Auxin Transporters—Why So Many?

Eva Zažímalová¹, Angus S. Murphy², Haibing Yang², Klára Hoyerová¹, and Petr Hošek^{1,3}

¹Institute of Experimental Botany AS CR, Rozvojová 263, CZ-165 02 Prague 6, Czech Republic

²Department of Horticulture, Purdue University, West Lafayette, Indiana 47907-2010

³Department of Biomedical Informatics, Faculty of Biomedical Engineering, Czech Technical University in Prague, Sítná 3105, CZ-272 01 Kladno, Czech Republic

Correspondence: zazimalova@ueb.cas.cz

Interacting and coordinated auxin transporter actions in plants underlie a flexible network that mobilizes auxin in response to many developmental and environmental changes encountered by these sessile organisms. The independent but synergistic activity of individual transporters can be differentially regulated at various levels. This invests auxin transport mechanisms with robust functional redundancy and added auxin flow capacity when needed. An evolutionary perspective clarifies the roles of the different transporter groups in plant development. Mathematical and functional analysis of elements of auxin transport makes it possible to rationalize the relative contributions of members of the respective transporter classes to the localized auxin transport streams that then underlie both pre-programmed developmental changes and reactions to environmental stimuli.

AUXIN AND ITS ROLE IN PLANT DEVELOPMENT

Auxin has a unique position among plant growth regulatory substances. A wide spectrum of developmental processes in plants is controlled by the differential distribution of its molecules (Benamins and Scheres 2008). The nonuniform levels of auxin in plant tissues or organs are created in response to transient exogenous stimuli and/or the internal developmental program, and thus they provide some kind of “interface” between environmental

and/or endogenous signals and signaling pathway(s), resulting in particular developmental event(s). In principle, there are two processes that have the potential to modify the level of any compound in a cell: metabolism and transport. Indeed, in the case of auxin, both metabolic changes of auxin as well as its transport have been shown to be involved in modulation of plant development (review by Vanneste and Friml 2009; Petrášek and Friml 2009). As is discussed here, multiple transport proteins are required to maintain directional auxin flows within organs and tissues.

E. Zažímalová et al.

AUXIN TRANSPORT: PHYSICAL-CHEMICAL BACKGROUND AND THE NEED FOR ACTIVE TRANSPORT

The distribution of native auxin (typically indole-3-acetic acid, IAA) in the plant body is realized over both short and long distances (e.g., between adjacent cells as well as between the shoot apex and sites of lateral root initiation, respectively). Auxin molecules can move over long distances through the vascular system (namely via the phloem from source tissues to the roots) by mass flow. However, there is also another system for auxin translocation—over both short and long distances. This system is

unique compared with other plant hormones; it involves a cell-to-cell mechanism and it is mostly polar. The key to understanding how auxin(s) can move across the plasma membrane (PM) lies in the physical-chemical nature of auxin molecules. Because all auxins are weak acids, their molecular form (proton-dissociated or nondissociated), and thus their ability to penetrate through the membrane, depends on pH. In plants, apoplastic pH is approximately 5.5 as a result of protons extruded by PM H^+ -ATPases (Fig. 1). At this pH, the equilibrium of IAA molecules ($pK_a = 4.85$) is calculated to be approximately 83% dissociated (anionic, A^-) and 17% proton-associated (HA , i.e., nondissociated).

Figure 1. Cellular auxin transport. The scheme shows the organization of proteins involved in auxin transport. PIN efflux carrier proteins depicted in red represent “long” PINs (PIN1, 2, 3, 4, and 7), whereas PINs marked in pink represent “short” PINs (PIN5, 6, and 8). ER marks endoplasmic reticulum, pale gray structures represent ER and endosomes, curved bold full arrows show constitutive protein cycling, and dashed arrows symbolize the process of transcytosis. Possible collaboration between ABCBs and PINs is suggested by placing the symbols close to each other.

Although the hydrophobic nature of the indole group in IAA allows association of the molecule with the PM surface, the negative charge of a dissociated carboxyl group on the molecule will prevent it from crossing the membrane. As such, only the proton-associated (i.e., nondissociated) IAA molecules can enter the cell by lipophilic diffusion (passive movement) across the plasma membrane without assistance of a carrier protein.

In the cytoplasm of plant cells, the pH is approximately 7; at this pH, the equilibrium of auxin molecules shifts almost entirely to the anionic, dissociated form. As anionic auxins cannot diffuse across the PM, cells function as weak acid anion traps unless efflux transporters are present to ameliorate this bottleneck. An obvious logical extension of this observation is that asymmetrical localization of relevant transporters can provide directional cellular auxin efflux and that coupled asymmetries in adjacent cells can establish observed polar flows. This concept is the basis of the chemiosmotic polar diffusion model or chemiosmotic hypothesis (Rubery and Shelldrake 1974; Raven 1975; Goldsmith 1977).

AUXIN TRANSPORTERS

The previously described physical-chemical background implies the need for active transport of auxin molecules (more precisely auxin anions) out from cells. Indeed, auxin efflux carriers were characterized within the last decade. However, surprisingly, there is not only one or a few auxin exporters, but there are at least two protein families, members of which possess auxin-exporting activity. These are from the plant-specific PIN family and from the ATP-binding cassette (ABC) superfamily of transporters, predominantly of its B type (ABCB/multidrug resistance [MDR]/phosphoglycoprotein [PGP]) (Fig. 1).

Members of PIN (PIN-FORMED) family were associated with polar auxin transport in the late 1990s (Gälweiler et al. 1998; Luschnig et al. 1998), although the first reference to the respective mutants (*pin-formed 1*, *pin1*) appeared in the 1950s (in Goto et al. 1987) and

these were characterized in the early 1990s (Okada et al. 1991). According to their amino acid sequence, PINs are integral membrane proteins with a topology similar to some transporter proteins. In *Arabidopsis*, the PIN family consists of eight members and divides into two basic subclades, differing in the length of a hydrophilic loop in the middle of their polypeptide chain. Canonical “long” PINs (PIN1–4 and 7) (reviews by Tanaka et al. 2006; Vieten et al. 2007; Zažímalová et al. 2007) show mostly polar PM-localization, and direct auxin transport function is strongly supported for PIN1, 2, 4, and 7 (Petrásek et al. 2006; Yang and Murphy 2009). The polar localization of these long PINs determines the direction of auxin flow (Wisniewska et al. 2006) and their roles in many auxin-dependent processes in plant development have been characterized (Luschnig et al. 1998; Friml et al. 2002a; 2002b; Blilou et al. 2005; Scarpella et al. 2006; Xu et al. 2006; Sauer et al. 2006). These canonical PINs, even though their cellular localization in domains in the PM is mostly polar, do not reside there statically. On the contrary, they undergo constitutive cycling between the PM and endosomal compartments (Geldner et al. 2001; Dhonukshe et al. 2007), and they can be relocalized rapidly to various parts of the cell via a transcytosis-like mechanism (Kleine-Vehn et al. 2008) in response to environmental signals. The polar localization of the long PIN proteins underlies the vectorial auxin transport required for embryo development, organogenesis, tropisms, and other developmental processes (Friml et al. 2002b; Friml et al. 2003; Benkova et al. 2003; Reinhardt et al. 2003; Blakeslee et al. 2005).

In contrast to long PINs, there are three members of the *AtPIN* family that have their central hydrophilic loop either partly (PIN6) or significantly (PIN5, 8) reduced. Recently (Mravec et al. 2009), it was shown that these “short” PINs do not localize to PM but to the endomembranes, namely—for PIN5 to the endoplasmic reticulum (ER). Given that PIN5 is a functional auxin transporter, its localization on the ER suggests a role in intracellular auxin distribution and regulation of cellular auxin

E. Zažímalová et al.

homeostasis, thus controlling availability of active auxin for various subcellular and cellular actions.

None of the PIN sequences contain an ATP-binding domain and they are considered to be secondary transporters. However, the source of energy for their operation is not known yet.

Probably all PINs directly transport auxins and, especially for long PINs, a wide functional redundancy has been described (Vieten et al. 2005). Unlike ABCB transporters, PIN-like proteins from other kingdoms exhibit very little sequence or functional similarity to plant PINs (Titapiwatanakun et al. 2009; Yang and Murphy 2009). Plant PINs exhibit approximately 15% sequence similarity with related proteins from yeasts and even less similarity to bacterial PIN-like proteins. Further, an auxin effluxer-like protein (AEL1) from *Schizosaccharomyces pombe* has been biochemically characterized and exhibits minimal auxin transport activity (Titapiwatanakun et al. 2009).

In contrast, the ABC superfamily is one of the largest and most ubiquitous transporter families, and ABC transporters are associated with the movements of a wide variety of small molecules, nutrients, and xenobiotics (Verrier et al. 2008). Recent structural solutions of the Sav1866 bacterial ABC transporter and the murine ABCB1 transporter indicate a remarkably high degree of structural conservation between bacterial and eukaryotic ABC transporters (Becker et al. 2009; H Yang, unpubl.). Despite this structural conservation, members of the ABC transporters exhibit narrow substrate specificity in some organisms and substrate promiscuity in others (Hrycyna et al. 1998; Geisler et al. 2005). Phylogenetic and structural analyses of plant ABC transporters indicates that a small subgroup of the ABCB subclass of ABC transporters, best represented by ABCB1 and 19 in *Arabidopsis*, function in auxin transport across plant species (Verrier et al. 2008). ABCB1/19 are the closest *Arabidopsis* orthologs of mammalian ABCB1 multidrug resistance transporters, but exhibit unique characteristics in their putative substrate binding sites and transport auxin with comparatively high substrate specificity (Titapiwatanakun et al. 2009; Yang and Murphy 2009). ABCB1/

PGP1 and ABCB19/PGP19 have been shown to act as auxin efflux carriers (reviews by Geisler and Murphy 2006; Titapiwatanakun and Murphy 2009), whereas ABCB4 exhibits more complex transport characteristics. When expressed in *S. pombe*, ABCB4 functions as auxin influx transporter under low concentrations of auxin and reverses to efflux as auxin concentrations increase (Yang and Murphy 2009). This activity is consistent with the demonstrated function of ABCB4 in auxin-dependent root hair elongation in *Arabidopsis* (Cho et al. 2007). ABCB1, 4, and 19 exhibit stable, primarily nonpolar PM-localization, although more polar localizations in some cells may contribute to tissue-specific function. PINs and ABCBs need not necessarily work independently of each other. On the contrary, namely ABCB19/PGP19 and PIN1 were shown to co-act (ABCB stabilizing PIN in PM microdomains) and enhance the substrate specificity of the overall transport in some tissues and under specific developmental situations (Mravec et al. 2008; Titapiwatanakun et al. 2009).

As mentioned previously, auxin molecules can enter cells passively; however, they can also be transported into cells via the H⁺-symport activity of the AUX1/LAX family of PM permeases (reviewed by Kerr and Bennett 2007) (Fig. 1). The *Arabidopsis* genome encodes one AUX1 and three Like AUX1 (LAX1, LAX2, and LAX3) proteins (Parry et al. 2001). AUX1 and the three LAX proteins share an amino acid sequence similarity of approximately 80% (Parry et al. 2001). The need for this “additional” active auxin-uptake process arises in cells where high and rapid auxin influx is needed, such as in the lateral root cap where AUX1 plays a major role in redirection of polar auxin streams (Kramer and Bennett 2006).

Although it is possible that some of the transporters described previously may be multifunctional and transport substrates other than auxins as well, the number of transporters involved in the process is initially striking. However, when the central role of auxin transport in programmed and plastic plant development is considered in an evolutionary context, the number and diversity of transport proteins involved is not surprising.

AN EVOLUTIONARY RATIONALE FOR OVERLAPPING AUXIN TRANSPORTER SYSTEMS

A design engineer would probably consider the three classes of auxin transporters comprising in summary at least 15 different carriers (in *A. thaliana*) an unnecessary redundancy. However, in biology, a combination of overlapping activities and functional redundancy in a robust transport network appears to be the norm. The movement of signaling molecules and vital mineral ions like calcium and nitrate is often mediated by multiple systems functioning in specific tissues, cells, and subcellular compartments (White and Broadley 2003; Miller et al. 2007). Transport of small molecules is often affected by a parallel gradient-driven cotransport and ATP-dependent pump systems (Gaxiola et al. 2007). Still, it is remarkable that auxin is mobilized by ATP-driven transporters (ABCBs), H^+ -symporters (AUX/LAXes), and gradient-driven carriers (PINs). The root of this diversity of transport systems appears to lie in the improvisational and adaptive processes that were integral to sessile vascular plant evolution, when mechanisms that control cellular division and expansion were augmented by new means of controlling polar development and the elaboration of complex reproductive/vegetative organs. The selective pressures favored an integrated network that actuates local responses but also provides for the transduction of a core integrative signal over distance. The vectorial movement of auxin may provide as much developmental information as does the level of auxin in a particular cell, and is regulated by a localized system of diversion, export, import, and metabolism. Information content in such a system is inherent in the signal, position, quantity of flow, and vector of movement rather than in an overarching central nervous system as seen in animals. In such a system, basal auxin concentrations, localized auxin sources and sinks, and auxin fluxes can all direct the position and polarity of growth (Benamins and Scheres 2008; Ikeda et al. 2009).

Phylogenetic analyses of PIN and ABCB transporters indicate that ABCBs constitute a more ancient transport system, as ABCB structure is

Auxin Transporters—Why So Many?

highly conserved throughout phyla (Peer and Murphy 2007), whereas PIN proteins appear to have arisen with vascular plants (Zažímalová et al. 2007). Further, endogenous plant flavonoids that emerged in early land plants interfere with the fundamental mechanism of ABCB transporters, whereas PIN proteins are only indirectly affected by these compounds (Rauscher 2006; Geisler et al. 2005; Blakeslee et al. 2007; Peer and Murphy 2007; Santelia et al. 2008; Sukumar et al. 2009). This suggests that presence of plant flavonoids was a selective factor in the evolution of PIN1/2-type PM efflux transporters and provides an evolutionary rationale for overlapping transporter function, as auxin transport must continue to function at basal levels in the presence of flavonoid production in response to herbivory and oxidative stress. The extent to which similar factors contributed to the emergence of AUX/LAX proteins from the ancient amino acid permease protein family is not clear.

However, the greater driving force for the elaboration of the PIN efflux system was apparently the more sophisticated developmental programming that appears in vascular plants. The generally apolar localization of ABCB transporters and their high degree of stability on the PM (Blakeslee et al. 2007; Titapiwatanakun et al. 2009; Titapiwatanakun and Murphy 2009) is not compatible with the plasticity and complexity of development required in terrestrial plants (Zažímalová et al. 2007) and the evolution of apical growth, vascular tissue, more complex organogenesis, and more refined polar auxin transport. Only singleton PIN-like genes with unknown function have been found in bacteria and yeasts (Paponov et al. 2005), and three PIN-like genes were identified in the moss *Physcomitrella patens* (Decker et al. 2006; Rensing et al. 2008). In contrast, the angiosperm *Arabidopsis* has eight PIN genes with a relatively high degree of homology (32%–45% mutual identity) (Paponov et al. 2005), even if they fall into two distinct subclades (“long” PINs and “short” PINs, see previous discussion), differing in the length of a central cytosolic loop (Zažímalová et al. 2007; Mravec et al. 2009). Some members of the short PIN subclade, namely PIN5 and 8 are similar in length to

E. Zažímalová et al.

bacterial and yeast PIN-like proteins and may represent ancestral plant PIN proteins. PIN5, 6, and 8, *PpPIN1A* from *P. patens*, and yeast AEL1 all localize to ER (Matsuyama et al. 2006; Mravec et al. 2009), whereas other *A. thaliana* PINs show PM localization, consistent with the hypothesis that the expansion of plant PIN proteins occurred during land plant radiation and evolution. The (mostly) polar localization of PIN proteins is also consistent with higher-plant polarity and organogenesis (Benjamins and Scheres 2008; Robert and Friml 2009). The recent evolution of PIN proteins coincides with the evolution of PINOID, a kinase regulating polar localization of PIN: No PINOID or PIN genes were found in green algal genomes of *Chlamydomonas* or *Ostreococcus*, although auxin transport seems to regulate the directional growth and patterning of brown algae, which is thought to be mediated by ABCB transporters (Cooke et al. 2002; Galvan-Ampudia and Offringa 2007). The coevolution of PINOID and PINs in land plants suggests that PINOID-kinase-regulated PIN-dependent polar auxin transport might play an important role in this transition (Galvan-Ampudia and Offringa 2007).

In addition to the auxin efflux carriers ABCBs and PINs, auxin influx carriers are represented by proteins from the AUX1/LAX family of PM permeases (Kerr and Bennett 2007) (see previous discussion). AUX1/LAX proteins from various species form two distinct subfamilies that differ in their amino acid sequences mainly in intracellular-oriented hydrophilic loops (Hoyerová et al. 2008) and they belong to the amino acid/auxin permease family of proton-driven transporters. They are thought to derive from the ancient amino acid permease family that mediates the uptake of tryptophan and other amino acids, although this has not been attributed to AUX1/LAX proteins (Bennett et al. 1996; Young et al. 1999).

FUNCTIONAL ANALYSIS OF AUXIN TRANSPORTERS

ABCB transport is a direct ATP-dependent activity that can function when chemiosmotic

gradients are decreased or when auxin must move against a gradient. An example is in cortical/epidermal tissues of the mature root where auxin flow against the stelar vector must be maintained to sustain root hair production and other growth phenomena (Terasaka et al. 2004; Geisler et al. 2005; Cho et al. 2007; Yang and Murphy 2009). However, a system dependent on direct ATP-driven transport is at a disadvantage in rapidly growing tissues where ATP has been depleted. In these tissues, gradient-driven transporters like PINs and AUX/LAX proteins would be expected to predominate.

A more important factor in determining auxin transporter use is a need for the polarity and plasticity of auxin transport. ABCB transporters characterized to date are highly stable on the PM and require activation/folding assisted by FKBP42 and other chaperone proteins (Bouchard et al. 2006; Rojas Pierce et al. 2007; Bailly et al. 2008; Titapiwatanakun et al. 2009). Any polarity seen in ABCB localization appears to be associated with secondary cell wall rather than cell plate formation and early events in cytokinesis, and is therefore not dynamic (Blakeslee et al. 2007; Titapiwatanakun et al. 2009). Consistent with these characteristics, *abcb* mutants exhibit elongation defects and reduced long-distance auxin transport, but only limited defects in organogenesis and embryo development (Noh et al. 2001; Geisler et al. 2003; Terasaka et al. 2005; Blakeslee et al. 2007).

In contrast, essential directional polar auxin transport required for embryogenesis and organogenesis is controlled by a subset of PIN proteins that are trafficked to the PM and that exhibit distinct polarized localizations (Benkova et al. 2003; Friml et al. 2003; Reinhardt et al. 2003; Dhonukshe et al. 2008). PIN1/PIN7 function in maintaining basipetal polar fluxes in the developing embryo and along the apical-basal embryonic axis throughout development (Friml et al. 2003; Jönsson et al. 2006; Kuhlemeier 2007), PIN1 and PIN2 mediate polar auxin fluxes in organogenesis (Benkova et al. 2003; Reinhardt et al. 2003), and PIN2 does so upwards from the root apex (Chen et al. 1998; Müller et al. 1998; Friml et al. 2004).

Plastic growth responses require dynamic redirection of auxin fluxes. PIN proteins appear to be the primary vehicles for the redirection of these fluxes (Friml et al. 2002b; Blakeslee et al. 2004; Robert and Friml 2009), but have not been shown to initiate these redirections. The speed of the initial auxin redirection response in gravi-responding roots suggests that asymmetric changes in pH and membrane potentials may precede PIN protein relocalization (review by Peer and Murphy 2007). Cellular trafficking of PIN proteins is often generalized as “dynamic” in tissues where the polar localization of the proteins is quite stable, as, e.g., PIN1 in mature vascular tissues (Friml et al. 2003), PIN7 in mature shoot tissue (Blakeslee et al. 2007), PIN3 in photo-responsive shoot tissues (Titapiwatanakun and Murphy 2009), and PIN2 in root epidermal tissues (Müller et al. 1998; Friml et al. 2004). On the other hand, PIN3 signal exhibits lateral reorientation in the root apex in response to gravistimulation (Friml et al. 2002b), dynamic PIN1 relocalization is observed in photo-responding shoot apical meristems (Blakeslee et al. 2004), PIN1 signals in the bundle sheath cells of dark-grown *Arabidopsis* hypocotyls rapidly disappear with the blue light treatment (Blakeslee et al. 2007), and PIN1 and PIN2 are able to undergo transcytosis in some cells (Kleine-Vehn et al. 2008). Although AUX1/LAX proteins are mobilized to and from the PM by dynamic processes similar but not identical to those mobilizing PINs (Friml et al. 2004; Dharmasiri et al. 2006; Kleine-Vehn et al. 2006), no evidence has been found for polarized relocalization of these proteins.

It is important to note, however, that little is known about regulatory processes that differentially control the activity of auxin transporters rather than their localization (Zažímalová et al. 2007; Vanneste and Friml 2009). The convenience of viewing changes in the subcellular localization of PIN proteins has led to the assumption that this is the only mechanism by which auxin transport streams are regulated. Recent evidence indicates that ABCB and PIN proteins are both regulated by complex and

partly conventional regulatory mechanisms (Cheng et al. 2008; Vanneste and Friml 2009; Zourelidou et al. 2009).

INDEPENDENT, BUT COORDINATED TRANSPORTER FUNCTIONS

One would prefer to have simple definitions of auxin transporter function that assign function by protein category. However, as the overlapping auxin transport mechanisms have developed in the context of plant evolution and natural selection, such approaches invoke so many exceptions that they may cause the generalities to fail. For instance, not all PIN proteins exhibit polar localizations in all tissues and ABCB localizations are not strictly apolar (Friml et al. 2002a; Terasaka et al. 2004; Geisler et al. 2005; Blakeslee et al. 2007; Wu et al. 2007; Titapiwatanakun et al. 2009). The same is true for AUX1 (Swarup et al. 2001; 2004). Further, complex interactions of some PIN and ABCB proteins based on indirect interactions (Blakeslee et al. 2007; Mravec et al. 2008; Titapiwatanakun et al. 2009) further limit this simplistic assignment of function to auxin transporters. A better approach might be to define basic auxin transport streams and view the role of the various transporters in that context (Kuhlemeier 2007).

Developmentally Essential Polar Auxin Streams

The polar auxin streams that function in the establishment of embryonic apical–basal polarity and organogenesis appear to be mediated almost exclusively by PIN1, PIN4, and, to a lesser extent, PIN7 (Friml et al. 2002b; Benkova et al. 2003; Friml et al. 2003; Reinhardt et al. 2003). Although *ABCB1* and *ABCB19* are expressed in late embryo development and the proteins can be visualized in the embryo (Mravec et al. 2008), loss of *ABCB* gene function does not appear to impact essential early developmental processes. Loss of *AUX1* function also has no obvious effect on embryonic development (Casimiro et al. 2001; Swarup et al. 2004). Analogous observations can

E. Zažímalová et al.

be made for vascular differentiation (Wenzel et al. 2007), organogenesis (Benkova et al. 2003; Reinhardt et al. 2003), and leaf margin development (Barkoulas et al. 2007; Rolland-Lagan 2008), but the phenotype, formation of auxin maxima, as well as reduced PIN polarization in quadruple *aux1/lax* mutants suggest a role for AUX1/LAX proteins in “buffering” the action of PINs in phyllotaxis (Bainbridge et al. 2008).

The auxin flow for lateral root development may be initiated by *AUX1* expression in lateral root primordia or relocalization of PIN1 in single protophloem cells, as in the case of mechanical induction (Marchant et al. 2002; Ditegou et al. 2008). However, *ABCB1* and *19* are also expressed in all stages of lateral root development, with a polar localization at early stages and an apolar one at later stages (Mravec et al. 2008). So, the coordinated action of *ABCB1*, *19*, *PIN1*, and *AUX1* are required for the normal lateral root initiation, *AUX1* generating a sink, and *ABCB1*, *19*, and *PIN1* providing the auxin source. The localization of *ABCB19* also suggested its role of auxin flow restriction in early lateral root development (Mravec et al. 2008). After later root emergence, auxin flows loaded by *ABCB1/19*, *AUX1*, and *PIN1* continue and resemble the main auxin streams in the primary root, as described below.

Auxin Loading and Restriction of Long-distance Auxin Transport Streams

The auxin long-distance stream from shoot apical meristem to root is loaded mainly by *PIN1*, *7*, and *ABCB1* and *19* (Gälweiler et al. 1998; Friml et al. 2003; Blakeslee et al. 2007). The expression of *PIN7* and *ABCB1* is more restricted in the shoot apex, consistent with their roles in auxin loading to the vascular stream. The expression of *PIN1* and *ABCB19* through the whole plant maintains the main auxin stream from shoot to root apices. A surprising number of auxin transport components were found in root tips; however, currently, it is easier to visualize the root tip than shoots and thus more auxin transport components may be identified in the shoots in the future. More

importantly, the growth of the root tip is highly organized to maintain an invariant cell organization, and it is very dynamic and flexible to circumvent unpredictable obstacles under the guidance of gravity and water and nutrient availability. Auxin is thought to be an internal messenger delivering these internal and external complex signals and the sonata of auxin flow requires multiple players to perform: Acropetal auxin flow (from the mature root to the root tip), driven mainly by *PIN1* and *ABCB19*, is redirected to the lateral root cap and epidermal cells (basipetal flows) through the concerted action of *AUX1*, *ABCB1*, *4*, *19*, and *PIN1*, *2*, *3*, and *4*. *AUX1*, *ABCB1*, *19*, and *PIN1*, *2*, *3*, and *4* function mainly in loading, whereas *AUX1*, *ABCB1*, *4*, and *PIN2* continue the stream along lateral root cap, the epidermal and cortical cells to guide cell division, cell elongation, and root hair development in the outer cell layers (Gälweiler et al. 1998; Müller et al. 1998; Swarup et al. 2001; Friml et al. 2002a; 2002b; Geisler et al. 2005; Terasaka et al. 2005; Blakeslee et al. 2007; Mravec et al. 2008). Rapid auxin influx via *AUX1* is consistent with the higher auxin concentration in lateral root cap cells compared with columella cells (Petersson et al. 2009).

Even if *ABCB19* and *PIN1* appear to be the principal mediators of polar auxin transport along the axis (Gälweiler et al. 1998; Blakeslee et al. 2007), retention of auxin in vascular transport streams in the shoot is more attributable to the combined export activities of *PIN3* and *ABCB19*, which are localized in bundle sheath cells (Friml et al. 2002b; Blakeslee et al. 2007). *ABCB19*, localized to the endodermis and pericycle, appears to have a similar function in the root (Blakeslee et al. 2007). Auxin flow against a concentration gradient would logically require an active and efficient transport system consistent with *ABCB19* function (Yang and Murphy 2009). *PIN2* localized to root cortical cells has also been proposed to function in auxin reflux back to the vascular cylinder at the root apex that is independent of *AUX1/PIN2/ABCB4*-mediated redirection of auxin out of the columella/lateral root cap region (Blilou et al. 2005). However, long-distance

auxin streams from the root apex upwards in epidermal cells above the elongation zone appear to be mediated by ABCB transporters (Terasaka et al. 2004; Geisler et al. 2005; Lewis et al. 2007; Titapiwatanakun et al. 2009).

Auxin Sinks Can Provide a Driving Force for Transport Streams

Although auxin transport in the vascular cylinder is primarily associated with xylem parenchyma cells in embryos and seedlings, phloem transport plays a more substantial role in the mature shoot and root (Ljung et al. 2005). Phloem transport was thus an early focus of investigation of the AUX1 uptake transporter and its potential role in generating auxin gradients that maintain cellular identity and the growth of the vasculature in post-meristematic cells (Swarup et al. 2001). However, more recent evidence has suggested the importance of auxin sinks generated by AUX/LAX proteins in the motivation of auxin transport streams in the stele cells to generate the auxin fluxes for lateral root development (Marchant et al. 2002; Swarup et al. 2005; 2008). Mathematical models that have been experimentally verified indicate that AUX1 uptake activity can contribute 10-times more to directional auxin movement than pH-driven passive movement alone (Tsurumi and Ohwaki 1978; Delbarre et al. 1996; Kramer 2004; Swarup et al. 2005; Li et al. 2005; Kramer and Bennett 2006). The contribution of AUX1 in generating auxin sinks is shown by the total loss of gravitropic responses in the *aux1* mutant (Mirza et al. 1984; Bennett et al. 1998). Loss of function of all other auxin transporters analyzed to date (except of PIN2) does not lead to such complete loss of gravitropic growth responses, even when multiple loss-of-function mutations are present. LAX3 has recently been shown to play a similar role in the accumulation of auxin in cortical and epidermal cells surrounding lateral root primordia to facilitate lateral root emergence (Swarup et al. 2008). These functions are all consistent with the evolutionary adaptation of permease proteins to auxin transport function.

Auxin Transporters—Why So Many?

Redirection of Auxin in Tropic Responses

Tropic growth such as gravitropism and phototropism is also guided by auxin flow. In response to gravity stimulation, symmetrically localized PIN3 in the root columella has been shown to relocate laterally and facilitate auxin transport to the lateral root cap (Friml et al. 2002b) and the AUX1/PIN2 systems that mobilize auxin away from the root apex. In response to unidirectional light, plants redistribute auxin laterally to the distal side with respect to the light, and growth is promoted there (Friml et al. 2002b). In the *Arabidopsis* hypocotyl, auxin flow is restricted in the vascular bundle by PIN3 and ABCB19 (Friml et al. 2002b; Blakeslee et al. 2007). Unidirectional light triggers its lateral auxin flow, which seems to be driven by PIN1, 3, and ABCB19. However, unlike in the gravitropic response, the relocation of PIN3 in the hypocotyls of dark grown seedlings was not seen with unidirectional blue light. Instead, PIN1 at the distal side immediately below the region of phototropic curvature is delocalized, which results in auxin accumulation above (Blakeslee et al. 2004).

Competitive Response Mechanisms

Although PINs, ABCBs, and AUX1/LAXes are the primary mediators of auxin transport in plants, it is likely that other plant anion transporters may exhibit lower levels of auxin transport activity. Competitive regulation of native auxin transport by nitrate transporters would explain the apparent induction of auxin uptake and lateral root formation in *Arabidopsis* grown under low N (Forde 2002; Hermans 2006; Desnos 2008; Vidal and Gutierrez 2008; Forde and Walch Liu 2009). Similar competitive mechanisms may exist with the malate transporter ABCB14 (Lee et al. 2008), as *abcb14* mutants exhibit reduced malate-inhibitable auxin transport in the shoot (Yang and Murphy, unpubl.), and possibly others as well. In all such cases, a localized increase in auxin transport in response to a shortage of the competing small molecule can be readily rationalized.

E. Zažímalová et al.

AUXIN TRANSPORTERS AND AUXIN HOMEOSTASIS

The role of transporters in regulating auxin homeostasis at the cellular level is often overlooked. The ABCB4 transporter appears to function in this capacity in root trichoblasts (Cho et al. 2008; Yang and Murphy 2009). As shown in yeast, ABCB4 has an importer activity at low auxin levels and converts to export activity as auxin levels increase (Yang and Murphy 2009). Such a reversible auxin transporter could maintain auxin homeostasis in the root hairs and elongating cells, where constant auxin levels are required and the AUX1 importer is not present (Swarup et al. 2001; Jones et al. 2009; Yang and Murphy 2009); in the lateral root cap and in a unique file of three cells in the central elongation zone of the root (Terasaka et al. 2004); and/or in gravi-responding *Arabidopsis* roots (Lewis et al. 2007). A different role in auxin homeostasis seems to be played by PIN5 and possibly also by other “short” PINs (6, 8), which localize to endomembranes (predominantly ER). These intracellular auxin transporters change the auxin distribution inside cells, thus exposing auxin molecules to different conjugating/degrading enzymes. Indeed, the overexpression of PIN5 results in dramatic change of profile of auxin metabolites (Mravec et al. 2009).

MATHEMATICAL MODELING AS A TOOL TO CREATE HYPOTHESES ABOUT THE INVOLVEMENT OF VARIOUS TRANSPORTERS

Auxin—with its specialized polar transport features—seems to be the most attractive for mathematical modeling of all plant hormones.

Indeed, observation that vascular development in plants is dependent on auxin flux that itself enhances transport capacity of cells further differentiating into vascular bundles (Sachs 1969) led to some of the first mathematical models of the auxin canalization hypothesis (Mitchison 1980, 1981). In Mitchison’s model (1981) cells exchanging auxin with adjacent cells increase permeability of their plasma membrane

on the side with large auxin flux. Models dealing with the presumption that cells are able to reinforce their capacity for auxin movement across PM by regulation of PIN localization evolved only during the last decade.

The necessity for PIN protein activity in the creation of the auxin maxima responsible for proper phyllotaxis (Jönsson et al. 2006; Smith et al. 2006; Merks et al. 2007) as well as the role of PINs in vein formation (Feugier 2005; Fujita and Mochizuki 2006; Prusinkiewicz and Rolland-Lagan 2006) have been explored in some detail, but the crucial question “what is the cellular mechanism that polarizes PIN proteins on PM?” still remains unanswered. The lack of a suitable auxin sensor motivated the search for another possible mechanism, i.e., the detection of auxin concentration in adjacent cells. Recent models of phyllotaxis presume that PINs polarize toward the cell with highest auxin concentration, thus pumping auxin against its concentration gradient and creating auxin maxima in the tissue (Jönsson et al. 2006; Smith et al. 2006).

The models became more precise with detailed characteristics of auxin carrier abundance on the PM, taking into account protein cycling, and also auxin-induced PIN expression (Merks et al. 2007). Besides auxin efflux carrier activity, the localization and participation of AUX/LAX influx carriers in auxin accumulation were shown in a model of simplified vein tissue (Kramer 2004). Although AUX1 was proposed to support phyllotaxis by maintaining high auxin concentration in outer layer of the shoot meristem (Reinhardt et al. 2003; Bainbridge et al. 2008), this hypothesis has not been reflected in the models yet. In spite of increasing knowledge about ABCB transporters, they have not been included in mathematical models of auxin flow so far. Interestingly, all current models are still focused on auxin transport at the tissue level and the basic transport element (a single cell) is very simplified there.

The advantage of mathematical modeling resides in the possibility to test a number of variants for auxin transport mechanisms, thus offering a tool to combine complex data sets

and test their congruence. Present models have the potential to support or not support biological hypotheses, and increasingly to suggest new hypotheses, such as the participation of new elements and processes. In this context, solid experimental data can substantially improve the usefulness of mathematical models, and thus support the characterization of the behavior of individual elements of the auxin transport network. Krupinski and Jönsson's article in this collection focuses on auxin transport modeling, which gives much more details related to this topic (Krupinski and Jönsson 2010).

ACKNOWLEDGMENTS

The authors acknowledge the support for their work from the Ministry of Education of the Czech Republic, project LC06034 (EZ, KH, PH), and from US Department of Energy (AM, HY). E.Z. and A.S.M. contributed to the paper equally.

REFERENCES

- Bailly A, Sovero V, Vincenzetti V, Santelia D, Bartnik D, Koenig BW, Mancuso S, Martinoia E, Geisler M. 2008. Modulation of P-glycoproteins by auxin transport inhibitors is mediated by interaction with immunophilins. *J Biol Chem* **283**: 21817–21826.
- Bainbridge K, Guyomarc'h S, Bayer E, Swarup R, Bennett M, Mandel T, Kuhlemeier C. 2008. Auxin influx carriers stabilize phyllotactic patterning. *Gene Dev* **22**: 810–823.
- Barkoulas M, Hay A, Kougioumoutzi E, Tsiantis M. 2008. A developmental framework for dissected leaf formation in the *Arabidopsis* relative *Cardamine hirsuta*. *Nat Genet* **40**: 1136–1141.
- Becker J-P, Depret G, Van Bambeke F, Tulkens PM, Prevost M. 2009. Molecular models of human P-glycoprotein in two different catalytic states. *BMC Struct Biol* **9**: doi:10.1186/1472-6807-9-3.
- Benjamins R, Scheres B. 2008. Auxin: The looping star in plant development. *Annu Rev Plant Biol* **59**: 443–465.
- 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.
- Bennett MJ, Marchant A, May ST, Swarup R. 1998. Going the distance with auxin: Unravelling the molecular basis of auxin transport. *Philos Trans R Soc Lond B Biol Sci* **353**: 1511–1515.
- Blakeslee JJ, Peer WA, Murphy AS. 2005. Auxin transport. *Curr Opin Plant Biol* **8**: 494–500.
- Blakeslee JJ, Bandyopadhyay A, Lee OR, Mravec J, Titapiwatanakun B, Sauer M, Makam SN, Cheng Y, Bouchard R, Adamec J, et al. 2007. Interactions among PIN-FORMED and P-glycoprotein auxin transporters in *Arabidopsis*. *Plant Cell* **19**: 131–147.
- Blakeslee JJ, Bandyopadhyay A, Peer WA, Makam SN, Murphy AS. 2004. Relocalization of the PIN1 auxin efflux facilitator plays a role in phototropic responses. *Plant Physiol* **134**: 28–31.
- 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, et al. 2006. Immunophilin-like TWISTED DWARF1 modulates auxin efflux activities of *Arabidopsis* P-glycoproteins. *J Biol Chem* **281**: 30603–30612.
- Casimiro I, Marchant A, Bhalarao RP, Beeckman T, Dhooge S, Swarup R, Graham N, Inzé D, Sandberg G, Casero PJ, et al. 2001. Auxin transport promotes *Arabidopsis* lateral root initiation. *Plant Cell* **13**: 843–852.
- Chen R, Hilson P, Sedbrook J, Rosen E, Caspar T, Masson PH. 1998. The *Arabidopsis thaliana* AGRATROPIC 1 gene encodes a component of the polar-auxin-transport efflux carrier. *Proc Natl Acad Sci* **95**: 15112–15117.
- Cheng Y, Qin G, Dai X, Zhao Y. 2008. NPY genes and AGC kinases define two key steps in auxin-mediated organogenesis in *Arabidopsis*. *Proc Natl Acad Sci* **105**: 21017–21022.
- Cho M, Lee SH, Cho HT. 2007. P-Glycoprotein4 displays auxin efflux transporter like action in *Arabidopsis* root hair cells and tobacco cells. *Plant Cell* **19**: 3930–3943.
- Cooke TJ, Poli D, Szein AE, Cohen JD. 2002. Evolutionary patterns in auxin action. *Plant Mol Biol* **49**: 319–338.
- Decker EL, Frank W, Sarnighausen E, Reski R. 2006. Moss systems biology en route: phytohormones in *Physcomitrella* development. *Plant Biol* **8**: 397–406.
- Delbarre A, Muller P, Imhoff V, Guern J. 1996. Comparison of mechanisms controlling uptake and accumulation of 2,4-dichlorophenoxy acetic acid, naphthalene-1-acetic acid, and indole-3-acetic acid in suspension-cultured tobacco cells. *Planta* **198**: 532–541.
- Desnos T. 2008. Root branching responses to phosphate and nitrate. *Curr Opin Plant Biol* **11**: 82–87.
- Dharmasiri S, Swarup R, Mockaitis K, Dharmasiri N, Singh SK, Kowalchuk M, Marchant A, Mills S, Sandberg G, Bennett MJ, et al. 2006. AXR4 is required for localization of the auxin influx facilitator AUX1. *Science* **312**: 1218–1220.
- Dhonukshe P, Aniento F, Hwang I, Robinson DG, Mravec J, Stierhof Y-D, 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, et al. 2008. Generation of cell polarity in plants links endocytosis, auxin distribution and cell fate decisions. *Nature* **456**: 962–966.

E. Zažímalová et al.

- Ditengou FA, Teale WD, Kochersperger P, Flittner KA, Kneuper I, van der Graaff E, Nziengui H, Pinosa F, Li XG, Nitschke R, et al. 2008. Mechanical induction of lateral root initiation in *Arabidopsis thaliana*. *Proc Natl Acad Sci* **105**: 18818–18823.
- Feugier FG, Mochizuki A, Iwasa Y. 2005. Self-organization of the vascular system in plant leaves: Inter-dependent dynamics of auxin flux and carrier proteins. *J Theoret Biol* **236**: 366–375.
- Forde BG. 2002. Local and long-range signaling pathways regulating plant responses to nitrate. *Annu Rev Plant Biol* **53**: 203–224.
- Forde BG, Walch-Liu P. 2009. Nitrate and glutamate as environmental cues for behavioral responses in plant roots. *Plant Cell Environ* **32**: 682–693.
- Friml J, Benková E, Blilou I, Wisniewska J, Hamann T, Ljung K, Woody S, Sandberg G, Scheres B, Jürgens G, et al. 2002a. AtPIN4 mediates sink-driven auxin gradients and root patterning in *Arabidopsis*. *Cell* **108**: 661–673.
- Friml J, Vieten A, Sauer M, Weijers D, Schwarz H, Hamann T, Offringa R, Jürgens G. 2003. Efflux-dependent auxin gradients establish the apical-basal axis of *Arabidopsis*. *Nature* **426**: 147–153.
- Friml J, Wiśniewska J, Benková E, Mendgen K, Palme K. 2002b. Lateral relocation of auxin efflux regulator PIN3 mediates tropism in *Arabidopsis*. *Nature* **415**: 806–809.
- Friml J, Yang X, Michniewicz M, Weijers D, Quint A, Tietz O, Benjamins R, Ouwerkerk PB, Ljung K, Sandberg G, et al. 2004. A PINOID-dependent binary switch in apical-basal PIN polar targeting directs auxin efflux. *Science* **306**: 862–865.
- Fujita H, Mochizuki A. 2006. Pattern formation by the positive feedback regulation between flow of diffusible signal molecule and localization of its carrier. *J Theoret Biol* **241**: 541–55.
- Galvan-Ampudia CS, Offringa R. 2007. Plant evolution: AGC kinases tell the auxin tale. *Trends Plant Sci* **12**: 541–547.
- Gälweiler L, Guan C, Müller A, Wisman E, Mendgen K, Yephremov A, Palme K. 1998. Regulation of polar auxin transport by AtPIN1 in *Arabidopsis* vascular tissue. *Science* **282**: 2226–2230.
- Gaxiola RA, Palmgren MG, Schumacher K. 2007. Plant proton pumps. *FEBS Lett* **581**: 2204–2214.
- Geisler M, Murphy AS. 2006. The ABC of auxin transport: The role of p-glycoproteins in plant development. *FEBS Lett* **580**: 1094–1102.
- Geisler M, Blakeslee JJ, Bouchard R, Lee OR, Vincenzetti V, Bandyopadhyay A, Titapiwatanakun B, Peer WA, Bailly A, Richards EL, et al. 2005. Cellular efflux of auxin catalyzed by the *Arabidopsis* MDR/PGP transporter AtPGP1. *Plant J* **44**: 179–194.
- Geisler M, Kolukisaoglu HU, Bouchard R, Billion K, Berger J, Saal B, Frangne N, Koncz-Kalman Z, Koncz C, Dudler R, et al. 2003. TWISTED DWARF1, a unique plasma membrane-anchored immunophilin-like protein, interacts with *Arabidopsis* multidrug resistance-like transporters AtPGP1 and AtPGP19. *Mol Biol Cell* **14**: 4238–4249.
- Geldner N, Friml J, Stierhof Y-D, Jürgens G, Palme K. 2001. Auxin transport inhibitors block PIN1 cycling and vesicle trafficking. *Nature* **413**: 425–428.
- Goldsmith MHM. 1977. The polar transport of auxin. *Annu Rev Plant Physiol* **28**: 439–478.
- Goto N, Starke M, Kranz AR. 1987. Effect of gibberellins on flower development of the pin-formed mutant of *Arabidopsis thaliana*. *Arabidopsis Information Service* **23**: 66–71.
- Hermans Ch, Hammond JP, White P, Verbruggen N. 2006. How do plants respond to nutrient shortage by biomass allocation? *Trends Plant Sci* **11**: 610–617.
- Hoyerová K, Perry L, Hand P, Laňková M, Kocáček T, May S, Kottová J, Pačes J, Napier R, Zažímalová E. 2008. Functional characterization of PaLAX1, a putative auxin permease, in heterologous plant systems. *Plant Physiol* **146**: 1128–1141.
- Hrycyna CA, Gottesman MM. 1998. Multidrug ABC transporters from bacteria to man: An emerging hypothesis for the universality of molecular mechanism and function. *Drug Resist Update* **1**: 81–83.
- Ikeda Y, Men S, Fischer U, Stepanova AN, Alonso JM, Ljung K, Grebe M. 2009. Local auxin biosynthesis modulates gradient-directed planar polarity in *Arabidopsis*. *Nat Cell Biol* **11**: 731–738.
- Jones AR, Kramer EM, Knox K, Swarup R, Bennett MJ, Lazarus CM, Leyser HM, Grierson CS. 2009. Auxin transport through non-hair cells sustains root-hair development. *Nat Cell Biol* **11**: 78–84.
- Jönsson H, Heisler MG, Shapiro BE, Meyerowitz EM, Mjolsness E. 2006. An auxin-driven polarized transport model for phyllotaxis. *Proc Natl Acad Sci* **103**: 1633–1638.
- Kerr ID, Bennett MJ. 2007. New insight into the biochemical mechanisms regulating auxin transport in plants. *Biochem J* **401**: 613–622.
- Kleine-Vehn J, Dhonukshe P, Sauer M, Brewer P, Wiśniewska J, Paciorek T, Benková E, Friml J. 2008. ARF GEF-dependent transcytosis and polar delivery of PIN auxin carriers in *Arabidopsis*. *Curr Biol* **18**: 526–531.
- 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.
- Kramer EM. 2004. PIN and AUX/LAX proteins: Their role in auxin accumulation. *Trends Plant Sci* **9**: 578–582.
- Kramer EM, Bennett MJ. 2006. Auxin transport: A field in flux. *Trends Plant Sci* **11**: 382–386.
- Krupinski P, Jönsson H. 2010. Modeling auxin-regulated development. *Cold Spring Harb Perspect Biol* **2**: a001560.
- Kuhlemeier C. 2007. Phyllotaxis. *Trends Plant Sci* **12**: 143–150.
- Lee M, Choi Y, Burla B, Kim YY, Jeon B, Maeshima M, Yoo JY, Martinoia E, Lee Y. 2008. The ABC transporter AtABCB14 is a malate importer and modulates stomatal response to CO₂. *Nat Cell Biol* **10**: 1217–1223.
- Lewis DR, Miller ND, Splitt BL, Wu G, Spalding EP. 2007. Separating the roles of acropetal and basipetal auxin transport on gravitropism with mutations in two *Arabidopsis* multidrug resistance-like ABC transporter genes. *Plant Cell* **19**: 1838–1850.
- Li J, Yang H, Peer WA, Richter G, Blakeslee J, Bandyopadhyay A, Titapiwatanakun B, Undurraga S, Khodakovskaya M, Richards EL, et al. 2005. *Arabidopsis* H⁺-PPase AVP1

Auxin Transporters—Why So Many?

- regulates auxin-mediated organ development. *Science* **310**: 121–125.
- 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.
- 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**: 2175–2187.
- Marchant A, Bhalerao R, Casimiro I, Eklof J, Casero PJ, Bennett M, Sandberg G. 2002. AUX1 promotes lateral root formation by facilitating indole-3-acetic acid distribution between sink and source tissues in the *Arabidopsis* seedling. *Plant Cell* **14**: 589–597.
- Marchant A, Kargul J, May ST, Muller P, Delbarre A, Perrot-Rechenmann C, Bennett MJ. 1999. AUX1 regulates root gravitropism in *Arabidopsis* by facilitating auxin uptake within root apical tissues. *EMBO J* **18**: 2066–2073.
- Matsuyama A, Arai R, Yashiroda Y, Shirai A, Kamata A, Sekido S, Kobayashi Y, Hashimoto A, Hamamoto M, Hiraoka Y, et al. 2006. ORFeome cloning and global analysis of protein localization in the fission yeast *Schizosaccharomyces pombe*. *Nat Biotechnol* **24**: 841–847.
- Merks RMH, Van de Peer Y, Inzé D, Beemster GTS. 2007. Canalization without flux sensors: A traveling-wave hypothesis. *Trends Plant Sci* **12**: 384–390.
- Miller AJ, Fan X, Orsel M, Smith SJ, Wells DM. 2007. Nitrate transport and signalling. *J Exp Bot* **58**: 2297–2306.
- Mirza JI, Olsen GM, Iversen TH, Maher EP. 1984. The growth and gravitropic responses of wild-type and auxin/resistant mutants of *Arabidopsis-thaliana*. *Physiol Plant* **60**: 516–522.
- Mitchison GJ. 1980. A model for vein formation in higher plants. *Proc R Soc Lond B* **207**: 79–109.
- Mitchison GJ. 1981. The polar transport of auxin and vein patterns in plants. *Philos Trans R Soc Lond B* **295**: 461–471.
- Mravec J, Kubeš M, Bielach A, Gaykova V, Petrášek J, Skůpa P, Chand S, Benková E, Zažímalová E, Friml J. 2008. Interaction of PIN and PGP transport mechanisms in auxin distribution-dependent development. *Development* **135**: 3345–3354.
- Mravec J, Skůpa P, Bailly A, Hoyerová K, Křeček P, Bielach A, Petrášek J, Zhang J, Gaykova V, Stierhof Y-D, et al. 2009. Subcellular homeostasis of phytohormone auxin is mediated by the ER-localized PIN5 transporter. *Nature* **459**: 1136–1140.
- Müller A, Guan C, Gälweiler L, Tänzler P, Huijser P, Marchant A, Parry G, Bennett M, Wisman E, Palme K. 1998. AtPIN2 defines a locus of *Arabidopsis* for root gravitropism control. *EMBO J* **17**: 6903–6911.
- 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.
- Okada K, Ueda J, Komaki MK, Bell CJ, Shimura Y. 1991. Requirement of the auxin polar transport system in the early stages of *Arabidopsis* floral bud formation. *Plant Cell* **3**: 677–684.
- Paponov IA, Teale WD, Trebar M, Blilou I, Palme K. 2005. The PIN auxin efflux facilitators: Evolutionary and functional perspectives. *Trends Plant Sci* **10**: 170–177.
- Parry G, Delbarre A, Marchant A, Swarup R, Napier R, Perrot-Rechenmann C, Bennett MJ. 2001. Novel auxin transport inhibitors phenocopy the auxin influx carrier mutation aux1. *Plant J* **25**: 399–406.
- Peer WA, Murphy AS. 2007. Flavonoids and auxin transport: Modulators or regulators? *Trends Plant Sci* **12**: 556–563.
- 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.
- Petrášek J, Friml J. 2009. Auxin transport routes in plant development. 2009. *Development* **136**: 2675–2688.
- Petrášek J, Mravec J, Bouchard R, Blakeslee JJ, Abas M, Šeifertová D, Wisniewska J, Tadele Z, Kubeš M, Čovanová M, et al. 2006. PIN proteins perform a rate-limiting function in cellular auxin efflux. *Science* **312**: 914–918.
- Prusinkiewicz P, Rolland-Lagan AG. 2006. Modeling plant morphogenesis. *Curr Opin Plant Biol* **9**: 83–88.
- 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.
- Rausher M. 2006. The evolution of flavonoids and their genes. In *The Science of Flavonoids* (ed. Grotenwald E), pp. 175–212, Springer, Heidelberg.
- 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.
- Rensing SA, Lang D, Zimmer AD, Terry A, Salamov A, Shapiro H, Nishiyama T, Perroud PF, Lindquist EA, Kamisugi Y, et al. 2008. The *Physcomitrella* genome reveals evolutionary insights into the conquest of land by plants. *Science* **319**: 64–69.
- Robert S, Friml J. 2009. Auxin and other signals on the move in plants. *Nat Chem Biol* **5**: 325–332.
- Rojas-Pierce M, Titapiwatanakun B, Sohn EJ, Fang F, Larive CK, Blakeslee J, Cheng Y, Cutler SR, Peer WA, Murphy AS, Raikhel NV. 2007. *Arabidopsis* P-glycoprotein19 participates in the inhibition of gravitropism by gravacin. *Chem Biol* **14**: 1366–1376.
- Rolland-Lagan AG. 2008. Vein patterning in growing leaves: Axes and polarities. *Curr Opin Genet Dev* **18**: 348–353.
- Rubery PH, Sheldrake AR. 1974. Carrier-mediated auxin transport. *Planta* **188**: 101–121.
- Sachs T. 1969. Polarity and the induction of organized vascular tissues. *Ann Bot Lond* **33**: 263.
- Santelia D, Henrichs S, Vincenzetti V, Sauer M, Bigler L, Klein M, Bailly A, Lee Y, Friml J, Geisler M, Martinoia E. 2008. Flavonoids redirect PIN-mediated polar auxin fluxes during root gravitropic responses. *J Biol Chem* **283**: 31218–31226.
- Sauer M, Balla J, Luschnig C, Wisniewska J, Reinohl V, Friml J, Benkova E. 2006. Canalization of auxin flow by Aux/

E. Zažímalová et al.

- 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.
- Smith RS, Guyomarc'h S, Mendel T, Reinhardt D, Kuhlemeier C. 2006. A plausible model of phyllotaxis. *Proc Natl Acad Sci* **103**: 1301–1306.
- Sukumar P, Edwards KS, Rahman A, Delong A, Muday G. 2009. PINOID kinase regulates root gravitropism through modulation of PIN2-dependent basipetal auxin transport in *Arabidopsis*. *Plant Physiol* **150**: 722–735.
- Swarup K, Benková E, Swarup R, Casimiro I, Péret B, Yang Y, Parry G, Nielsen E, De Smet I, Vanneste S, et al. 2008. The auxin influx carrier LAX3 promotes lateral root emergence. *Nat Cell Biol* **10**: 946–954.
- 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, Kargul J, Marchant A, Zadik D, Rahman A, Mills R, Yemm A, May S, Williams L, Millner P, et al. 2004. Structure-function analysis of the presumptive *Arabidopsis* auxin permease AUX1. *Plant Cell* **16**: 3069–3083.
- Swarup R, Kramer EM, Perry P, Knox K, Leyser HMO, Haseloff J, Beemster GTS, Bhalerao R, Bennett MJ. 2005. Root gravitropism requires lateral root cap and epidermal cells for transport and response to a mobile auxin signal. *Nat Cell Biol* **7**: 1057–1065.
- 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.
- Terasaka K, Sato F, Yazaki K. 2004. Expression analyses of a P-glycoprotein-like (PGP) ABC protein gene, Atpgp4, in *Arabidopsis thaliana*. *Plant Cell Physiol* **45**: S176–S176.
- Terasaka K, Blakeslee JJ, Titapiwatanakun B, Peer WA, Bandyopadhyay A, Makam SN, Lee OR, Richards EL, Murphy AS, Sato F, Yazaki K. 2005. PGP4, an ATP binding cassette P-glycoprotein, catalyzes auxin transport in *Arabidopsis thaliana* roots. *Plant Cell* **17**: 2922–2939.
- Titapiwatanakun B, Murphy AS. 2009. Post-transcriptional regulation of auxin transport proteins: Cellular trafficking, protein phosphorylation, protein maturation, ubiquitination, and membrane composition. *J Exp Bot* **60**: 1093–1107.
- Titapiwatanakun B, Blakeslee JJ, Bandyopadhyay A, Yang H, Mravec J, Sauer M, Cheng Y, Adamec J, Nagashima A, Geisler M, et al. 2009. ABCB19/PGP19 stabilises PIN1 in membrane microdomains in *Arabidopsis*. *Plant J* **57**: 27–44.
- Tsurumi S, Ohwaki Y. 1978. Transport of ¹⁴C-labeled indoleacetic acid in *Vicia* root segments. *Plant Cell Physiol* **19**: 1195–1206.
- Vanneste S, Friml J. 2009. Auxin: A trigger for change in plant development. *Cell* **136**: 1005–1016.
- Vidal EA, Gutierrez RA. 2008. A systems view of nitrogen nutrient and metabolite responses in *Arabidopsis*. *Curr Opin Plant Biol* **11**: 521–529.
- Vieten A, Sauer M, Brewer PB, Friml J. 2007. Molecular and cellular aspects of auxin-transport-mediated development. *Trends Plant Sci* **12**: 160–168.
- 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.
- Verrier PJ, Bird D, Buria B, Dassa E, Forestier C, Geisler M, Klein M, Kolukisaoglu U, Lee Y, Martinoia E, et al. 2008. Plant ABC proteins—a unified nomenclature and updated inventory. *Trends Plant Sci* **13**: 151–159.
- Wenzel CL, Schuetz M, Yu Q, Mattsson J. 2007. Dynamics of MONOPTEROS and PIN-FORMED1 expression during leaf vein pattern formation in *Arabidopsis thaliana*. *Plant J* **49**: 387–98.
- White PJ, Broadley MR. 2003. Calcium in plants. *Ann Bot—London* **92**: 487–511.
- Wiśniewska J, Xu J, Seifertová D, Brewer PB, Růžicka 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 J, Hofhuis H, Heidstra R, Sauer M, Friml J, Scheres B. 2006. A molecular framework for plant regeneration. *Science* **311**: 385–388.
- Yamamoto M, Yamamoto KT. 1998. Differential effects of 1-naphthalene acetic acid, indole-3-acetic acid and 2,4-dichlorophenoxyacetic acid on the gravitropic response of roots in an auxin resistant mutant of *Arabidopsis*, Aux1. *Plant Cell Physiol* **39**: 660–664.
- Yang H, Murphy AS. 2009. Functional expression and characterization of *Arabidopsis* ABCB, AUX1 and PIN auxin transporters in *Schizosaccharomyces pombe*. *Plant J* **59**: 179–191.
- Young GB, Jack DL, Smith W, Saier MH Jr. 1999. The amino acid/auxin:Proton symport permease family. *Biochim Biophys Acta* **1415**: 306–322.
- Zažímalová E, Křeček P, Skůpa P, Hoyerová K, Petrášek J. 2007. Polar transport of the plant hormone auxin - the role of PIN-FORMED (PIN) proteins. *Cell Mol Life Sci* **64**: 1621–1637.
- Zourelidou M, Müller I, Willige BC, Nill C, Jikumaru Y, Li H, Schwechheimer C. 2009. The polarly localised D6 PROTEIN KINASE is required for efficient auxin transport in *Arabidopsis thaliana*. *Development* **136**: 627–636.

Cold Spring Harbor Perspectives in Biology

Auxin Transporters—Why So Many?

Eva Zazímalová, Angus S. Murphy, Haibing Yang, Klára Hoyerová and Petr Hosek

Cold Spring Harb Perspect Biol 2010; doi: 10.1101/cshperspect.a001552 originally published online December 16, 2009

Subject Collection [Auxin Signaling](#)

Fourteen Stations of Auxin

Jirí Friml

Computational Models of Auxin-Driven Patterning in Shoots

Mikolaj Cieslak, Andrew Owens and Przemyslaw Prusinkiewicz

Auxin Transporters—A Biochemical View

Ulrich Z. Hammes, Angus S. Murphy and Claus Schwechheimer

Structural Aspects of Auxin Signaling

Nicholas Morffy and Lucia C. Strader

The Story of Auxin-Binding Protein 1 (ABP1)

Richard Napier

Noncanonical Auxin Signaling

Heather Marie McLaughlin, Aaron Chun Hou Ang and Lars Østergaard

Casting the Net—Connecting Auxin Signaling to the Plant Genome

Yanfei Ma, Sebastian Wolf and Jan U. Lohmann

Auxin Plays Multiple Roles during Plant–Pathogen Interactions

Barbara N. Kunkel and Joshua M.B. Johnson

Auxin in Root Development

Suruchi Roychoudhry and Stefan Kepinski

Modeling Auxin Signaling in Roots: Auxin Computations

Jaap Rutten, Thea van den Berg and Kirsten ten Tusscher

The Systems and Synthetic Biology of Auxin

R. Clay Wright, Britney L. Moss and Jennifer L. Nemhauser

Auxin Does the SAMba: Auxin Signaling in the Shoot Apical Meristem

Markéta Pernisová and Teva Vernoux

Chemical Biology in Auxin Research

Ken-ichiro Hayashi

Uncovering How Auxin Optimizes Root Systems Architecture in Response to Environmental Stresses

Nicola Leftley, Jason Banda, Bipin Pandey, et al.

Auxin Interactions with Other Hormones in Plant Development

Serina M. Mazzoni-Putman, Javier Brumos, Chengsong Zhao, et al.

No Time for Transcription—Rapid Auxin Responses in Plants

Shiv Mani Dubey, Nelson B.C. Serre, Denisa Oulehlová, et al.

For additional articles in this collection, see <http://cshperspectives.cshlp.org/cgi/collection/>

**All Modifications and
Oligo Types Synthesized**
Long Oligos • Fluorescent • Chimeric • DNA • RNA • Antisense

Oligo Modifications?
Your wish is our command.

For additional articles in this collection, see <http://cshperspectives.cshlp.org/cgi/collection/>

**All Modifications and
Oligo Types Synthesized**

Oligo Modifications?
Your wish is our command.

Long Oligos • Fluorescent • Chimeric • DNA • RNA • Antisense