Polar Auxin Transport: Cell Polarity to Patterning

Kalika Prasad and Pankaj Dhonukshe

Abstract Auxin is a signaling molecule with profound influence on plant morphogenesis. Because of its activity gradient-related effects on plant development and response programs, it is considered as a plant morphogen. Auxin displays a spectacular ability to mobilize in a cell-to-cell and polar fashion. Auxin efflux carrier PIN proteins direct this intercellular flow of auxin and thus bear a rate-limiting effect on the formation of auxin activity gradients. With this influence on directionality and amount of auxin transport, PINs play crucial roles in plant body organization and connect cell polarity to plant patterning. As a consequence, mechanisms regulating the localization of PINs are widely investigated. Recent work uncovers the roles of vesicle trafficking regulator ARF–GEF GNOM, a kinase PINOID, a SNX1–VPS29 retromer complex, ROP-GTPases, Rab-GTPases, endocytosis regulator clathrin, membrane sterol composition, and cytoskeleton for subcellular PIN trafficking and their polar localization. In this chapter, we cover the state of the art of polar auxin transport and its impact on plant morphogenesis.

K. Prasad (✉)
School of Biology, Indian Institute of Science Education and Research, Thiruvananthapuram 695016, Kerala, India
e-mail: kalika@iisertvm.ac.in

P. Dhonukshe (✉)
Section of Cell Biology, Faculty of Science, Department of Biology, Utrecht University, Kruyt Building (5th Floor), Padualaan 8, 3584 CH Utrecht, The Netherlands
e-mail: P.B.Dhonukshe@uu.nl

R. Chen and F. Baluška (eds.), *Polar Auxin Transport*,
Signaling and Communication in Plants 17, DOI 10.1007/978-3-642-35299-7_2,
© Springer-Verlag Berlin Heidelberg 2013

1 Auxin: A Plant Signaling Molecule with Morphogen-Like Characters

The forms and functions of multicellular organisms are not possible without effective communication between cells, tissues, and organs. Due to cell immobility, the regulation of morphogenesis in plants occurs in a coordinated fashion, and it predominantly depends on spatially instructive chemical signals called hormones. Auxin was the first hormone to be discovered in plants with some morphogen-like characteristics. In a classical book "The Power of Movement in Plants" in 1880, Charles Darwin described the effects of light on movement of canary grass (*Phalaris canariensis*) coleoptiles. Upon application of unidirectional light on the coleoptile, it bends in the direction of the light. Darwin's experiment suggested that the tip of the coleoptile was the tissue responsible for perceiving the light and producing some signal, which was transported to the lower part of the coleoptile where bending occurred. In 1926, Fritz Went isolated a plant growth substance by placing agar blocks under coleoptile tips and then removing and placing them on decapitated Avena stems (Went 1926).

Indole-3-acetic acid (IAA) is the most important member of signaling molecules from the auxin family. The production and persistence of IAA in the plants are controlled in many ways. By functioning as a mobile signal that connects cells, tissues, and organs, auxin coordinates plant morphogenesis and response programs. It plays crucial roles in many growth and developmental processes and thus shapes plant architecture. Interestingly, auxin is the only hormone transported in a polar manner. The pattern of auxin distribution within the plant navigates plant growth (Friml et al. 2003; Benkova et al. 2003). The long-distance auxin transport occurs via the stream of fluid in phloem vessels, and the short-distance auxin transport exhibits cell-to-cell movement. Auxin regulates transcription of various downstream genes in auxin-signaling pathway (Abel and Theologis 1996; Hagen and Guilfoyle 2002). Auxin binds and activates the TIR1 F-box component of the SCFTIR1 E3 ubiquitin ligase, which then ubiquitinates the auxin-signaling repressor AUX/IAA proteins, targeting them for destruction by the proteasome (Dharmasiri et al. 2005; Kepinski and Leyser 2005). This releases the auxin response factor (ARF) from the repressing influence of AUX/IAA to activate transcription of downstream genes (Tiwari et al. 2001). Plant, such as *Arabidopsis*, possesses 29 AUX/IAAs and 23 ARFs representing a complex matrix of auxin-signaling-dependent transcriptional network. In addition, functions of the ARF proteins are variable and also a high degree of functional redundancy is seen among the family members (Rademacher et al. 2011). Together, this poses tremendous challenges to untangle the circuitry of auxin-signaling machinery. A transcription-based synthetic reporter, DR5, consisting of multiple tandem repeats of ARF binding site (TGTCTC) is generally used to detect auxin activity in plant cells (Ulmasov et al. 1997a, b). Recently, more sensitive and repression-based auxin sensor has been developed, exploiting the auxin perception-related degradation properties of IAA (Vernoux et al. 2011;

Brunoud et al. 2012). Additional experimental methods such as microdissection, mass spectroscopy, and immuno-localization are used to measure the auxin level, and its distribution can be correlated with DR5 reporter.

Auxin regulates cell expansion, cell growth, and cell division, in a concentration and context-dependent manner. Auxin concentration along with other local factors contributes to cell specification, differentiation, and dedifferentiation. Depending on the specific tissue, auxin may stimulate axial elongation, lateral expansion, or isodiametric expansion. In addition to the differential distribution of auxin-signaling machinery, auxin biosynthesis is also spatiotemporally regulated. Once auxin is synthesized, it is transported to sites of its action. Auxin is generally transported from shoot apex to root apex. For a long-distance auxin transport, phloem vessels act as the highway, but for a short-distance auxin transport, a unique system of cell-to-cell polar transport is exploited. As auxin signaling relates to auxin distribution patterns and as auxin distribution patterns are regulated by the landscape of cell-to-cell polar auxin transport canals, it is considered that polar auxin transporters directly feed on the auxin-regulated programs. Thus, the directional signaling of auxin depends on the subcellular localizations of plasma membrane-associated auxin efflux and influx carriers. As auxin can enter into the cell either with the help of influx carrier or in a passive manner and as auxin requires efflux carriers for its exit out of the cell, the efflux carrier bears critical rate-limiting and directional influence on auxin transport. Efflux carrier PIN-FORMED (PIN) proteins have been established to be the main actors for the directional auxin transport. The localization of PIN proteins determines the direction of auxin flow and the choreographed relay of PIN activity along the auxin passage generates auxin gradients (Benkova et al. 2003; Petrásek et al. 2006; Paponov et al. 2005).

Auxin biosynthesis-, auxin conjugation-, and polar auxin transport-achieved auxin accumulation provides spatial coordinates for navigating plant organ formation, organ growth, and organ response programs (Benjamins and Scheres 2008). Auxin contributes to apical dominance. The apical bud synthesizes auxin and it diffuses downward to suppress lateral bud dominance. As shoot tip forms an auxin source and root tip an auxin sink, cutting of shoot tip impairs auxin supply to the roots and leads to inhibition of root growth and the formation of lateral roots (Sassi et al. 2012). In contrast, shoot decapitation leads to the development of lateral stems that allows gardeners to practice pruning in order to promote formation of extra shoots. Further, auxin participates in phototropism, geotropism, hydrotropism, and other developmental modifications. Cell division increases the number of cells and cell expansions, and growth is ultimately reflected in the tissue morphology, organ shape, and plant architecture. Differential auxin distribution modifies the coordination between cell division and expansion and as a consequence leads towards differential growth, triggering the shoots bending towards light or the root bending towards gravity (Peer et al. 2011). Auxin is necessary for fruit development and it delays fruit senescence. Exogenous application of auxin in fruits with removed seeds initiates fruit growth. When polar auxin transport is disrupted, it leads to abnormal

fruit morphologies. Auxin also plays a role in flower initiation and development of reproductive organs (Sundberg and Østergaard 2009). Auxin also plays an instrumental role in regeneration process (Duclercq et al. 2011). Furthermore, auxin promotes organization and development of xylem and phloem (Ye 2002).

1.1 Morphogenic Properties of Auxin

A morphogen is generally thought of as a chemical whose concentration varies in space, and for which varying threshold concentrations direct qualitatively different cellular responses or fates. Auxin acts in the micro- to nano-molar range. Graded concentration of auxin is essential for embryonic patterning and root and shoot organogenesis. Total amount of auxin arriving from the shoot to the root influences the degree of root growth. Auxin appears to dictate cell fates in the embryo in a concentration-dependent manner. Through regulated transport, it can accumulate in a spatially asymmetric concentration gradient and acts as a transcriptional regulator. Varying concentrations of auxin could result in different degrees of AUX/IAA degradation, thus releasing variable amounts of ARF proteins that could then activate downstream targets in an ARF concentration-dependent manner. However, a direct correspondence between the cellular auxin concentration gradient and the development of discrete cell types or regions in the embryo has yet to be proved (Bhalerao and Bennett 2003; Friml et al. 2003).

1.2 Auxin Efflux Carrier PINs

The PIN proteins have been identified as the key regulators of auxin-mediated developmental processes including growth, tropism, embryogenesis, and organogenesis (Friml et al. 2002, 2003; Friml 2003). PIN proteins are plasma membrane-located proteins that act as efflux carriers (Petrásek et al. 2006; Paponov et al. 2005). The polar localization of PIN proteins determines the direction of auxin flow (Wisniewska et al. 2006). There are eight PIN genes in the genome of Arabidopsis and encode for protein between 351 and 647 amino acids. PIN1 and PIN4 are involved in organogenesis; PIN1, PIN3, PIN4, and PIN7 are involved in embryogenesis; PIN2 and PIN3 are involved in gravitropism; and PIN1 and PIN3 are involved in phototropism (Paponov et al. 2005). The Arabidopsis PIN proteins are functionally characterized and found to be localized in a polar fashion either at different sides of various cell types or within the cell organelle (Blilou et al. 2005; Vieten et al. 2005; Mravec et al. 2009; Ding et al. 2012). The PIN proteins constitute prominent cell polarity markers in plants. The analysis of intron–exon structures of Arabidopsis thaliana (At) AtPIN family members reveals the relationship between AtPIN1, AtPIN4, and AtPIN7. Five of the eight AtPINs are located in the duplicated blocks. AtPIN3 and AtPIN7 share the same location. AtPIN1 is more closely related

Table 1 Members of the PIN protein family and their respective roles for various plant developmental and response programs

Auxin efflux carrier	Role in development
AtPIN1	Phyllotaxy, vein formation. Embryogenesis, lateral organ formation. Vascular development
AtPIN2	Organ development, root gravitropism
AtPIN3	Gravitropism, phototropism, organ development
AtPIN4	Root patterning, embryogenesis
AtPIN5	Regulation of intracellular auxin metabolism
AtPIN6	Transport activity
AtPIN7	Embryogenesis, root development

to AtPIN3, AtPIN4, and AtPIN7 as compared to AtPIN2 (Paponov et al. 2005). The diversity of hydrophilic central regions of AtPIN shows that there is a degree of functional variation in this family. Genes homologous to Arabidopsis PIN family are identified in most of the plants. With the divergence of both monocot and dicot plants, there have been significant changes in the number and the structure of PINs. The plants *Medicago* and potato contain five PIN sequences similar to one of the eight PINs of Arabidopsis. The low identity between PIN5 genes and the hydrophilic domains in the proteins reveals that PIN5 diverged from the ancestral PIN gene from early stage of development. Not surprisingly, unlike other PIN proteins, PIN5 is not localized at the PM and is not involved in cell-to-cell polar auxin transport. Instead, it resides at the endoplasmic reticulum and regulates intracellular auxin homeostasis and metabolism (Mravec et al. 2009; Table 1).

Auxin efflux is proportional to the degree of PIN expression and its polar localization, and the entire process is sensitive to polar auxin transport inhibitors. Auxin abundance regulates *PIN* gene expression, localization, and degradation, forming a complex feedback loop between auxin and its transport amount and directionality. Besides the changes in PIN expression and localization in response to developmental cascades, PIN polarity switches can also occur in response to environmental stimuli (Friml et al. 2002, 2003; Benkova et al. 2003; Reinhardt et al. 2003; Scarpella et al. 2006).

2 Role of PINs in Plant Development

2.1 Auxin and Embryogenesis

Polar auxin transport has long been suggested to play a principal role in plant embryogenesis. It has been shown that the hypocotyl of mature embryos of both angiosperms and gymnosperms transports auxin in the direction of the root (Greenwood and Goldsmith 1970; Fry and Wangermann 1976). Fry and Wangermann

(1976) were the first to propose that the commencement of polarized auxin transport in globular embryo might facilitate the morphological polarity expressed in subsequent stages of plant embryogenesis. The probable role of polar auxin transport in somatic embryogenesis was confirmed by Schiavone and Cooke (1987) who treated different phases of carrot somatic embryos by TIBA and NPA. Both auxin transport inhibitors at a concentration of 1 μM are capable of blocking the ability of somatic embryos to go through morphogenetic change to the successive phases: globular embryo goes through persistent spherical expansion, oblong embryos (an intermediary phase in somatic embryogenesis) carry on axis elongation devoid of any cotyledon initiation, and heart embryo grows additional growth axis on their hypocotyls. The embryonic pattern formation is in fact well maintained by two coinciding mechanisms:

1. A positional mechanism that rises as a maternal consequence from the ovular tissue surrounding the zygotic embryo or as a result of the polarized location of the egg cell and/or early embryo inside the embryo sac
2. Auxin-mediated mechanisms that are established right since beginning of polar auxin transport in the late globular embryo

The route of auxin transport is determined by the polar plasma membrane localization of PIN proteins. Even before the identification of the PIN proteins, it was revealed that pharmacological inhibition of auxin transport obstructs normal embryo patterning in numerous plant species (Liu et al. 1993; Hadfi et al. 1998), indicating a role for auxin transport in embryo patterning. In *Arabidopsis*, four PIN proteins are dynamically expressed throughout the embryogenesis (Friml et al. 2003). Once the first division of the zygote is over, PIN7 is confined to the apical side of the basal cell and probably driving auxin transport into the apical cell. At the 32-cell phase, PIN7 polarity reverses to the basal membranes of the suspensor cells, possibly causing transport of auxin away from the suspensor cells. PIN1 gets expressed from the two-cell onwards to the 16-cell stage well before the establishment of its polarity in the embryo. At the 32-cell stage, PIN1 becomes polarly confined to the basal membranes in the provascular cells adjacent to the hypophysis and helps the transport of auxin into the hypophysis (Fig. 1). At the transition stage of embryogenesis, PIN1 gets polarly localized near the flanks of the apical embryo domain, which possibly results in auxin maxima at these convergent points. The PIN4 protein gets expressed in the hypophysis cell and following division, in its topmost daughter cell. The expression of PIN3 commences fairly late at the heart stage in the columella precursors.

The direction of auxin flow indicated by the localization of PIN proteins corresponds well to the expression pattern of the auxin response reporter, suggesting that auxin response maxima are likely to reflect the concentration of auxin and that the auxin response pattern is a result of active transport. Certainly, *pin7* mutant embryos are affected in the DR5 action in the early embryo and show related cell division perturbations, signifying that an appropriate auxin circulation and response is required for exact cell specification in the early embryo. *pin1* mutant shows defects in patterning of apical half of embryo, eventually resulting either into fused cotyledon or into creation of tri-cotyledon or single-cotyledon seedlings

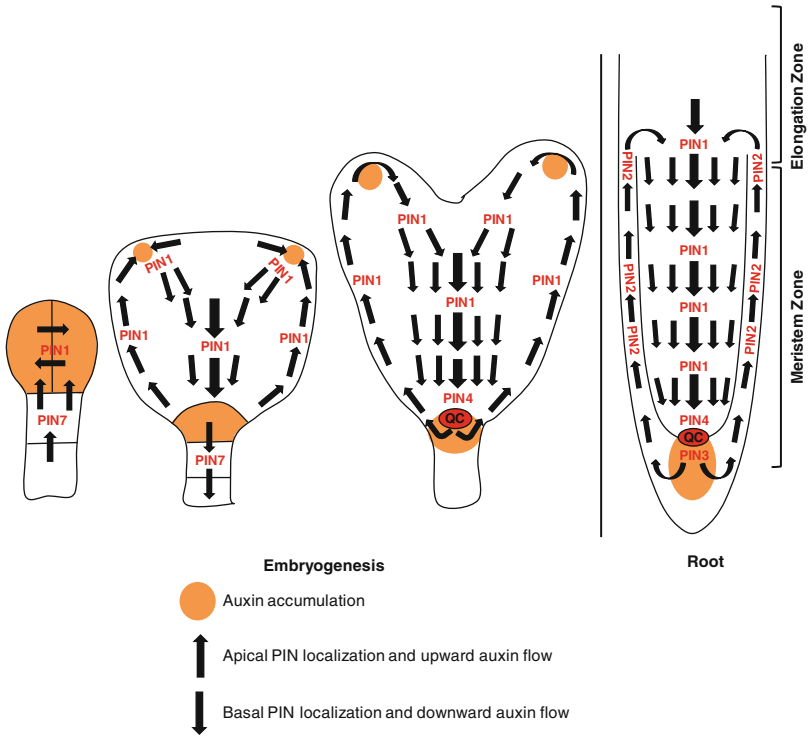

Fig. 1 Polar-localized auxin efflux carrier PIN proteins direct auxin flow during embryogenesis and root meristem growth to generate local auxin accumulation foci responsible for organ growth. This figure is adapted from Scientific World Journal (2012) 981658

(Aida et al. 2002). Single *pin* mutant does not display dramatic defects in embryonic patterning, suggesting redundant roles of PIN gene family during embryogenesis. Indeed, multiple *pin* mutant combinations show severe root and shoot pole defects (Friml et al. 2003; Blilou et al. 2005).

2.2 *Auxin and Root Development*

Auxin plays major roles in root development. Its concentration determines various aspects of root growth such as length of the epidermal-derived root hairs, the increase in quantity of lateral root primordia, and the response to gravity (Pitts et al. 1998; Rahman et al. 2002; Ishida et al. 2008; Péret et al. 2009). Auxin is synthesized in young leaves and cotyledons (Ljung et al. 2005) and transported to the root tip which represents the major sink tissue. Sorting of the root cells and measuring auxin concentration among them provide the most direct evidence of auxin gradients in the root, including the expected maxima in the quiescent center

(Petersson et al. 2009). In silico modeling of diffusion and PIN-facilitated auxin transport in and across root cells suggests that a robust auxin gradient associated with the maximum is able to explain the formation, maintenance, and growth of meristematic and elongation zones (Fig. 1) (Grieneisen et al. 2007). The local control of auxin levels creates regional concentration gradients and local maxima that are vital for establishing and sustaining a root primordium (Reviewed by Benjamins and Scheres 2008). The cellular auxin level in turn dictates the regulation of gene expression, which defines cell fate. Pharmacological or genetic interruptions of auxin transport intensely impact root patterning.

Several lines of experimental evidence support the idea that root-derived auxin contributes to establishment and maintenance of auxin gradient in the root and thus root growth. First, evidence for the role of auxin biosynthesis pathways in roots came from the characterization of the weak ethylene-insensitive (*wei2* and *wei7*) and transport inhibitor response 7 (*tir7-1*) mutants (Ljung et al. 2005; Stepanova et al. 2005). These mutants suppress the high-auxin phenotypes of the auxin-overproducing superroot (*sur1 or sur2*) and interrupt one of the two subunits of anthranilate synthase, an enzyme that catalyzes the rate-limiting step of anthranilate from chorismate during tryptophan synthesis. The failure of specific cells to yield tryptophan lowers their capacity to yield indole-3-acetic acid, which impairs root growth (Ljung et al. 2005; Stepanova et al. 2005).

2.3 Auxin and Lateral Root Development

Auxin is the key regulator of lateral root development (reviewed by Benkova and Hejatko 2009; Fukaki and Tasaka 2009). In many dicot plants as well as *Arabidopsis*, lateral roots originate from root pericycle cells adjacent to the protoxylem poles of the parent root (Beeckman et al. 2001). Initial events of lateral root formation are the divisions of a few pericycle cells positioned adjacent to a protoxylem pole. These cell division events are commonly designated as "lateral root initiation." Because the whole protoxylem pole pericycle displays a strong cell proliferation capability (Beeckman et al. 2001) and every pericycle cell adjacent to a xylem pole shows the ability to divide in response to elevated auxin levels (Himanen et al. 2002, 2004; Dubrovsky et al. 2008), it is believed that spatiotemporal control exists to limit lateral root initiation to certain sites and time points for the duration of root growth.

The even spacing and arrangement of lateral root primordia draws a parallel with a priming event that targets only a few pericycle cells as they depart the basal meristem (De Smet et al. 2007). These cells become primed due to an auxin response maximum that arises in the adjacent protoxylem cells. The auxin response maximum in the basal meristem and the simultaneous priming is not continuous but oscillates with a period of 15 h, which is in turn reflected in the regular spacing of lateral root along the root axis. The uniform spacing of lateral root primordia arises from pulses of auxin signaling in the basal meristem. The basal meristem includes

the set of cells that transit from the meristematic zone into the elongation zone and thus comprises of cells that undergo division as well as elongation. In seedlings that are grown in constant light, pulses of auxin signaling occur with a periodicity of 15 h. The response to auxin signaling in the xylem cells primes the adjacent pericycle cells so that they are competent to develop lateral root founder cells upon a second, auxin-dependent signal in the differentiation zone (De Smet et al. 2007). As such, the pulses of auxin in the basal meristem together with the uninterrupted growth of the root lead to the observed regular arrangement of lateral root primordia.

Auxin derived from both root and shoot is essential for the initiation and development of lateral roots. Passage of IAA via phloem from the leaf to root at the seedling stage is essential for emergence but not initiation of lateral roots in *Arabidopsis*. Genetic and pharmacological manipulation of this auxin movement interrupts lateral root formation (Reed et al. 1998; Bhalerao et al. 2002; Wu et al. 2007). Furthermore, experiments with the IAA transport inhibitor NPA demonstrate that IAA movement through the root tip is important for lateral root initiation, whereas shoot-derived transport is necessary for lateral root emergence (Casimiro et al. 2001).

Mutations or transgenes disturbing auxin biosynthesis, auxin metabolism, auxin transport, and auxin signaling affect the ability to form lateral roots. A gain-of-function mutation in a member of Aux/IAA protein family, IAA14/SOLITARY-ROOT, blocks the lateral root initiation (Fukaki et al. 2002). The presence of several auxin-related mutations specifically inhibiting lateral root initiation, lateral root morphogenesis, or lateral root emergence shows that auxin is essential not only for lateral root initiation but also for lateral root primordium development and morphology (Fukaki and Tasaka 2009). Mutations in several auxin efflux carrier *PIN* family members interrupt auxin-induced lateral root primordium development (Benkova et al. 2003). In addition, auxin influx carrier *AUX1/LAX* family members play important roles in lateral root initiation (Swarup et al. 2001; De Smet et al. 2007). These studies indicate that the regulation of distinct dynamic auxin transport systems is crucial for lateral root formation and development.

2.4 Auxin and Shoot Morphogenesis

Recent cellular and genetic data point towards the importance of auxin transport (Reinhardt et al. 2000, 2003) along with mechanical strains (Heisler et al. 2010; Peaucelle et al. 2011) in shaping phyllotactic patterns. These data allow incorporating in vivo PIN dynamics and auxin distribution details into computational models to theoretically test their relevance for phyllotaxis. Many models that attempt to understand the ability of auxin transport and mechanical stresses to capture leaf and even floral (van Mourik et al. 2012-considered for its similarity to purely phyllotactic models) patterns arising from cell-level simulations have

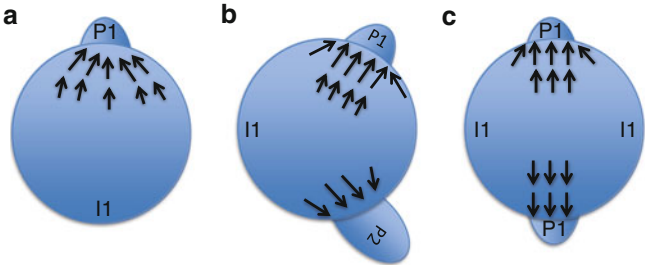

Fig. 2 Polar auxin transport based schematic representation of different patterns arising at the shoot apex; (**a**) Distichous (alternate), (**b**) Spiral, (**c**) Decussate (opposite) pattern. I (Incipient primordium), P (Primordium). The schematic representation is based on Reinhardt et al. (2003)

been proposed (de Reuille et al. 2006; Heisler et al. 2010; Stoma et al. 2008; Smith et al. 2006; Jönsson et al. 2006; Bayer et al. 2009).

Role of auxin in defining the initiation pattern of lateral organs sparks from sufficiency of auxin in triggering organ initiation when applied at the tip of naked meristem of *pin1* mutant, which is defective in polar auxin transport (Reinhardt et al. 2000). The presumed model suggests that auxin is transported to the site of lateral organ primordia inception by polar auxin efflux carrier PIN1. Growing lateral organ primordium acts as sink and this leads to depletion of auxin from surrounding cells, creating an inhibitory field which in turn controls the spacing between lateral organs to define a specific phyllotactic pattern (Reinhardt 2003; Fig. 2). While role of PIN1 was predicted earlier, only recently it has been shown that *pin1* hypomorphs result in switch of spiral pattern to opposite pattern (Prasad et al. 2011). Several computational models attempt to explain how a defined pattern is initiated. There is some consensus of assumptions found in various models made at the cellular and/or tissue level. The commonly used assumptions are:

(a) All decisive events that determine phyllotaxis occur in the outermost L1 layer except (Bayer et al. 2009) all models reviewed here limit themselves to the study of a single sheet of cells that form the shoot apical meristem.
(b) Movement of auxin through the apoplast is not considered.
(c) Auxin flows between cells by active transport and diffusion. Active transport is mediated by membrane proteins (PIN proteins) that show dynamic localization which is dictated by the specific polar auxin mechanism being modeled.
(d) Primordia are formed when auxin levels increase beyond a certain threshold, and once initiated, it is irreversible.
(e) Nascent and growing primordia act as auxin sinks (the empirical counterpart of the "inhibitory field"), modeled by the induction of another layer of "vascular tissue" beneath them that funnels auxin away from surrounding regions (de Reuille et al. 2006; Stoma et al. 2008), or an increased rate of degradation (van Mourik et al. 2012), both of which have equivalent mathematical formulations.

The distinct consequences of these sinks arise when applied over time. In some models, the converse is assumed, with developing primordia producing more auxin (Smith et al. 2006); in this case also, primordia act as sinks because of the concentration-based feedback involved in this model.

3 PIN Trafficking and Localization

Subcellular polarity of PINs determines the direction of auxin efflux out of that cell and thus coordinated PIN localizations along the chain of cells channelize directionality of auxin transport (Wisniewska et al. 2006; Dhonukshe et al. 2008, 2010). Subcellular analyses of PIN trafficking suggest that the PINs are not statically localized at the plasma membrane but undergo rapid endocytic cycling involving PIN internalization from the plasma membrane via clathrin-mediated endocytic pathway and PIN recycling back to the plasma membrane via ARF–GEF-regulated polar recycling (Geldner et al. 2001; Friml et al. 2002; Dhonukshe et al. 2007, 2008; Kleine-Vehn et al. 2008). The protein phosphatase 2A and Ser/Thr protein kinase PINOID are one of the major determinants of polar PIN localization (Christensen et al. 2000; Benjamins et al. 2001; Michniewicz et al. 2007; Dhonukshe et al. 2010). Apically localized PIN2 or basally localized PIN1 in the root meristem seems to be delivered originally in a nonpolar fashion after the de novo synthesis. Their apical or basal polarity is then established in the next step involving internalization from plasma membrane and phosphorylation-state-based polar recycling (Dhonukshe et al. 2008, 2010; Fig. 3). Additionally, molecules involved in intracellular trafficking of PIN and other proteins include endocytosis regulators, endosomal sorting/recycling regulators, and transcytosis regulators which are all associated in modulating polar localization of auxin transporters (Geldner et al. 2003; Kleine-Vehn et al. 2008).

3.1 Action of ARF–GEF GNOM in Polar Recycling of PINs

The *gnom* mutant phenocopies *pin1* mutant during early embryogenesis in Arabidopsis (Steinmann et al. 1999). GNOM encodes a BFA (brefeldin A)-sensitive guanine nucleotide exchange factor (GEF) for ARF GTPases that control vesicle budding (Steinmann et al. 1999; Geldner et al. 2003; Robert et al. 2008). GNOM protein resides in both the plasma membrane and endosomes (Steinmann et al. 1999; Geldner et al. 2003) and co-localizes with PIN1. In *gnom* mutant, PIN1 recycling is abnormal and as a result coordinated polar localization of PIN1 (and perhaps other PIN family members) is perturbed, leading towards drastic auxin transport-related

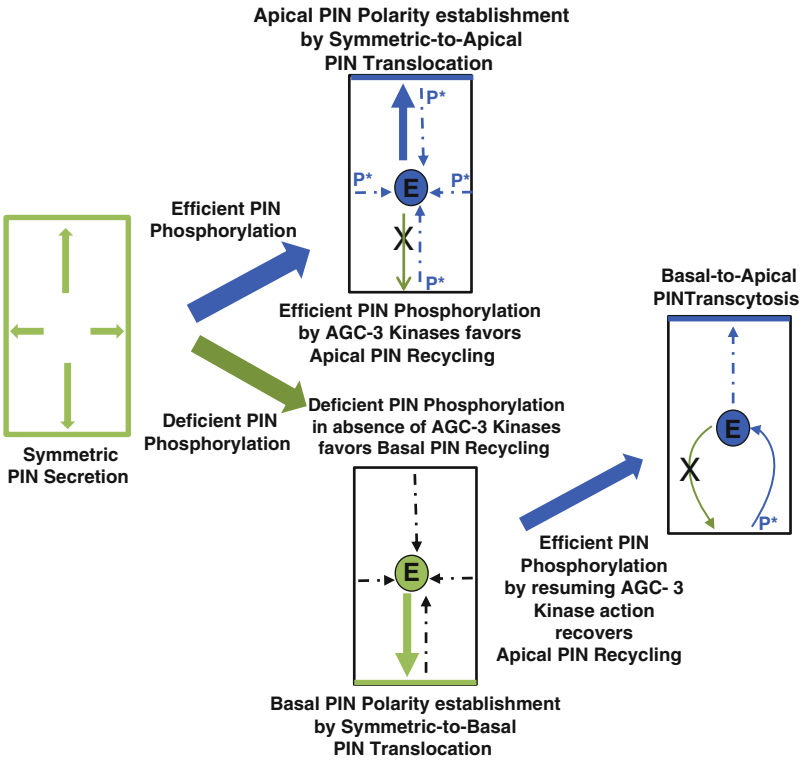

Fig. 3 Mechanism of establishment of PIN polarity by AGC-3 kinases. Non polar PIN gets translocated to apical upon phosphorylation by AGC-3 kianses and to basal in absence of phosphorylation. This figure is adapted from (Dhonukshe et al. 2012)

morphogenic defects. Transcytosis refers to the translocation of cargos from one polar domain to another. GNOM regulates basal PIN1 recycling and thus its basal localization. PINs have discrete polar plasma membrane localization on the apical, basal, or lateral side of cells, based on specific cell types (Kaplinsky and Barton 2004). GNOM-dependent transcytosis is crucial for early cell polarization. In the developmental progression of provascular cells, PIN1 can effectively shift from the apical side to the basal side in a wild-type line. In contrast, the *gnom* mutant fails to transport PIN1, localized on the apical side, to the basal side and shows abnormal embryo patterning. The BFA-treated wild-type seedlings also display *gnom*-like phenotypes (Geldner et al. 2001; Friml et al. 2003). In roots, PIN1, which is found in the stele, and PIN2, which is found in the cortex, are localized at the basal side of cells. However, PIN2 is localized at the apical side of epidermal cells. It was revealed that the BFA-sensitive GNOM ARF–GEF is obligatory for the basal but not apical localization of PINs. Loss-of-function mutations in *GNOM* or BFA treatment affected the basal PINs by recruiting them to the apical side. The shift in polarity of PINs due to the introduction of BFA was reversible in a protein

synthesis-independent manner. It was thus resolved that transcytosis through endosome-mediated trafficking plays a significant role in modulating PIN polarity (Kleine-Vehn et al. 2008).

3.2 Action of PID (PINOID) Kinase Providing Phosphorylation Bias for PIN Recruitment into Inverse Recycling Pathways

The *pid* mutant shows shoot phenotype similar to that of *pin1* mutants, displaying pin-like inflorescence, which indicates PID's role in auxin transport and/or signaling (Christensen et al. 2000; Benjamins et al. 2001). PID encodes a plant-specific serine/threonine protein kinase (Christensen et al. 2000). Interestingly, upon PID overexpression, PIN1 localization shifts from basal to apical cell side of root stele cells. The PID protein kinase directly phosphorylates PIN1, and the status of PID-mediated PIN1 phosphorylation governs its apical or basal distribution via its differential recruitment into apical or basal recycling pathway (Fig. 3). In addition to PID, WAG1 and WAG2, other two members of the AGC kinase protein family, instruct recruitment of PINs into the apical recycling pathway by phosphorylating the middle serine in three conserved TPRXS(N/S) motifs within the PIN central hydrophilic loop (Dhonukshe et al. 2010).

3.3 PP2A: A Protein Phosphatase Counteracting to the Action of PID Kinase

Given the significance of PIN phosphorylation status in determining PIN polar localization, PIN dephosphorylation is predicted to bear counteractive role. Recent study demonstrates that the PP2A phosphatase activity is essential for appropriate polar PIN localization and auxin transport-dependent plant development (Michniewicz et al. 2007). Mutations in PP2As (in Arabidopsis, there are three closely linked PP2A, including PP2A1, PP2A2, and PP2A3) cause various developmental abnormalities consistent with defective polar auxin transport. Genetic studies pointed out that PP2A (Ser/Thr phosphatases) and PID (Ser/Thr kinase) have counteracting roles in regulating auxin-dependent embryo and root development. A confined signaling pathway operates probably at the plasma membrane where PINs, PID, and PP2A all co-localize. Calcium also seems to play a role in PID/PP2A-mediated PIN phosphorylation/dephosphorylation. PID phosphorylation activity is negatively controlled by calcium and is concentration dependent. Auxin-related cell elongation and root alignment have been suggested to respond to changes in cytoplasmic calcium levels, and PINOID-mediated auxin signaling includes calcium-binding protein (Benjamins et al. 2003).

3.4 Role of SNX1 and VSP29 for PIN Trafficking and Localization

A retromer is a heteropentameric compound comprising of a sorting nexin dimer (through indefinite grouping of SNX1, SNX2, SNX5, or SNX6) and a trimer made up of VPS26, VPS29, and VPS35 in mammalian cells (Jürgens and Geldner 2007; Jaillais et al. 2006, 2007). The SNX dimers accomplish the recruitment of the pentameric retromer to endosomes, while the VPS22–VPS29–VPS35 triple subcomplex is thought to be binding cargos, which travel between endosomes and the *trans*-Golgi network (TGN). An *Arabidopsis thaliana* sorting nexin 1 (AtSNX1) was discovered in a novel endosomal compartment (Jaillais et al. 2006). AtSNX1 exists along with endosomal markers RABF1 and RABF2b in endosomes but not in Golgi, in the *trans*-Golgi network (TGN), and in endosomes having GNOM in them (Jaillais et al. 2006). Wortmannin, a PI3K (phosphatidylinositol 3-kinase) inhibitor deregulating the SNX localization in mammalian cells, also induced the generation of an enlarged AtSNX1-containing compartment in Arabidopsis. Wortmannin along with cycloheximide (protein synthesis inhibitor) triggered PIN2 accumulation in wortmannin-induced compartments. Based on these observations, it was established that polar PIN2 distribution is maintained by a novel AtSNX1-mediated endosomal pathway that is dissimilar to the GNOM-dependent PIN1-trafficking pathway, and it was suggested that two distinct populations of endosomes are involved in PIN1 and PIN2 trafficking, respectively.

3.5 Role of Rab5 for PIN Trafficking and Localization

Rab5 affects endocytosis via regulation of clathrin-coated vesicle formation at the plasma membrane, fusion of vesicles to endosomes, and fusion between endosomes (van der Bliek 2005). Rab5 localizes in the endosome and acts as a molecular switch by cycling between GDP-bound and GTP-bound states (Vitale et al. 1995). Arabidopsis possesses two direct homologues of Rab5 proteins. They are Ara7 and Rha1. The double mutant of ara7rha1 is gametophytic lethal (Dhonukshe et al. 2008). AtVps9a is the activator of Ara7 and Rha1. In Arabidopsis Rab5 homologues are identified in the endosomes. PINs are internalized largely by clathrin-mediated endocytosis pathway. The Rab5 interference affects PIN endocytosis. Interference with PIN endocytosis by manipulating the Arabidopsis Rab5 GTPase pathway prevents PIN polarization. Intriguingly, symmetric PIN1 leads to abnormal auxin distribution and one of the outcomes of such distribution causes enhanced local accumulation of auxin at cotyledon primordia eventually leading to root formation from places of cotyledon emergence (Dhonukshe et al. 2008), suggesting that maintenance of distinct polarity is prerequisite to specify cell fate and organ identity.

3.6 Role of ROP/RAC GTPases for PIN Trafficking and Localization

The plant-specific ROP/Rac subfamily of the extremely conserved Rho-family GTPases modulates signaling to set up the cell polarity in yeast and animal cells. Interestingly, it has also been shown to control cell polarity in several cell types of plants (Yang 2008). GFP–ROP2 was shown to be polarly confined to the plasma membrane in a similar manner to that of PIN2–GFP (Li et al. 2005). Gravity stimulation was found to induce vectorial re-localization of GFP–ROP2 in a way analogous to PIN2 re-localization. Furthermore, ROP2 overexpression intensifies PIN2–GFP polar localization and amplifies gravity responsiveness (Li et al. 2005). Further, auxin transported by PIN1 at the site of ROP2 activation induces ROP2 and ROP6 to regulate interdigitated growth of *Arabidopsis* leaf epidermal pavement cells (Xu et al. 2010).

3.7 Importance of Sterol Composition for PIN Localization

Sterols are integral components of the plasma and endomembranes. Sterols play a central role in the formation of membrane microdomains such as lipid rafts in the plasma membrane, which have a noteworthy effect on cell polarity. The chemical structure of plant sterol is similar to animal cholesterol. Polar delivery of cargos in plants also depends on the sterol composition of plasma membrane (Grebe et al. 2003; Willemsen et al. 2003; Kleine-Vehn et al. 2006). Recent work uncovers the major role of sterol in reiteration of PIN2 polarity after the division of polarized cells (Men et al. 2008). Sterols are also dynamic in endocytic trafficking among the plasma membrane and endosomal compartments, which is sensitive to the application of BFA.

4 Perspective

Recently, there has been a burst of literature on plant growth hormone auxin. We have begun to understand the mechanisms of polar localization and subcellular trafficking of auxin efflux carrier PINs. Precise localization of PINs, together with auxin influx carriers AUXs, shapes up the local accumulation of auxin during plant development. Recent experimental studies have allowed laying down conceptual models that can explain polar auxin transport and dynamics of PINs in generating the patterns. A major challenge ahead is to integrate the auxin-signaling pathways to the mechanistic aspects of polar auxin transport. How do auxin-signaling pathways instruct the subcellular trafficking of PINs and their polar localization and thus influence the polar auxin transport? Intriguingly, cell polarity is

instrumental to set the organ polarity. How the polar localization of PIN in individual cell and thus auxin transport from one cell to other gets communicated at the multicellular level to generate the polar organ remains largely unknown. However, gaining collaborations between in silico modeling studies and in vivo lab experiments are beginning to uncover the details of the morphogenetic auxin gradients in orchestrating various developmental processes.

Acknowledgements P.D. is supported by the Utrecht University Starting Independent Investigator Grant and Netherlands Organisation for Scientific Research's VIDI grant. KP is supported by IISER TVM startup grant.

References

Abel S, Theologis A (1996) Early genes and auxin action. Plant Physiol 111:9–17

Aida M, Vernoux T, Furutani M, Traas J, Tasaka M (2002) Roles of PIN-FORMED1 and MONOPTEROS in pattern formation of the apical region of the Arabidopsis embryo. Development 129:3965–3974

Bayer EM, Smith RS, Mandel T, Nakayama N, Sauer M, Prusinkiewicz P, Kuhlemeier C (2009) Integration of transport-based models for phyllotaxis and midvein formation. Genes Dev 23:373–384

Beeckman T, Burssens S, Inze D (2001) The peri-cell-cycle in Arabidopsis. J Exp Bot 52:403–411

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 CS, Hooykaas PJ, Offringa R (2003) PINOID-mediated signaling involves calcium-binding proteins. Plant Physiol 132:1623–1630

Benkova E, Hejatko J (2009) Hormone interactions at the root apical meristem. Plant Mol Biol 69:383–396

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

Bhalerao RP, Bennett MJ (2003) The case for morphogens in plants. Nat Cell Biol 5:939–943

Bhalerao RP, Eklof J, Ljung K, Marchant A, Bennett M, Sandberg G (2002) Shoot-derived auxin is essential for early lateral root emergence in Arabidopsis seedlings. Plant J 29:325–332

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

Brunoud G, Wells DM, Oliva M, Larrieu A, Mirabet V, Burrow AH, Beeckman T, Kepinski S, Traas J, Bennett MJ, Vernoux T (2012) A novel sensor to map auxin response and distribution at high spatio-temporal resolution. Nature 482:103–106

Casimiro I, Marchant A, Bhalerao RP, Beeckman T, Dhooge S, Swarup R, Graham N, Inze D, Sandberg G, Casero PJ et al (2001) Auxin transport promotes Arabidopsis lateral root initiation. Plant Cell 13:843–852

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

de Reuille PB, Bohn-Courseau I, Ljung K, Morin H, Carraro N, Godin C, Traas J (2006) Computer simulations reveal properties of the cell-cell signaling network at the shoot apex in Arabidopsis. Proc Natl Acad Sci USA 103:1627–1632

De Smet I, Tetsumura T, De Rybel B, Frey NF, Laplaze L, Casimiro I, Swarup R, Naudts M, Vanneste S, Audenaert D et al (2007) Auxin-dependent regulation of lateral root positioning in the basal meristem of Arabidopsis. Development 134:681–690

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

Dhonukshe P (2012) Mechanistic framework for establishment, maintenance, and alteration of cell polarity in plants. ScientificWorldJournal 2012:981658

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 (2008) Generation of cell polarity in plants links endocytosis, auxin distribution and cell fate decisions. Nature 456:962–966

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

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

Dubrovsky JG, Sauer M, Napsucialy-Mendivil S, Ivanchenko MG, Friml J, Shishkova S, Celenza J, Benkova E (2008) Auxin acts as a local morphogenetic trigger to specify lateral root founder cells. Proc Natl Acad Sci USA 105:8790–8794

Duclercq J, Sangwan-Norreel B, Catterou M, Sangwan RS (2011) De novo shoot organogenesis: from art to science. Trends Plant Sci 16:597–606

Friml J (2003) Auxin transport - shaping the plant. Curr Opin Plant Biol 2003(6):7–12

Friml J, Wiśniewska J, Benková E, Mendgen K, Palme K (2002) 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

Fry SC, Wangermann E (1976) Polar transport of auxin through embryos. New Phytol 77:313–317

Fukaki H, Tasaka M (2009) Hormone interactions during lateral root formation. Plant Mol Biol 69:437–449

Fukaki H, Tameda S, Masuda H, Tasaka M (2002) Lateral root formation is blocked by a gain-of-function mutation in the SOLITARY-ROOT/IAA14 gene of Arabidopsis. Plant J 29:153–168

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, KornbergerW MP, 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

Grebe M, Xu J, Möbius 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

Greenwood MS, Goldsmith MHM (1970) Polar transport and accumulation of indole-3-acetic acid during root regeneration by *Pinus lambertiana* embryos. Planta 95:297–313

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

Hadfi K, Speth V, Neuhaus G (1998) Auxin-induced developmental patterns in Brassica juncea embryos. Development 125:879–887

Hagen G, Guilfoyle T (2002) Auxin-responsive gene expression: genes, promoters and regulatory factors. Plant Mol Biol 49:373–385

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: e1000516

Himanen K, Boucheron E, Vanneste S, De Almeida EJ, Inze D, Beeckman T (2002) Auxin-mediated cell cycle activation during early lateral root initiation. Plant Cell 14:2339–2351

Himanen K, Vuylsteke M, Vanneste S, Vercruysse S, Boucheron E, Alard P, Chriqui D, Van Montagu M, Inze D, Beeckman T (2004) Transcript profiling of early lateral root initiation. Proc Natl Acad Sci USA 101:5146–5151

Ishida T, Kurata T, Okada K, Wada T (2008) A genetic regulatory network in the development of trichomes and root hairs. Annu Rev Plant Biol 59:365–386

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

Jönsson H, Heisler MG, Shapiro BE, Meyerowitz EM, Mjolsness E (2006) An auxin-driven polarized transport model for phyllotaxis. Proc Natl Acad Sci USA 103:1633–1638

Jürgens G, Geldner N (2007) The high road and the low road: trafficking choices in plants. Cell 130:977–979

Kaplinsky NJ, Barton MK (2004) Plant biology: plant acupuncture: sticking PINs in the right places. Science 306:822–823

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

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, Wisniewska J, Paciorek T, Benkova E, Friml J (2008) ARF GEF-dependent transcytosis and polar delivery of PIN auxin carriers in Arabidopsis. Curr Biol 18:526–531

Li L, Xu J, Xu ZH, Xue HW (2005) Brassinosteroids stimulate plant tropisms through modulation of polar auxin transport in Brassica and Arabidopsis. Plant Cell 17:2738–2753

Liu C, Xu Z, Chua NH (1993) Auxin polar transport is essential for the establishment of bilateral symmetry during early plant embryogenesis. Plant Cell 5:621–630

Ljung K, Hull AK, Celenza J, Yamada M, Estelle M, Normanly J, Sandberg G (2005) Sites and regulation of auxin biosynthesis in Arabidopsis roots. Plant Cell 17:1090–1104

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

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 (2007) Antagonistic regulation of PIN phosphorylation by PP2A and PINOID directs auxin flux. Cell 130:1044–1056

Mravec J, Skůpa P, Bailly A, Hoyerová K, Krecek P, Bielach A, Petrásek J, Zhang J, Gaykova V, Stierhof YD, Dobrev PI, Schwarzerová K, Rolcík J, Seifertová D, Luschnig C, Benková E, Zazímalová E, Geisler M, Friml J (2009) Subcellular homeostasis of phytohormone auxin is mediated by the ER-localized PIN5 transporter. Nature 459:1136–1140

Paponov IA, TealeWD TM, Blilou I, Palme K (2005) The PIN auxin efflux facilitators: evolutionary and functional perspectives. Trends Plant Sci 10:170–177

Peaucelle A, Braybrook SA, Le Guillou L, Bron E, Kuhlemeier C, Höfte H (2011) Pectin-induced changes in cell wall mechanics underlie organ initiation in Arabidopsis. Curr Biol 21:1720–1726

Peer WA, Blakeslee JJ, Yang H, Murphy AS (2011) Seven things we think we know about auxin transport. Mol Plant 4:487–504

Péret B, De Rybel B, Casimiro I, Benková E, Swarup R, Laplaze L, Beeckman T, Bennett MJ (2009) Arabidopsis lateral root development: an emerging story. Trends Plant Sci 14:399–408

Petersson SV, Johansson AI, Kowalczyk M, Makoveychuk A, Wang JY, Moritz T, Grebe M, Benfey PN, Sandberg G, Ljung K (2009) An auxin gradient and maximum in the Arabidopsis root apex shown by high-resolution cell-specific analysis of IAA distribution and synthesis. Plant Cell 21:1659–1668

Petrásek J, Mravec J, Bouchard R, Blakeslee JJ, Abas M, Seifertová D, Wisniewska J, Tadele Z, Kubes M, Covanová M, Dhonukshe P, Skupa P, Benková E, Perry L, Krecek P, Lee OR, Fink GR, Geisler M, Murphy AS, Luschnig C, Zazímalová E, Friml J (2006) PIN proteins perform a rate-limiting function in cellular auxin efflux. Science 312:914–918

Pitts RJ, Cernac A, Estelle M (1998) Auxin and ethylene promote root hair elongation in Arabidopsis. Plant J 16:553–560

Prasad K, Grigg SP, Barkoulas M, Yadav RK, Sanchez-Perez GF, Pinon V, Blilou I, Hofhuis H, Dhonukshe P, Galinha C, Mähönen AP, Muller WH, Raman S, Verkleij AJ, Snel B, Reddy GV, Tsiantis M, Scheres B (2011) Arabidopsis PLETHORA transcription factors control phyllotaxis. Curr Biol 21:1123–1128

Rademacher EH, Möller B, Lokerse AS, Llavata-Peris CI, van den Berg W, Weijers D (2011) A cellular expression map of the Arabidopsis AUXIN RESPONSE FACTOR gene family. Plant J 68:597–606

Rahman A, Hosokawa S, Oono Y, Amakawa T, Goto N, Tsurumi S (2002) Auxin and ethylene response interactions during Arabidopsis root hair development dissected by auxin influx modulators. Plant Physiol 130:1908–1917

Reed RC, Brady SR, Muday GK (1998) Inhibition of auxin movement from the shoot into the root inhibits lateral root development in Arabidopsis. Plant Physiol 118:1369–1378

Reinhardt D (2003) Vascular patterning: more than just auxin? Curr Biol 13:R485–R487

Reinhardt D, Mandel T, Kuhlemeier C (2000) Auxin regulates the initiation and radial position of plant lateral organs. Plant Cell 12:507–518

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

Robert S, Chary SN, Drakakaki G, Li S, Yang Z, Raikhel NV, Hicks GR (2008) Endosidin1 defines a compartment involved in endocytosis of the brassinosteroid receptor BRI1 and the auxin transporters PIN2 and AUX1. Proc Natl Acad Sci USA 105:8464–8469

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

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

Schiavone FM, Cooke TJ (1987) Unusual patterns of somatic embryogenesis in domesticated carrot: developmental effects of exogenous auxins and auxin transport inhibitors. Cell Diff 21:53–62

Smith RS, Guyomarc'h S, Mandel T, Reinhardt D, Kuhlemeier C, Prusinkiewicz P (2006) A plausible model of phyllotaxis. Proc Natl Acad Sci USA 103:1301–1306

Steinmann T, Geldner N, Grebe M, Mangold S, Jackson CL, Paris S, Gälweiler L, Palme K, Jürgens G (1999) Coordinated polar localization of auxin efflux carrier PIN1 by GNOM ARF GEF. Science 286:316–318

Stepanova AN, Hoyt JM, Hamilton AA, Alonso JM (2005) A Link between ethylene and auxin uncovered by the characterization of two root-specific ethylene-insensitive mutants in Arabidopsis. Plant Cell 17:2230–2242

Stoma S, Lucas M, Chopard J, Schaedel M, Traas J, Godin C (2008) Flux-based transport enhancement as a plausible unifying mechanism for auxin transport in meristem development. PLoS Comput Biol 4:e1000207

Sundberg E, Østergaard L (2009) Distinct and dynamic auxin activities during reproductive development. Cold Spring Harb Perspect Biol 6:a001628

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

Tiwari SB, Wang XJ, Hagen G, Guilfoyle TJ (2001) AUX/IAA proteins are active repressors, and their stability and activity are modulated by auxin. Plant Cell 13:2809–2822

Tiwari SB, Hagen G, Guilfoyle TJ (2004) Aux/IAA proteins contain a potent transcriptional repression domain. Plant Cell 16:533–543

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

Ulmasov T, Murfett J, Hagen G, Guilfoyle TJ (1997b) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. Plant Cell 9:1963–1971

van der Bliek AM (2005) A sixth sense for Rab5. Nat Cell Biol 7:548–550

van Mourik S, Kaufmann K, van Dijk AD, Angenent GC, Merks RM, Molenaar J (2012) Simulation of organ patterning on the floral meristem using a polar auxin transport model. PLoS One 7:e28762

Vernoux T, Brunoud G, Farcot E, Morin V, Van den Daele H, Legrand J, Oliva M, Das P, Larrieu A, Wells D, Guédon Y, Armitage L, Picard F, Guyomarc'h S, Cellier C, Parry G, Koumproglou R, Doonan JH, Estelle M, Godin C, Kepinski S, Bennett M, De Veylder L, Traas J (2011) The auxin signalling network translates dynamic input into robust patterning at the shoot apex. Mol Syst Biol 7:508

Vieten A, Vanneste S, Wisniewska 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

Vitale G, Alexandrov K, Ullrich O, Horiuchi H, Giner A, Dobson C, Baykova O, Gournier H, Stenmark H, Zerial M (1995) The GDP/GTP cycle of Rab5 in the regulation of endocytotic membrane traffic. Cold Spring Harb Symp Quant Biol 60:211–220

Went FW (1926) On growth-accelerating substances in the coleoptile of *Avena sativa*. Proc Kon Ned Akad Wet 30:10–19

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

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

Wu G, Lewis DR, Spalding EP (2007) Mutations in Arabidopsis multidrug resistance-like ABC transporters separate the roles of acropetal and basipetal auxin transport in lateral root development. Plant Cell 19:1826–1837

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 interdigitation in Arabidopsis. Cell 143:99–110

Yang Z (2008) Cell polarity signaling in Arabidopsis. Annu Rev Cell Dev Biol 24:551–575

Ye ZH (2002) Vascular tissue differentiation and pattern formation in plants. Annu Rev Plant Biol 53:183–202