, Friml J, Kleine-Vehn J (2011) Feedback models for polarized auxin transport: an emerging trend. Mol Biosyst 7:2352

Wan Y, Jasik J, Wang L, Hao H, Volkmann D, Menzel D, Mancuso S, Baluška F, Lin J (2012) The signal transducer NPH3 integrates the phototropin1 photosensor with PIN2-based polar auxin transport in *Arabidopsis* root phototropism. Plant Cell 24:551–565

Wang B, Henrichs S, Geisler M (2012) The AGC kinase, PINOID, blocks interactive ABCB/PIN auxin transport. Plant Signal Behav 7(12):1515–1517 (Epub ahead of print)

Wang B, Bailly A, Zwiewka M, Henrichs S, Azzarello E, Mancuso S, Maeshima M, Friml J, Schulz A, Geisler M (2013) *Arabidopsis* TWISTED DWARF1 functionally interacts with auxin exporter ABCB1 on the root plasma membrane. Plant Cell 25:202–214

Wells CD, Fawcett JP, Traweger A, Yamanaka Y, Goudreault M, Elder K, Kulkarni S, Gish G, Virag C, Lim C, Colwill K, Starostine A, Metalnikov P, Pawson T (2006) A Rich1/Amot complex regulates the Cdc42 GTPase and apical-polarity proteins in epithelial cells. Cell 125:535–548

Whitford R, Fernandez A, Tejos R, Pérez AC, 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. Dev Cell 22:678–685

Willemsen V, Friml J, Grebe M, van den Toorn A, Palme K, Scheres B (2003) Cell polarity and PIN protein positioning in *Arabidopsis* require STEROL METHYLTRANSFERASE1 function. Plant Cell 15:612–625

Willige BC, 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

Winter V, Hauser MT (2006) Exploring the ESCRTing machinery in eukaryotes. Trends Plant Sci 11:115–123

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

Wollert T, Wunder C, Lippincott-Schwartz J, Hurley JH (2009) Membrane scission by the ESCRT-III complex. Nature 458:172–177

Xu T, Wen M, Nagawa S, Fu Y, Chen JG, Wu MJ, Perrot-Rechenmann C, Friml J, Jones AM, Yang Z (2010) Cell surface- and rho GTPase-based auxin signaling controls cellular interdigitationin *Arabidopsis*. Cell 143:99–110

Yang Y, Hammes UZ, Taylor CG, Schachtman DP, Nielsen E (2006) High-affinity auxin transport by the AUX1 influx carrier protein. Curr Biol 16:1123–1127

Žárský V, Cvrčková F, Potocký M, Hála M (2009) Exocytosis and cell polarity in plants – exocyst and recycling domains. New Phytol 183:255–272

Zažímalová E, Krecek P, Skůpa P, Hoyerová K, Petrásek J (2007) Polar transport of the plant hormone auxin—the role of PIN-FORMED (PIN) proteins. Cell Mol Life Sci 64:1621–1637

Zegzouti H, Anthony RG, Jahchan N, Bögre L, Christensen SK (2006) Phosphorylation and activation of PINOID by the phospholipid signaling kinase 3-phosphoinositide-dependent protein kinase 1 (PDK1) in *Arabidopsis*. Proc Natl Acad Sci U S A 103:6404–6409

Zhang J, Nodzynski T, Pencík A, Rolcík J, Friml J (2010) PIN phosphorylation is sufficient to mediate PIN polarity and direct auxin transport. Proc Natl Acad Sci U S A 107:918–922

Zhang J, Vanneste S, Brewer PB, Michniewicz M, Grones P, Kleine-Vehn J, Löfke C, Teichmann T, Bielach A, Cannoot B, Hoyerová K, Chen X, Xue HW, Benková E, Zažímalová E, Friml J (2011a) Inositol trisphosphate-induced Ca2+ signaling modulates auxin transport and PIN polarity. Dev Cell 20:855–866

Zhang W, To JP, Cheng CY, Eric Schaller G, Kieber JJ (2011b) Type-A response regulators are required for proper root apical meristem function through post-transcriptional regulation of PIN auxin efflux carriers. Plant J 68:1–10

Zourelidou M, Müller I, Willige BC, 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

Zwiewka M, Feraru E, Müller B, Hwang I, Feraru MI, Kleine-Vehn J, Weijers D, Friml J (2011) The AP-3 adaptor complex is required for vacuolar function in *Arabidopsis*. Cell Res 21:1711–1722