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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 preprogrammed 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 (Benjamins 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.

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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).

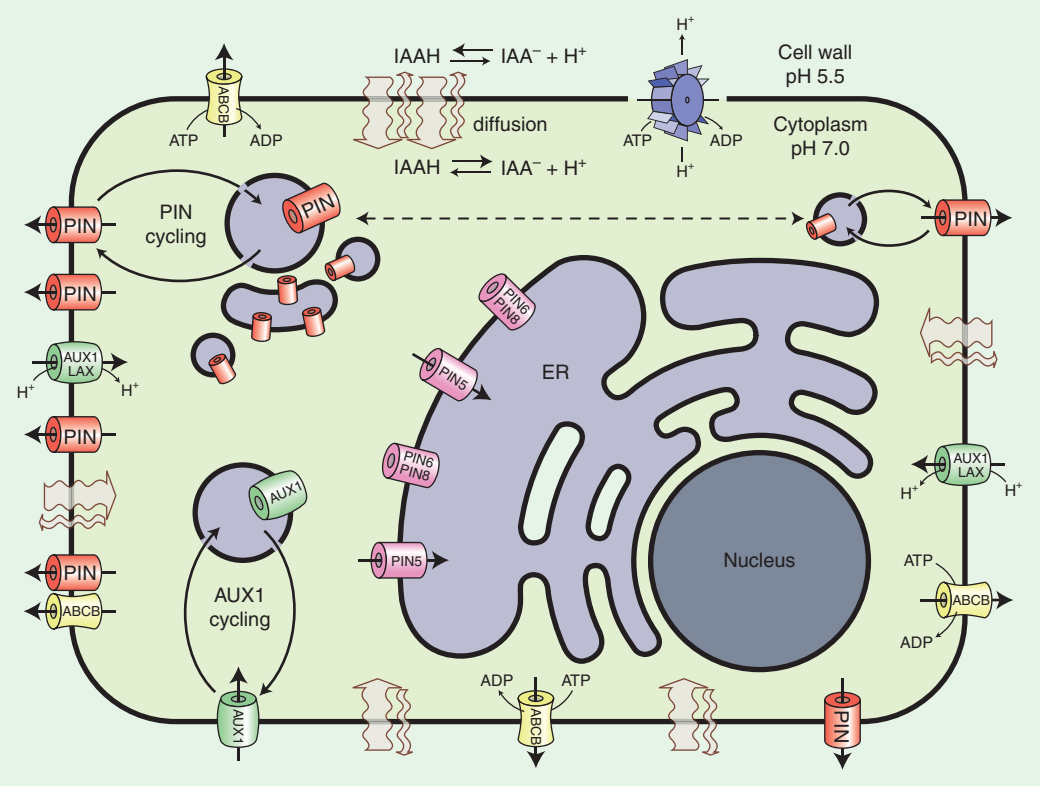

Figure 1. Cellular auxin transport. The scheme shows the organization of proteins involved in auxin transport. PIN efflux carrier proteins depicted in red represent "long" PINs (PIN1, 2, 3, 4, and 7), whereas PINs marked in pink represent "short" PINs (PIN5, 6, and 8). ER marks endoplasmic reticulum, 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 assymetries in adjacent cells can establish observed polar flows. This concept is the basis of the chemiosmotic polar diffusion model or chemiosmotic hypothesis (Rubery and Sheldrake 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 (Petrášek 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 *At*PIN 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

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 (Benjamins 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 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 (Rausher 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

bacterial and yeast PIN-like proteins and may represent ancestral plant PIN proteins. PIN5, 6, and 8, *Pp*PINA 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

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; Ditengou 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 PIN 3,4 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.

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 relocalize 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 relocalization 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.

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).

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