Auxin transport routes in plant development

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The differential distribution of the plant signaling molecule auxin is required for many aspects of plant development. Local auxin maxima and gradients arise as a result of local auxin metabolism and, predominantly, from directional cell-to-cell transport. In this primer, we discuss how the coordinated activity of several auxin influx and efflux systems, which transport auxin across the plasma membrane, mediates directional auxin flow. This activity crucially contributes to the correct setting of developmental cues in embryogenesis, organogenesis, vascular tissue formation and directional growth in response to environmental stimuli.

Introduction

The plant hormone auxin (the predominant form of which is indole-3-acetic acid; IAA) is a major coordinating signal in the regulation of plant development. Many aspects of auxin action depend on its differential distribution within plant tissues, where it forms local maxima or gradients between cells. Besides local biosynthesis and the release of active forms from inactive precursors, the major determinant of differential auxin distribution is its directional transport between cells. This regulated polar auxin transport (PAT) within plant tissues appears to be unique to auxin, as it has not been detected for any other signaling molecule. Molecular biology and genetics approaches in the model system *Arabidopsis thaliana* have contributed fundamentally to our understanding of the mechanisms of auxin transport. Currently, a large body of evidence supports the concept that intercellular auxin movement depends on several auxin-transporting mechanisms, which include both passive and active processes that transport auxin over long and short distances. Of these, the major mechanism for controlling auxin distribution during plant development appears to be the active directional cell-to-cell movement of auxin that is mediated by plasma membrane-based influx and efflux carriers (see Glossary, Box 1). Here, we summarize the present state of knowledge on how the various auxin transport mechanisms cooperate during plant development to fine-tune auxin distribution. We describe the basic pathways of auxin transport and discuss auxin transport routes during diverse developmental processes, such as embryogenesis, root and shoot organogenesis, vascular tissue formation and tropisms (see Glossary, Box 1).

Auxin transport systems in plants

In plants, auxin is generally transported by two distinct pathways. Throughout the plant, most IAA is probably transported away from the source tissues (young leaves and flowers) by an unregulated bulk flow in the mature phloem (see Glossary, Box 1). In addition, a slower, regulated, carrier-mediated cell-to-cell directional transport moves auxin in the vascular cambium from the shoot towards the root apex (Goldsmith, 1977), and also mediates short-range auxin movement in different tissues. These two pathways seem to be connected at the level of phloem loading in leaves (Marchant et al., 2002) and phloem unloading in roots (Swarup et al., 2001).

A series of classical physiological experiments (Box 2) predicted the existence of carrier-type auxin influx and efflux components that mediate PAT. The asymmetric cellular localization of these transporters has been proposed to determine the direction of auxin flow. During the past two decades, candidates for auxin carrier proteins and for the relevant regulatory mechanisms have been identified (Fig. 1). Heterologous expression experiments in cultured plant cells, yeast, *Xenopus laevis* oocytes and mammalian cells have demonstrated the auxin-transporting capacity of these carrier proteins (Vieten et al., 2007). Expression and localization studies of auxin carrier proteins, as well as specific defects in differential auxin distribution (Box 3) in plants that lack the function of these carriers, established that carrier-dependent PAT is absolutely required for the generation and maintenance of local auxin maxima and gradients.

Influx carriers

For auxin influx, the characterization of an agravitropic (see Glossary, Box 1) *auxin resistant 1* mutant (*aux1*) of *Arabidopsis* that shows resistance to an exogenous synthetic auxin, 2,4-D, led to the identification of the AUX1/LIKE AUX1 (AUX1/LAX) family of transmembrane proteins, which are similar to amino acid permeases, a group of proton-gradient-driven transporters (Bennett et al., 1996; Swarup et al., 2008). To date, four auxin influx carriers with specific functions have been described in *Arabidopsis*, and the functions of some homologs in other plants have also been studied (Table 1). Recently, AUX1 and LAX3 has been shown to mediate IAA uptake when heterologously expressed in *Xenopus* oocytes (Yang et al., 2006; Swarup et al., 2008), which provides biochemical evidence for their role as auxin influx carriers.

Efflux carriers

The investigation of several *Arabidopsis* mutants, namely of the allelic root mutants *agravitropic 1* (*agr1*), *wavy roots 6* (*wav6*) (Bell and Maher, 1990; Okada and Shimura, 1990) and *ethylene insensitive root 1* (*eir1*) (Roman et al., 1995), and the floral mutant *pin-formed1* (*pin1*) (Okada et al., 1991), resulted in the identification of auxin efflux carrier candidates. The root agravitropic phenotypes, as well as the *pin1* phenotype with defects in organ initiation and phyllotaxy (see Glossary, Box 1), can be phenocopied by the pharmacological inhibition of auxin efflux. Additionally, these mutants display decreased PAT in shoots and roots. The corresponding *PIN1* gene encodes a plant-specific protein with two transmembrane regions separated by a hydrophilic loop (Gälweiler et al., 1998). Concomitantly, the *agr1*, *wav6* and *eir1* mutants have been shown to be allelic with a mutant that carries a mutation in another PIN family member, *PIN2*. The *AGR1*, *WAV6*, *EIR1* and *PIN2* genes encode a homologous protein designated PIN2 (Chen et al., 1998; Luschnig

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Box 1. Glossary

Acropetal transport: Transport of various compounds (including auxin) towards the tip of a particular organ (stem or root).

Agravitropic: Having defects in response to gravity. This defect might be a result of a specific mutation. Horizontally placed agravitropic roots are unable to grow downwards, agravitropic stems are unable to grow upwards.

Anticlinal division: Cell division in a layer of cells that occurs perpendicular to the plane of the cell layer.

Apical: 'Upper' side of the cell, facing the shoot apical meristem.

Auxin influx and efflux carriers: Integral plasma membrane proteins that transport auxin molecules into and out of the cell, respectively.

Basal: 'Lower' side of the cell, facing the root tip.

Basipetal transport: Transport of various compounds (including auxin) from the tip towards the basis of the particular organ (stem or root).

Columella: Group of cells in the central root cap, which contain plastids with starch; the site of root gravity perception.

Cotyledons: Embryonic leaves formed during embryonic development. In dicotyledon plants, cotyledons are typically positioned symmetrically.

Flavonoids: Plant secondary polyphenolic metabolites. Besides playing a role in defense responses to environmental impact, they have been shown to modulate auxin transport by their preferential effect on ABCB auxin transporters.

Hypophysis: The most apical cell of the suspensor, which forms the attachment between the suspensor and the developing embryo. It gives rise to the embryonic root of a plant, the radicle, which develops into the primary root.

Periclinal division: Cell division in a layer of cells that occurs parallel to the plane of the cell layer.

Phloem: Part of the vasculature that transports metabolites from the source tissues (leaves) to other tissues.

Phyllotaxy: The typically regular arrangement of leaves or floral organs, which initiates at the shoot apical meristem.

Primary root: The first root that develops from the embryonic root of a plant embryo, the radicle.

Stele: The central part of the root or stem that contains the vascular tissue.

Suspensor: Single cell file formed from the zygote daughter basal cell by transverse divisions. This cell file connects the embryo with mother tissues and later degenerates.

Tropisms: Directional plant growth responses to various environmental stimuli, such as light (phototropism) or gravity (gravitropism). The response always depends on the direction of the stimulus and could therefore be positive or negative (towards or away from the stimulus, respectively).

Vasculature: Complex conductive tissue that consists of specialized cells that transport water and nutrients from roots (xylem), cells that transport products of photosynthesis and other metabolites from source tissue (phloem) and several other cell types that form supporting tissues. In both xylem and phloem, various plant hormones have been detected.

Box 2. Physiology-based models of auxin transport across the plasma membrane

A model for the mechanism that underlies the directionality of cell-to-cell auxin transport was proposed simultaneously by Rubery and Sheldrake (Rubery and Sheldrake, 1974) and Raven (Raven, 1975), and is known as the chemiosmotic polar diffusion model (Goldsmith, 1977). According to this model, an undissociated lipophilic form of the native auxin molecule (IAA) can easily enter the cell cytoplasm from a slightly acidic extracellular environment (pH 5.5) by passive diffusion. As the pH of the cytoplasm is more alkaline (pH 7) than the extracellular environment, a difference that is maintained by the plasma membrane-located H^+-ATPase, more IAA molecules dissociate after entering the cells, and the resulting hydrophilic auxin anions (IAA^-) are trapped in the cytosol. The exit of IAA^- was therefore proposed to be assisted by active auxin anion efflux carriers that constitute the limiting step and the major control units of auxin transport. The directionality of auxin transport was postulated to be attributable to the asymmetric distribution of such carriers at a particular side of the cell (Goldsmith, 1977), which would steer auxin flow in the direction of the predominant localization of the transporters. Early experiments with suspension-cultured crown gall cells of *Parthenocissus tricuspidata* (Rubery and Sheldrake, 1974) also suggested the existence of active auxin anion uptake carriers that probably act as $2H^+$ cotransporters.

PIN homologs in other plants have also been identified (Zažímalová et al., 2007), and some of them have been functionally characterized (Table 1).

Other proteins that play a role in auxin efflux are plant orthologs of the mammalian ATP-binding cassette subfamily B (ABCB)-type transporters of the multidrug resistance/phosphoglycoprotein (ABCB/MDR/PGP) protein family (Noh et al., 2001; Verrier et al., 2008). Some of these (ABCB1, ABCB4 and ABCB19) have been identified as proteins with binding affinity to the auxin transport inhibitor 1-naphthylphthalamic acid (NPA) (Murphy et al., 2002; Noh et al., 2001). The biochemical evidence for these ABCB proteins having a role in auxin transport has been provided by heterologously expressing them in tobacco cells, HeLa cells and yeast (Geisler et al., 2005; Petrášek et al., 2006; Santelia et al., 2005; Terasaka et al., 2005). The importance of the ABCB proteins for auxin transport-related development has been also documented in other higher plants (Table 1).

Recently, a system for comparative analyses of transport activities and the structure of all three groups of auxin transporters (AUX1/LAX, PIN and ABCB) has been established in *Schizosaccharomyces pombe* (Yang and Murphy, 2009). It represents a valuable tool for testing the cooperation between these transporters, as well as with other regulatory proteins.

Other auxin transporter candidates exist, for example the members of a group of aromatic and neutral amino acid transporters in *Arabidopsis* (Chen et al., 2001) or the transmembrane protein TM20 in maize (*Zea mays*) (Jahrmann et al., 2005). However, their contribution to the intercellular transport of auxin is still unclear.

Auxin transport regulation

Various aspects of plant development are mediated by transport-dependent differential auxin distribution within tissues. Conceptually, multiple signals can be integrated to modulate auxin-dependent development, which highlights the importance of regulating each auxin-transporting system individually. Auxin itself seems to be one of the most important regulators of its own transport. Earlier physiological observations on the role of auxin in
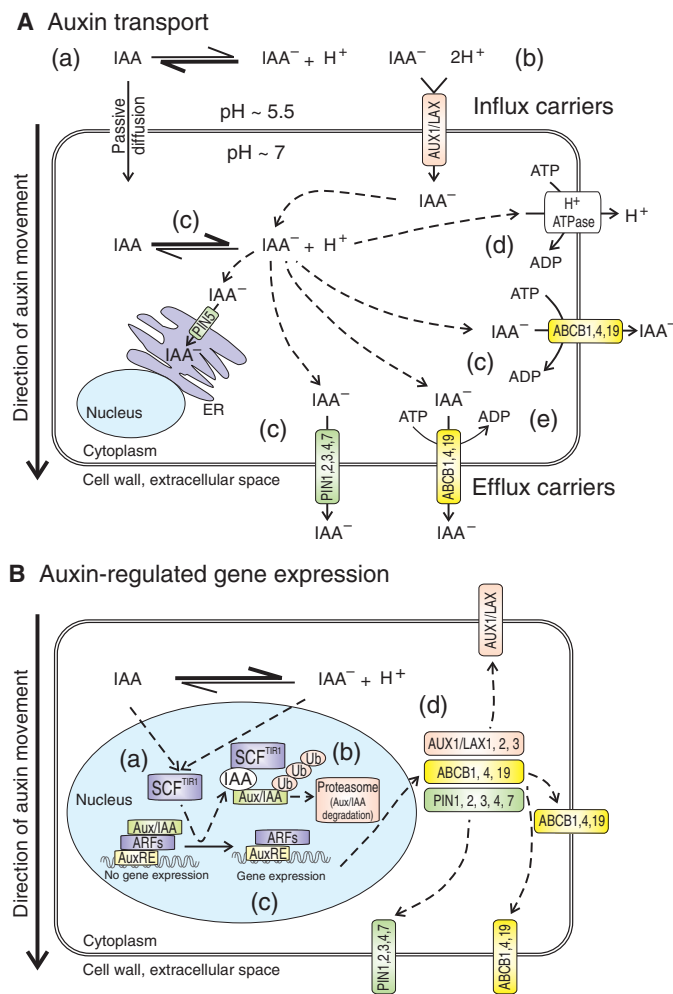
et al., 1998; Müller et al., 1998; Utsuno et al., 1998). Until now, eight members of the PIN protein family have been isolated in *Arabidopsis* and are commonly referred to as PIN1 to PIN8 (Vieten et al., 2007; Zažímalová et al., 2007). A subgroup comprising PIN5, PIN6 and PIN8 has a reduced middle hydrophilic loop and presumably regulates the auxin exchange between the endoplasmic reticulum and the cytosol (Mravec et al., 2009). The PIN1, PIN2, PIN3, PIN4 and PIN7 proteins, by contrast, are localized at the plasma membrane, where they act as auxin efflux carriers (Mravec et al., 2008; Petrášek et al., 2006).

A Auxin transport

B Auxin-regulated gene expression

Fig. 1. Auxin transport across the plasma membrane and auxin-regulated gene expression. (**A**) Schematic depiction of auxin transport across the plasma membrane. Both passive diffusion and specific auxin influx and efflux carriers are involved in the transport of auxin (IAA) across the plasma membrane. Undissociated IAA molecules enter cells by passive diffusion (a), whereas the less lipophilic, and therefore less permeable, dissociated auxin anions (IAA⁻) are transported inside via auxin influx $2H^+$ cotransporters of the AUX1/LAX family (b). In the more basic intracellular environment (c), IAA dissociates and requires active transport through the PIN or ABCB efflux transporter proteins to exit the cell. Some cytosolic IAA is transported by PIN5 into the lumen of the endoplasmic reticulum (ER). This compartmentalization presumably serves to regulate auxin metabolism (Mravec et al., 2009). Whereas PIN transporter activity is supposed to use a H^+ gradient that is maintained by the action of the plasma membrane H^+-ATPase (d), and possibly also the vacuolar H^+ pyrophosphatase (Li et al., 2005), ABCB transporters have ATPase activity (e). (**B**) Schematic depiction of auxin-regulated gene expression. Intracellular auxin binds to its nuclear receptor from the TRANSPORT INHIBITOR RESPONSE 1/AUXIN SIGNALING F-BOX (TIR1/AFB) family of F-box proteins, which are subunits of the SCF E3-ligase protein complex (a). This leads to the ubiquitylation and the proteasome-mediated specific degradation of auxin Aux/IAA transcriptional repressors (b). Subsequently, the auxin response factors (ARFs) are derepressed and activate auxin-inducible gene expression (c) (Dharmasiri et al., 2005; Kepinski and Leyser, 2005). Among other auxin-responsive genes, all known auxin transporters are regulated by this feedback mechanism (d). Ub, ubiquitin.

the formation and regeneration of vascular tissues led to the formulation of the canalization hypothesis, which postulates that auxin acts to polarize its own transport (Sachs, 1981). This theory proposes that the initial diffusion of auxin away from a source positively reinforces its own transport, which ultimately leads to the distribution of auxin into narrow canals, and that this canalization is an important part of the mechanism that underlies coordinated tissue polarization.

In general, the carrier-mediated transport of auxin can be regulated at three levels: by the regulation of (1) the abundance of a carrier (by regulating its transcription, translation and degradation); (2) subcellular trafficking and targeting of auxin carriers to a specific position on the plasma membrane; and (3) transport activity (e.g. through the post-translational modification of carriers, the levels and activity of endogenous inhibitors, the regulation of the plasma membrane pH gradient, the composition of the plasma membrane and the interactions among individual transporters or transport systems).

Indeed, the transcription of all known carrier proteins (PIN, ABCB and AUX1/LAX) is influenced by auxin triggering a signaling cascade that involves the F-box protein TRANSPORT INHIBITOR RESPONSE 1 (TIR1) auxin receptor (Fig. 1) (Geisler et al., 2005; Noh et al., 2001; Terasaka et al., 2005; Vanneste et al., 2005; Vieten et al., 2005). Variable timing of the transcriptional response, as well as its modulation by the developmental context, has been reported. In the case of the PIN proteins, the auxin-dependent regulation of transcription might play an important role in the extensive functional redundancy within the PIN family, which becomes apparent in the specific upregulation of other PINs in the expression domain of a PIN gene that is affected by a mutation (Blilou et al., 2005; Vieten et al., 2005). Other plant hormones, such as ethylene or cytokinins, might also modulate the expression of PIN and AUX1 proteins (Dello Ioio et al., 2008; Pernisová et al., 2009; Růžička et al., 2007; Růžička et al., 2009).

In addition, the abundance of some PIN proteins is further controlled by degradation via the vacuolar targeting pathway (Kleine-Vehn et al., 2008b; Laxmi et al., 2008), which requires proteasome-mediated steps (Abas et al., 2006) and is regulated by the MODULATOR OF PIN (MOP) proteins (Malenica et al., 2007).

Transport can also be controlled by the incidence of transporters at the plasma membrane (Box 4). This mode of regulation has been demonstrated for some PIN proteins that undergo constitutive internalization and recycling back to the cell surface (Dhonukshe et al., 2007; Geldner et al., 2001). It is probably important for the establishment of (Dhonukshe et al., 2008b), and for dynamic changes in (Kleine-Vehn et al., 2008a), PIN subcellular localization. Importantly, auxin inhibits PIN internalization by an unknown mechanism, thus increasing the amount and the activity of PIN proteins at the cell surface (Paciorek et al., 2005). This constitutes another, possibly non-transcriptional, mechanism for the feedback regulation of auxin transport.
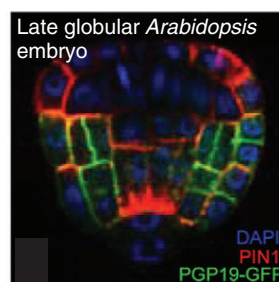
The regulation of PIN subcellular targeting is an effective way to modulate auxin distribution because, consistent with classical predictions (Box 2), the polar subcellular localization of the PIN auxin efflux carriers has been shown to be important for the directionality of auxin fluxes (Wiśniewska et al., 2006). Little is known about the mechanisms that control cell polarity in plants; nonetheless, the phosphorylation of PIN is important for decisions on PIN polar targeting. Analyses of *Arabidopsis* mutants that have phenotypes typical for altered auxin transport, namely *roots curl in NPA 1* (*rcn1*) and *pinoid* (*pid*), have led to the identification of the

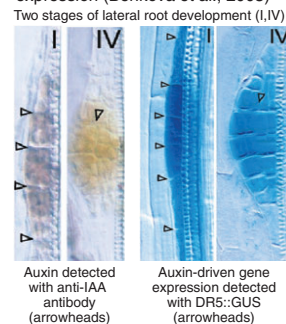## Box 3. Tracking auxin distribution and transport in plants

A GC-MS (gas chromatography-mass spectrometry) determination of endogenous auxin (IAA) levels in *Arabidopsis* leaf (Ljung et al., 2001)
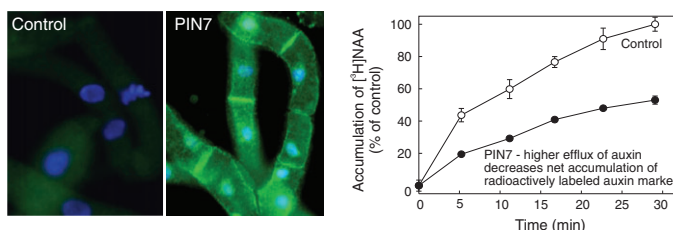
Petiole

15 mm

Tip

IAA (pg mg⁻¹ FW)
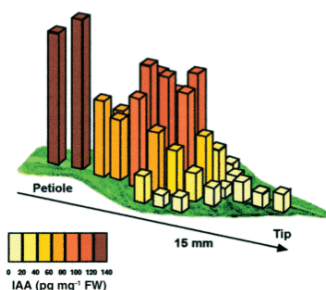
B Vizualization of auxin carriers using antibodies or GFP-tagged fusion proteins (Mravec et al., 2008)

Late globular *Arabidopsis* embryo

DAPI
PIN1
PGP19-GFP

C Vizualization of auxin using antibodies and auxin-inducible gene expression (Benková et al., 2003)
Two stages of lateral root development (I,IV)

I   IV   I   IV

Auxin detected with anti-IAA antibody (arrowheads)

Auxin-driven gene expression detected with DR5::GUS (arrowheads)

D Auxin transport assay in a simplified cell culture model. Tobacco cells expressing the PIN7 auxin efflux carrier from *Arabidopsis* (Petrášek et al., 2006)

Control   PIN7

Control

PIN7 - higher efflux of auxin decreases net accumulation of radioactively labeled auxin marker

Accumulation of [³H]NAA (% of control)

Time (min)

Despite the fact that auxin (indole-3-acetic acid; IAA) distribution plays an important morphoregulatory role in plants, scientists still have no direct method for tracking it in vivo at the cellular level and, instead, have to rely on a set of more or less indirect techniques. For directly measuring the endogenous IAA content, even in very small samples of plant tissue, gas chromatography-mass spectrometry (GC-MS) is the most frequently employed method (panel A) (Ljung et al., 2005), but this technique lacks cellular resolution. To track auxin distribution at the cellular level, antibodies against auxin carriers (panel B) or IAA (panel C) are used (Benková et al., 2003; Friml et al., 2003a). However, immunohistochemical staining procedures often suffer from technical problems connected with the fixation of the rather diffusive IAA molecules, as well as with the specificity of anti-IAA antibodies. Therefore, for noninvasive in vivo tracking of auxin activity, synthetic promoters based on auxin-inducible genes are employed (panel C) (Ulmasov et al., 1997). These consist of multiple TGTCTC repeats of the auxin-responsive element (designated DR5 or DR5rev in reverse orientation) and can be coupled to markers, such as *Escherichia coli* β-D-glucuronidase (GUS) (Sabatini et al., 1999), endoplasmic reticulum-localized *Aequorea victoria* green fluorescent protein (GFP) (Friml et al., 2003b), and a nucleus-localized version of GFP or the modified yellow fluorescent protein (YFP) version VENUS-N7 (Heisler et al., 2005), to track their activity in plant tissues. Auxin-responsive reporter constructs are widely used to get a preliminary impression of the distribution of auxin activity, but their efficiency is limited by their dependence on a comparable availability of the auxin signaling machinery in all cells, nonlinear signal output, a relatively narrow concentration range for detection, the time requirements of the transcription and protein folding process, as well as the stability of the reporter molecules. For measurements of auxin flow in plants, microscale assays with radiolabeled IAA have been successfully adapted for *Arabidopsis* stem and root segments, and even for whole seedlings (Lewis and Muday, 2009; Murphy et al., 2000). More detailed information on the kinetic parameters of auxin transporters can be obtained with the same technique in plant suspension cultures (panel D) (Delbarre et al., 1996; Petrášek et al., 2006). An alternative, but yet not well established, approach for measuring the actual flow of IAA at the tissue level utilizes vibrating IAA-selective microelectrodes (Mancuso et al., 2005), which offer the advantage of noninvasive and continual recording of auxin flow. Images are reproduced, with permission, from (A) Ljung et al. (Ljung et al., 2001), (B) Mravec et al. (Mravec et al., 2008), (C) Benková et al. (Benková et al., 2003) and (D) Petrášek et al. (Petrášek et al., 2006).

regulatory subunit of protein phosphatase 2A (PP2A) (Deruére et al., 1999) and the serine/threonine protein kinase PID (Christensen et al., 2000) as factors that are important for PIN targeting. The current model is that PID phosphorylates PIN proteins, thus supporting their apical targeting, and that PP2A antagonizes this action, thus promoting basal PIN delivery (Friml et al., 2004; Michniewicz et al., 2007). Moreover, the *Arabidopsis* 3-PHOSPHOINOSITIDE-DEPENDENT PROTEIN KINASE 1 (PDK1) has been shown to stimulate the activity of PID kinase, which provides evidence for a role of upstream phospholipid signaling in the control of auxin transport (Zegzouti et al., 2006). Similarly, the transcription factor INDEHISCENT (IND) regulates PID expression, thus mediating auxin distribution-dependent fruit development (Sorefan et al., 2009). Conceptually, one can imagine that any signaling pathway upstream of PID/PP2A has the capacity to modulate the transport-dependent distribution of auxin by changing the balance between phosphorylation and dephosphorylation. Interestingly, auxin itself regulates *PID* expression (Benjamins et al., 2001) and PIN polarity through TIR1-mediated signaling (Sauer et al., 2006).

The composition of the plasma membrane provides the appropriate environment for protein-protein interactions and can thereby determine how effective the auxin flux across the membrane will be. Indeed, the sterol composition of membranes, which depends on the activity of the enzymes STEROL METHYL TRANFERASE 1 (SMT1) and CYCLOPROPYL ISOMERASE 1 (CPI1) has been shown to be crucial for the positioning of certain PIN proteins in the plasma membrane (Men et al., 2008; Willemsen et al., 2003). Plasma membrane composition is also important for the localization of ABCB19, which has been found to be present in

**Table 1. Selected auxin carriers with established developmental roles**

Gene	Role in development	Key references
Auxin influx carrier		
AtAUX1	Root gravitropism, lateral root formation, phloem loading in leaves and unloading in roots, root hair development, phyllotaxis, hypocotyl phototropism	Bainbridge et al., 2008; Bennett et al., 1996; Jones et al., 2009; Marchant et al., 2002; Stone et al., 2008; Swarup et al., 2001
AtLAX1	Phyllotaxis	Bainbridge et al., 2008
AtLAX2	Phyllotaxis	Bainbridge et al., 2008
AtLAX3	Phyllotaxis, lateral roots emergence	Bainbridge et al., 2008; Swarup et al., 2008
PttLAX1-3	Vascular cambium development in wood-forming tissues	Schrader et al., 2003
PaLAX	Root gravitropism	Hoyerová et al., 2008
MtLAX1-5	Early nodule development	de Billy et al., 2001; Schnabel and Frugoli, 2004
CsAUX1	Root gravitropism	Kamada et al., 2003
LaAUX1	Etiolated hypocotyl growth	Oliveros-Valenzuela et al., 2007
CgAUX1, CgLAX3	Actinorhizal nodule formation after *Frankia* infection	Peret et al., 2007
ZmAUX1	Root development	Hochholdinger et al., 2000
Auxin efflux carrier		
AtPIN1	Vascular development, phyllotaxis, vein formation, embryogenesis, lateral organ formation	Benková et al., 2003; Gälweiler et al., 1998; Reinhardt et al., 2003; Scarpella et al., 2006; Weijers et al., 2005
AtPIN2 (EIR1, AGR1, WAV6)	Root gravitropism, lateral organ development	Benková et al., 2003; Chen et al., 1998; Luschnig et al., 1998; Müller et al., 1998; Utsuno et al., 1998
AtPIN3	Shoot and root gravitropism and phototropism, lateral organ development	Benková et al., 2003; Friml et al., 2002b
AtPIN4	Embryogenesis, root patterning	Benková et al., 2003; Friml et al., 2002a; Friml et al., 2003b; Weijers et al., 2005
AtPIN5	Regulation of the intracellular auxin homeostasis and metabolism	Mravec et al., 2009
AtPIN6	Transport activity demonstrated in tobacco cells, in planta function unknown	Benková et al., 2003; Petrášek et al., 2006
AtPIN7	Embryogenesis, root development	Benková et al., 2003; Friml et al., 2003b; Blilou et al., 2005
PttPIN1-3	Vascular cambium development in wood-forming tissues	Schrader et al., 2003
CsPIN1	Gravitropism	Kamada et al., 2003
LaPIN1,3	Etiolated hypocotyl growth	Oliveros-Valenzuela et al., 2007
ZmPIN1	Inflorescence branching	Carraro et al., 2006
BjPIN1-3	Differential expression in various tissues, vascular development	Ni et al., 2002a; Ni et al., 2002b
OsPIN1	Adventitious root emergence	Xu et al., 2005
AtPGP1 (ABCB1)	Embryogenesis, lateral root organogenesis, hypocotyl and plant growth	Geisler et al., 2005; Lin and Wang, 2005; Mravec et al., 2008; Noh et al., 2001
ZmPGP1 (br2; brachytic) SbPGP1 (dw3; dwarf)	Elongation growth	Multani et al., 2003
AtABCB19 (MDR1, MDR11, PGP19)	Embryogenesis, lateral root formation, root gravitropism, hypocotyl phototropism and gravitropism, leaf shape	Lewis et al., 2007; Mravec et al., 2008; Nagashima et al., 2008a; Nagashima et al., 2008b; Noh et al., 2001; Petrášek et al., 2006; Wu et al., 2007
AtABCB4 (MDR4, PGP4)	Basipetal transport in root epidermis, lateral root and root hair development, gravitropism	Cho et al., 2007; Lewis et al., 2007; Santelia et al., 2005; Terasaka et al., 2005; Wu et al., 2007
ZmTM20	Vasculature development	Jahrmann et al., 2005; Stiefel et al., 1999

At, Arabidopsis thaliana; Bj, Brassica juncea; Cg, Casuarina glauca; Cs, Cucumis sativus; La, Lupinus albus; Mt, Medicago truncatula; Os, Oryza sativa; Pa, Prunus avium; Ptt, Populus tremula x tremuloides; Sb, Sorghum bicolor; Zm, Zea mays.

the detergent-resistant microsomal protein fractions of *Arabidopsis* seedling tissue lysates (Titapiwatanakun et al., 2009). Such sterol- and sphingolipid-rich plasma membrane microdomains presumably constitute important specialized sites at which ABCB19 and PIN1 might interact physically (Blakeslee et al., 2007). Moreover, ABCB19 stabilizes PIN1 in these domains, and presumably influences the rate of PIN1 endocytosis and thus its incidence at the plasma membrane (Titapiwatanakun et al., 2009) (Box 4).

Box 4. Intracellular trafficking of auxin transporters
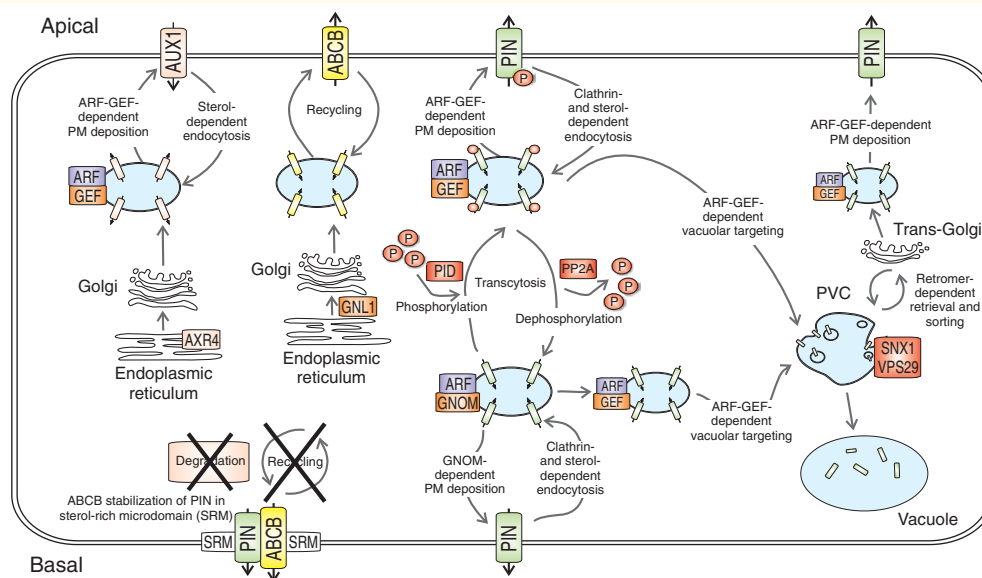
The developmentally regulated formation of auxin gradients depends largely on the fine-tuning of auxin flow polarity by means of the differential subcellular trafficking and targeting of the AUX1/LAX, PIN and ABCB auxin transporters. As all of these transporters are integral plasma membrane (PM) proteins, they are distributed by the general mechanisms of vesicle trafficking. All auxin transporters have been shown to be constitutively recycled between the plasma membrane and endosomal compartments (shown in blue). The endocytosis step of PIN1 and PIN2 recycling depends on clathrin (Dhonukshe et al., 2007) and on the sterol composition of the plasma membrane (Men et al., 2008; Willemsen et al., 2003), which also influences AUX1 trafficking (Kleine-Vehn et al., 2006). It is also crucial for the interaction between ABCB19 and PIN1 proteins (Titapiwatanakun et al., 2009). This interaction seems to be important for the stabilization of PIN1 at the plasma membrane sterol-rich microdomain (SRM), with the subsequent enhancement of its auxin transporting activity. Compared with ABCB1, ABCB19 is a rather stable plasma membrane protein, and its trafficking requires the activity of the GNOM-LIKE 1 (GNL1) guanine nucleotide exchange factor for ADP-ribosylation factors (ARF-GEF) (Titapiwatanakun et al., 2009). PIN1 targeting to the basal plasma membrane is regulated by GNOM, another ARF-GEF (Geldner et al., 2003), whereas one or more additional ARF-GEFs mediate PIN targeting to the apical plasma membranes (Kleine-Vehn et al., 2008a). The apical localization of AUX1 is maintained by the activity of another ARF-GEF and is assisted by the endoplasmic reticulum accessory protein AXR4 (Dharmasiri et al., 2006). ARF-GEF-dependent endosomal sorting is also involved in the trafficking of PIN2 to the lytic vacuolar pathway through the prevacuolar compartment (PVC), from which PIN proteins might be retrieved again into the trans-Golgi network through the assistance of the retromer complex subunits SORTING NEXIN 1 (SNX1) and VACUOLAR PROTEIN SORTING 29 (VPS29) (Jaillais et al., 2006; Kleine-Vehn et al., 2008b). The ubiquitylation of PIN2 potentially plays a role in the subcellular trafficking of PIN2 and further regulates the amount of PIN2 at the plasma membrane (Abas et al., 2006). Additionally, PIN proteins are targets of phosphorylation by PINOID (PID) kinase and of dephosphorylation by protein phosphatase 2A (PP2A) (Michniewicz et al., 2007); their phosphorylation state might be crucial for determining PIN recruitment into the apical or basal targeting pathways.

Little is known about the mechanisms that might regulate the activity of auxin transporters directly. It is possible that an additional phosphorylation of PIN, distinct from PID-dependent action and mediated by D6 protein kinases, controls PIN auxin efflux activity (Zourelidou et al., 2009). Alternatively, PIN auxin transport activity might be regulated by chemical inhibitors. These exogenous compounds, which have been known for decades, have been valuable tools in physiological studies on auxin transport and include a well-known inhibitor of auxin efflux, NPA (Rubery, 1990), as well as a well-known inhibitor of auxin influx, 1-naphthoxyacetic acid (1-NOA) (Parry et al., 2001). Detailed knowledge about the mechanisms by which NPA and similar compounds inhibit auxin efflux is still lacking. NPA has a high affinity for binding ABCB-type auxin carriers, but low-affinity binding sites have also been found (Murphy et al., 2002). This low-affinity binding might be related to the more general inhibitory effects of some efflux inhibitors on actin cytoskeleton dynamics and PIN trafficking processes (Dhonukshe et al., 2008a; Geldner et al., 2001). A group of naturally occurring substances that might act analogously to auxin transport inhibitors are

the flavonoids (see Glossary, Box 1), endogenous polyphenolic compounds that modulate auxin transport and tropic responses (Murphy et al., 2000; Santelia et al., 2008). Both NPA and flavonoids regulate the activity of ABCB1 and ABCB19 (Bailly et al., 2008; Geisler et al., 2003; Murphy et al., 2002; Noh et al., 2001; Rojas-Pierce et al., 2007), possibly through influencing interaction with the peripheral plasma membrane protein TWISTED DWARF 1 (TWD1) (Geisler et al., 2003; Bailly et al., 2008).

The above-mentioned examples only constitute glimpses into how the auxin distribution network might be regulated at different levels. Nonetheless, they demonstrate the potential for various internal and external signals to influence the throughput and the direction of intercellular auxin fluxes, and thus to regulate auxin-dependent development.

Auxin transport routes during embryogenesis

Auxin and auxin transport is already important at the earliest stages of plant development. The analysis of *Arabidopsis* mutants, combined with the visualization of the auxin response by means
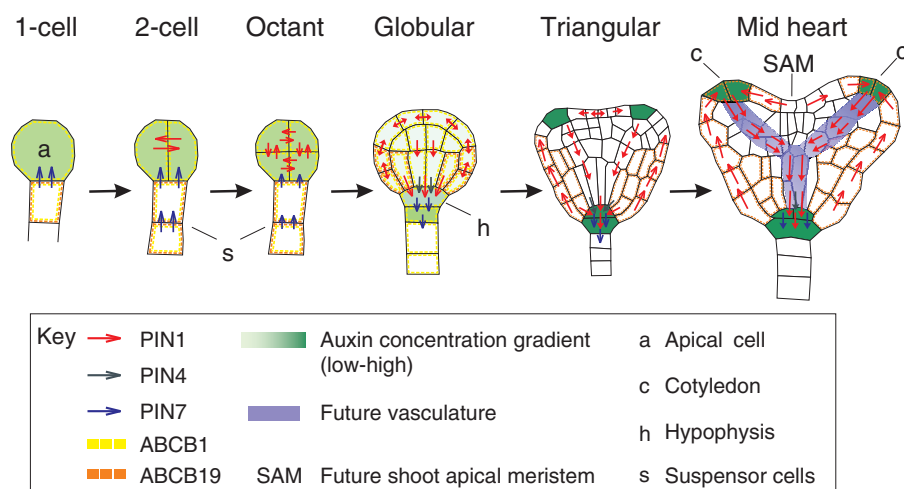
Fig. 2. Auxin gradients and auxin transporters during embryogenesis. Schematic depiction of the auxin distribution and the localization of auxin transporters during early plant embryonic development. Auxin distribution (depicted as a green gradient) has been inferred from DR5 activity and IAA immunolocalization (Benková et al., 2003; Friml et al., 2002a; Friml et al., 2003a). The localization of the efflux transporters PIN1, PIN4 and PIN7, as well as that of ABCB1 and ABCB19, is based on immunolocalization studies and on in vivo observations of green fluorescent protein (GFP)-tagged proteins (Dhonukshe et al., 2008b; Friml et al., 2003b; Mravec et al., 2008). Arrows indicate auxin flow mediated by a particular transporter; dotted lines indicate the cell type-specific localization of particular auxin transporters with no obvious polarity. PIN7, localized at the apical sides of the suspensor cells (s), transports auxin towards the apical cell (a) that forms the pro-embryo; there, PIN1, which is localized at all inner cell sides, distributes auxin homogenously. ABCB1 and ABCB19 cooperate during this initial stage and are localized apolarly in all cells or only in the uppermost suspensor cell, respectively. The crucial moment in the setting of the basal end of the apical-basal embryonic axis occurs during the early globular stage, when PIN1 starts to be localized basally in the pro-embryonal cells, and PIN7 is simultaneously shifted from the apical to the basal plasma membrane of suspensor cells. These PIN polarity rearrangements reverse the auxin flow downwards and, with the aid of PIN4, lead to auxin accumulation in the forming hypophysis (h) (see Glossary, Box 1). At this stage, ABCB19 helps to maintain the auxin distribution in the outer layers of the embryo. In triangular- and heart-stage embryos, bilateral symmetry is established through auxin maxima at the incipient cotyledon (c) primordia. These auxin maxima are generated by PIN1 activity in the epidermis; in the inner cells of cotyledon primordia, however, PIN1 mediates basipetal auxin transport towards the root pole. SAM, future shoot apical meristem.

of auxin-inducible promoters, demonstrated that differential auxin distribution mediates important steps during embryogenesis, such as apical-basal axis specification and embryonic leaf formation. The concerted action of PIN1, PIN4 and PIN7 efflux carriers (Friml et al., 2002a; Friml et al., 2003b) is required for the differential auxin distribution in embryogenesis (Fig. 2). Individual PIN proteins act redundantly, given that single pin mutants can still complete embryogenesis, whereas *pin1 pin3 pin4 pin7* quadruple mutants are strongly defective in the overall establishment of apical-basal polarity (Benková et al., 2003; Friml et al., 2003b). In contrast to pin mutants, mutants in other auxin transport components, such as the *abcb* and *aux1/lax* mutants, are not defective in embryogenesis, which suggests a major role for PIN-dependent auxin transport in patterning the embryo.

Soon after the first anticlinal division (see Glossary, Box 1) of a fertilized zygote, increased auxin accumulation can be detected in the apical cell by the activity of the auxin-inducible element DR5 or by IAA immunolocalization (Box 3). This differential distribution results from the activity of PIN7 that is localized apically in the adjacent suspensor cells. At this stage, PIN1 presumably mediates the uniform distribution of auxin between cells of the forming pro-embryo (Fig. 2, Box 4). Both ABCB1 and ABCB19 contribute to auxin transport during the early stages of pro-embryo formation (Mravec et al., 2008). ABCB1 is localized to all suspensor cells (see Glossary, Box 1) and pro-embryonal cells, and ABCB19 localization is restricted to the suspensor-forming cells. Both proteins are localized without obvious polarity. Later, during the

early globular stage, PIN1 gradually relocalizes to the bottom plasma membranes of the embryo cells that face the uppermost suspensor cell, the hypophysis (Kleine-Vehn et al., 2008a). Simultaneously, the polarity of PIN7 shifts from apical to basal in the suspensor cells (Fig. 2, Box 4). These coordinated PIN polarity rearrangements, which are later also supported by the action of PIN4, lead to an apical-to-basal flow of auxin and to auxin accumulation in the hypophysis. At this stage, the auxin distribution and response are crucial for the specification of the hypophysis as the precursor of the root meristem. Accordingly, mutants of the auxin-binding F-box proteins TIR1 and AFB (Dharmasiri et al., 2005), and of the downstream transcriptional regulators MONOPTEROS (MP, also known as ARF5) and BODENLOS (BDL, also known as IAA12) (Hamann et al., 2002; Hardtke and Berleth, 1998), show pronounced defects in embryonic root formation.

Afterwards, during the development of the heart stage of the *Arabidopsis* embryo, additional auxin maxima are formed at the positions of the two initiating cotyledons (see Glossary, Box 1), mainly through the action of PIN1 (Benková et al., 2003). At this stage, the ABCB19 expression pattern is largely complementary to that of PIN1 and shows the highest expression in endodermal and cortical tissues (Fig. 2). The *pin1 abcb1 abcb19* triple mutants, in contrast to the single *pin1* or double *abcb1 abcb19* mutants, are severely defective in establishing auxin maxima and show fused cotyledons, which hints at a synergistic genetic interaction between PIN1 and ABCB proteins (Mravec et al., 2008). These results indicate a role for both the ABCB-mediated
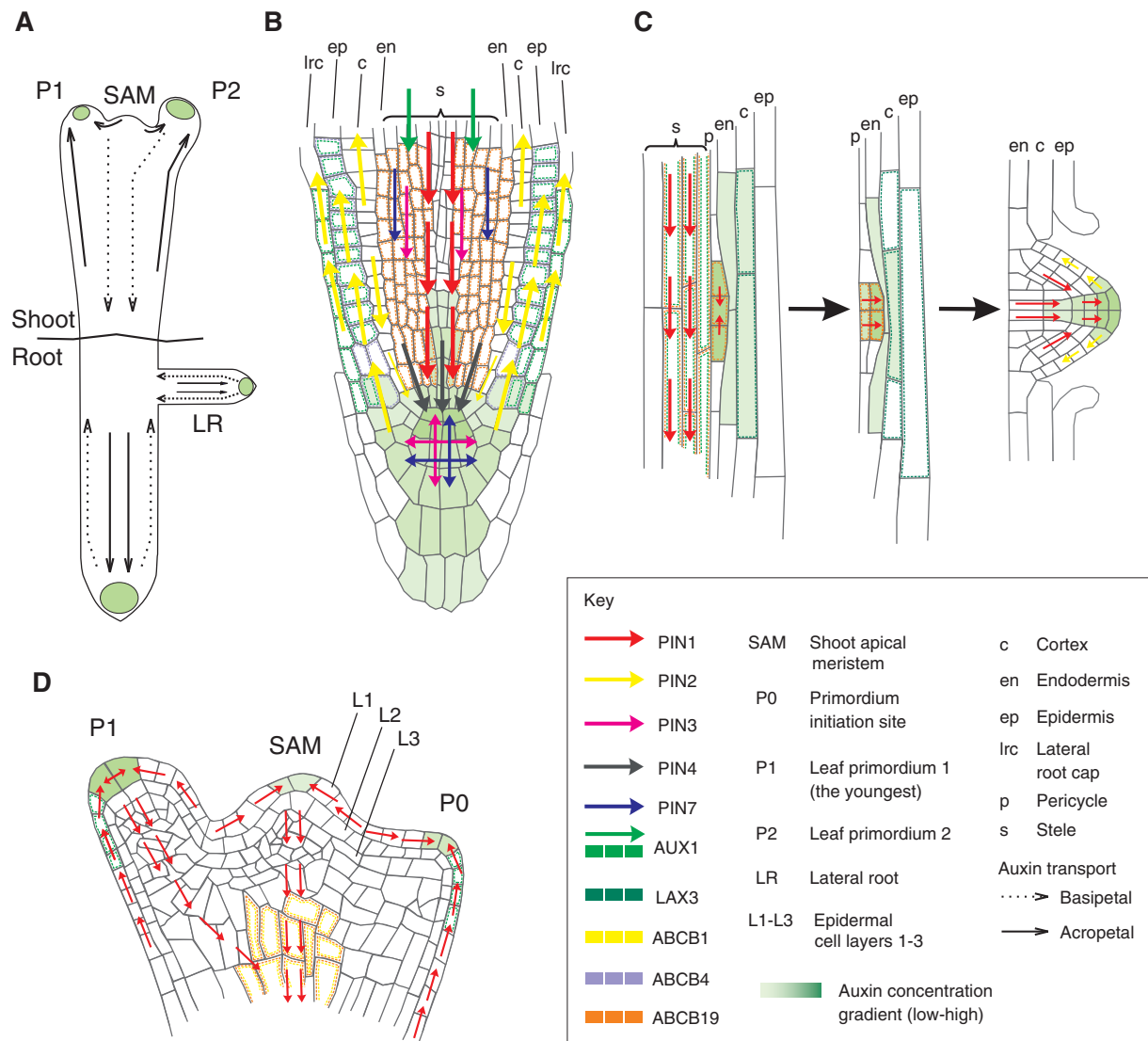
Fig. 3. Auxin gradients and auxin transporters in root and shoot morphogenesis. (A) Schematic overview of the directional flow of auxin in the shoot and root of *Arabidopsis thaliana*. Auxin maxima in shoot- and root-derived primordia and the root apex (green) are maintained by auxin flow towards the root and shoot apices (solid arrows) and reverse flow towards the root and shoot basis (dashed arrows). In the shoot and in shoot-derived organs (leaf primordia P1 and P2), auxin is transported towards the tip in the epidermal layers and refluxed back through inner tissues (future vasculature). In the root and in root-derived organs (lateral root, LR), auxin is transported towards the tip through the interior of the primordium and refluxed back through the epidermis. **(B,C)** Auxin transporters in the root tip (B) and developing lateral roots (C). **(D)** Auxin transport in the shoot apical meristem (SAM) and during phyllotaxis. See the main text for details on the role of each individual transporter. Auxin distribution (depicted as a green gradient) has been inferred from DR5 activity and IAA immunolocalization. The localization of auxin transporters is based on immunolocalization studies and on in vivo observations of GFP-tagged proteins (Benková et al., 2003; Blakeslee et al., 2007; Friml et al., 2002a,b; Friml et al., 2003b; Heisler et al., 2005; Lewis et al., 2007; Reinhardt et al., 2003; Swarup et al., 2008; Swarup et al., 2001; Wu et al., 2007). Arrows indicate auxin flow mediated by a particular transporter; dotted lines depict the cell type-specific localization of particular auxin transporters with no obvious polarity.

and PIN-dependent auxin transport pathways in the generation of differential auxin distribution at different stages of embryogenesis.

Auxin and postembryonic root and shoot development

Auxin plays an important role in the patterning of both shoot and root apices, as well as in the initiation and the subsequent development of root and shoot organs. Increased auxin levels at the incipient positions of the primary root and the cotyledons (see

Glossary, Box 1) during embryogenesis are reflected in postembryonic development. Auxin maxima always mark the positions of organ initiation and, later, of the tips of developing organ primordia (Benková et al., 2003). Correspondingly, the local application and production of auxin triggers the formation of leaves or flowers (Reinhardt et al., 2000) and of lateral roots (Dubrovsky et al., 2008). Auxin fluxes and maxima in root- and shoot-derived organ primordia are similar and can be described in terms of fountain and reverse fountain models, respectively (Benková et al., 2003) (Fig. 3A). In general, all three auxin transport systems,
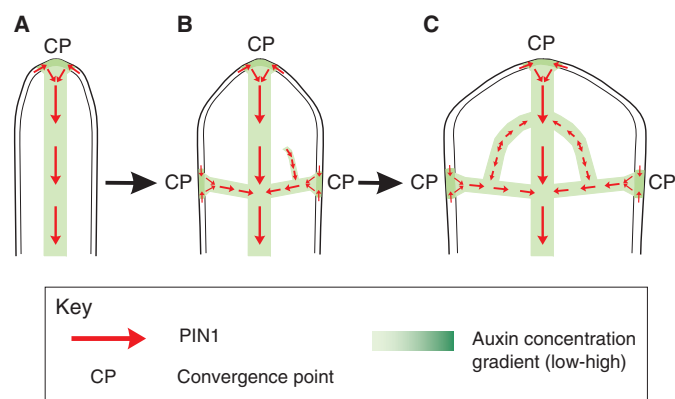
Fig. 4. Auxin distribution and localization of auxin transporters in vascular tissue formation. Three developmental stages of leaf primordia according to Scarpella et al. (Scarpella et al., 2006). (**A**,**B**) Polarly localized PIN1 in the epidermis directs auxin flow towards the convergence point (CP). (**C**) Gradual PIN1 polarization and establishment of auxin channels away from the CPs determine the future development of the venation pattern. Arrows indicate auxin flow mediated by PIN1.

using PIN, ABCB and AUX1/LAX proteins, contribute to postembryogenic auxin transport, although the exact contribution of each of these cooperating transport systems to total auxin transport remains unresolved.

Auxin transport routes during root development

In the primary root, auxin is transported acropetally (see Glossary, Box 1) towards the root tip by a PIN-dependent route through the vascular parenchyma and through the phloem, with subsequent AUX1-dependent unloading into protophloem cells (Friml et al., 2002a; Swarup et al., 2001). Auxin flow towards the tip is maintained by the action of basally localized PIN1, PIN3 and PIN7 in the stele (see Glossary, Box 1) (Blilou et al., 2005; Friml et al., 2002a). In the columella (see Glossary, Box 1), the action of PIN3 and PIN7 redirects auxin flow laterally to the lateral root cap and the epidermis, where the apically localized PIN2 mediates the upward flow of auxin to the elongation zone (Friml et al., 2003a; Müller et al., 1998) (Fig. 3B). The PIN2-based epidermal auxin flow is further supported by the action of AUX1 (Swarup et al., 2001) and ABCB4 (Terasaka et al., 2005; Wu et al., 2007), whereas PIN1, PIN3 and PIN7 recycle some auxin from the epidermis back to the vasculature (Blilou et al., 2005). The concerted action of the PIN auxin efflux carriers is one of the major determinants of pattern formation in root tips (Fig. 3B). By concentrating auxin in the quiescent center, the columella initiates, whereas surrounding stem cells (Sabatini et al., 1999) restrict, the expression domain of the auxin-inducible PLETHORA (PLT) transcription factors. PLTs are the master regulators of root fate and, in turn, are required for PIN transcription (Blilou et al., 2005). The ABCB1 and ABCB19 auxin transporters seem to play a supportive role in controlling how much auxin is available for each PIN-based transport flow. *ABCB1* is expressed in all root cells, except for the columella (Mravec et al., 2008), whereas *ABCB19* expression is restricted to the endodermis and the pericycle, which might help to separate the acropetal and basipetal auxin fluxes in the stele and the epidermis, respectively (Blakeslee et al., 2007; Mravec et al., 2008; Wu et al., 2007).

Auxin transport is also crucial for lateral root initiation and development (Fig. 3C). In pericycle cells, auxin maxima specify the founder cells for lateral root initiation (Dubrovsky et al., 2008).

Subsequent rounds of coordinated divisions form the lateral root primordium, from which the lateral root emerges later. Indeed, the functionally redundant network of PIN efflux carriers facilitates the auxin transport that is needed for the correct development of lateral root primordia (Benková et al., 2003). During the initiation phase, PIN1 is localized at the anticlinal membranes. The switch of the pericycle cell division plane from anticlinal to periclinal (see Glossary, Box 1) is accompanied by the redistribution of PIN1 to the outer lateral plasma membranes of inner cells (Benková et al., 2003). This guanine nucleotide exchange factor for ADP-ribosylation factors (ARF-GEF)-dependent, transcytosis-like PIN1 polarity switch (Kleine-Vehn et al., 2008a) mediates the auxin flow towards the primordium tip, where an auxin maximum is formed. At later stages, the PIN2-mediated auxin transport away from the tip through the outer layers is established.

AUX1 significantly contributes to lateral root formation, probably by controlling the overall auxin levels in the root tip (by unloading auxin from the phloem) and its availability in the region of lateral root initiation (by basipetal transport from the tip) (Marchant et al., 2002). An interesting role is reserved for LAX3, which is induced in cells around the developing primordium, where it establishes the auxin maxima needed for the specific production of cell-wall-remodeling enzymes, which is necessary for lateral root emergence (Swarup et al., 2008). The ABCB1 and ABCB19 proteins are also expressed and required for lateral root formation, as indicated by the defects in the *abcb* and *pin abcb* mutants (Mravec et al., 2008; Petrášek et al., 2006).

Auxin transport routes during shoot development

In the shoot apical meristem (SAM), the main source of auxin is unclear, but auxin is probably partly supplied by the phloem (as in the case of roots) and by young developing organs in the vicinity. Auxin fluxes are largely reversed in shoots when compared with roots. Auxin arrives at the organ initiation sites through the epidermis layer L1 and is canalized through the interior of developing primordia into the basipetal stream of the main shoot (Fig. 3D). This stream is mostly maintained by the activities of PIN1, localized basally in xylem parenchyma cells (Gälweiler et al., 1998), and of ABCB19 (Noh et al., 2001), which, together with ABCB1, helps to concentrate auxin flux in the vascular parenchyma (Blakeslee et al., 2007; Geisler et al., 2005).

Shoot lateral organs (leaves and flowers) are generated from the SAM in a highly periodic phyllotactic pattern. In *Arabidopsis* phyllotaxis, the 137° angle between developing primordia is marked by auxin maxima at the position of incipient primordia (Benková et al., 2003; Heisler et al., 2005). This highly organized auxin distribution is maintained by the cooperative action of AUX1, LAX1, LAX2 and LAX3 (Bainbridge et al., 2008), as well as that of PIN1. PIN1 polarities in the L1 layer, which also undergo complex rearrangements relative to auxin maxima, appear to be responsible for generating the phyllotactic pattern of auxin distribution, whereas auxin influx activities largely restrict auxin to the L1 layer (Reinhardt et al., 2003). Not only the positioning, but also the development of shoot lateral organs is regulated by auxin distribution, with the maximum concentration at the primordium tip, where it is maintained mainly by the activity of PIN1, which transports auxin through the epidermis towards the tip. From there, a new basipetal, PIN1-dependent, transport route is gradually established through the interior of the primordium. This marks future developing vascular tissues that will connect new organs with the pre-existing vascular network (Benková et al., 2003; Heisler et
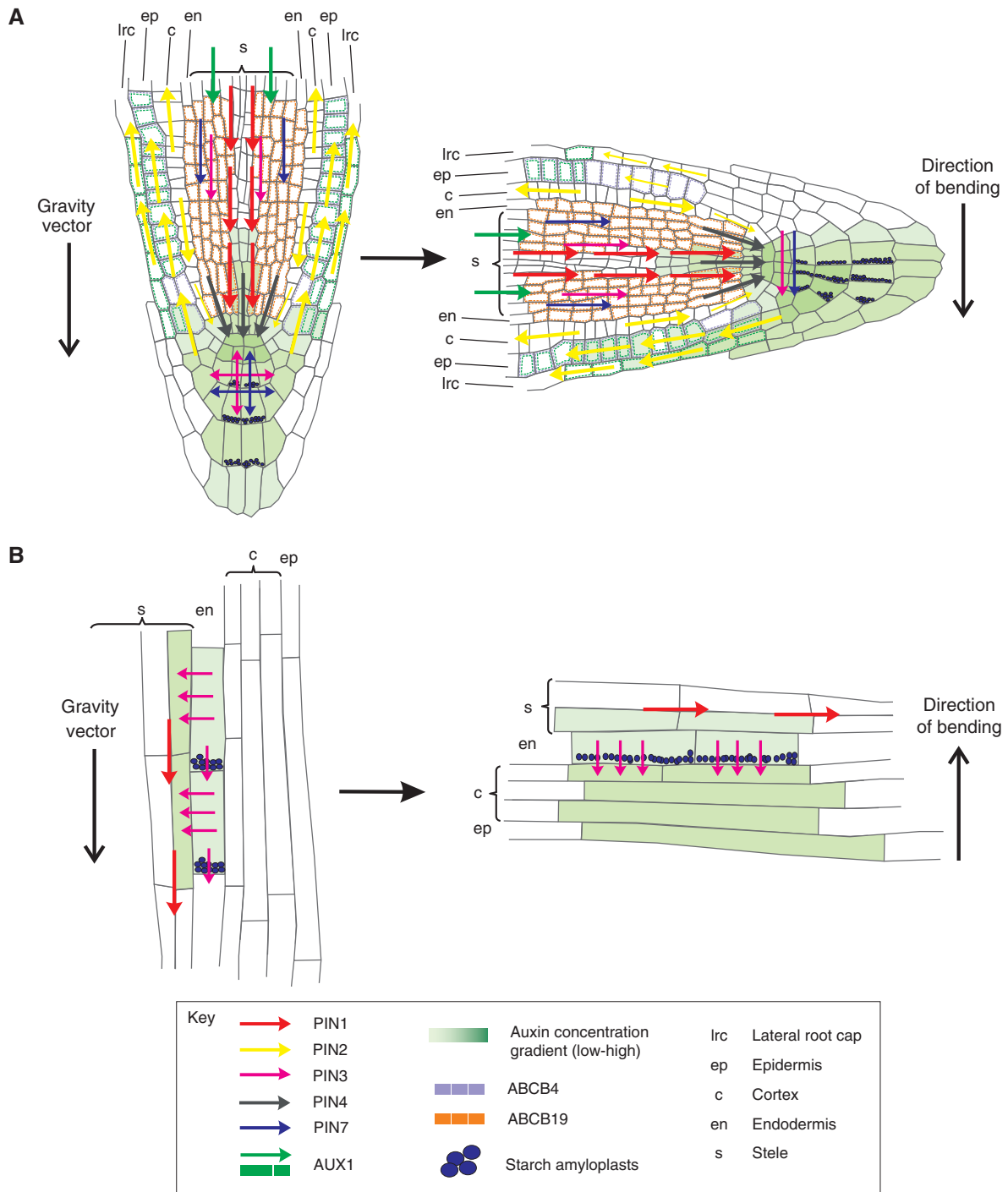
Fig. 5. Auxin gradients and auxin transporters during gravitropic response. (**A**) Positive root gravitropism. In starch-containing, gravity-sensing root cap cells, PIN3 is relocalized from a symmetric distribution towards the newly established bottom side after gravistimulation (Friml et al., 2002b) (left). The auxin that is redirected to the lower side of the root tip is further transported to the elongation zone by epidermal PIN2/AUX1-mediated flow, where it inhibits cell growth and causes the downward bending of the root (Luschnig et al., 1998; Müller et al., 1998; Bennett et al., 1996; Swarup et al., 2001; Swarup et al., 2005) (right). (**B**) Negative shoot gravitropism. Gravity is detected in starch-containing endodermal cells, where PIN3 is supposed to redirect auxin laterally to the vasculature (stele) (left). After gravistimulation (right), PIN3 is presumably (in analogy to the situation in roots) relocated to the new basal side of endodermal cells, and auxin flow is redirected to the outer cell layers along the bottom side of the shoot, where auxin stimulates cell elongation and the subsequent upward bending of the stem (Friml et al., 2002b). Arrows indicate auxin flow mediated by a particular transporter; dotted lines indicate the cell type-specific localization of particular auxin transporters with no obvious polarity; black arrows indicate the gravity vector (left) and the direction of bending (right).

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al., 2005). ABCB1 and ABCB19 also contribute to the establishment of this auxin sink (Noh et al., 2001) (Fig. 3D). Observations regarding the localization of the components of different auxin transport systems, combined with the defects in the corresponding mutants, show that all the transport systems that depend on ABCB, AUX1/LAX and PIN proteins are involved in shoot-derived organogenesis.

Auxin in vascular tissue development

As indicated already by the role of PIN1-dependent auxin flow in the establishment of new vasculature from shoot-derived organs, auxin and auxin transport are among the major determinants of the organized development of vascular tissues, which serve as the main distribution route for water and nutrients. Auxin seems to be a major positional signal for vascular tissue formation, because local auxin applications to responsive tissues are sufficient to trigger the de novo formation of vasculature (Sachs, 1991). As stated before, the canalization model of auxin flow predicts a feedback regulation of the auxin transport rate and polarity by a localized auxin source. Such a mechanism would be adequate to gradually generate more concentrated auxin channels that would determine the position of the new vasculature and explain the vasculature formation seen in leaves after wounding or in newly initiated organs. Indeed, multiple feedback regulatory loops of PIN-dependent auxin transport have been identified. Auxin modulates PIN transcription (Vieten et al., 2005), PIN incidence at the plasma membrane (Paciorek et al., 2005) and also PIN polar localization (Sauer et al., 2006). For example, during the formation of vascular veins in leaves, PIN1 directs auxin towards a convergence point in the leaf epidermis, from where veins are being initiated and where *PIN1* expression and polar localization mark the position of all future veins (Scarpella et al., 2006) (Fig. 4). Similarly, after wounding, PIN1 is repolarized, and a new transport route is set up that determines the position of the regenerating vasculature. Importantly, local auxin application is sufficient to induce *PIN1* expression, polarization and the subsequent establishment of PIN1-based auxin channels, thus essentially specifying the future vasculature (Sauer et al., 2006). These observations provide strong support for the canalization hypothesis and suggest that the auxin-dependent polarization of PIN1 is a key event in vascular tissue formation during a variety of developmental processes.

The role of other auxin transport mechanisms in this process is unclear, but they might have supporting functions. For example, AUX1 presumably facilitates auxin loading into and out of the phloem component of the vascular transport system (Marchant et al., 2002) (Fig. 4). ABCB19 is mostly localized in the vascular bundle sheet cells and potentially prevents auxin leakage from the vascular flow (Blakeslee et al., 2007).

Auxin routes in tropisms

The role of auxin and auxin transport in the directional growth responses of plants to light (phototropism) and to gravity (gravitropism) played a major role in the discovery of auxin and in the formulation of the concept of plant hormones (Darwin, 1880). The negative gravitropism of stems, the positive gravitropism of roots and the positive phototropic curvature of stems are characterized by the uneven distribution of auxin at the different sides of stimulated organs. This differential auxin distribution activates asymmetric growth and subsequent organ bending (Went, 1974) in a context-specific manner: whereas higher intracellular auxin concentrations trigger elongation in shoots, they are inhibitory in roots.

In roots, gravity is detected in the starch-containing root cap cells, in which PIN3 is relocalized from its originally uniform distribution to the bottom plasma membranes after gravistimulation (Friml et al., 2002b). Auxin flow is redirected towards the lower side of the root tip, from where it is transported through the lateral root cap and epidermal cells towards the elongation zone, where growth-inhibitory auxin responses are induced (Swarup et al., 2005). This basipetal transport route requires both the epidermally localized PIN2 (Luschnig et al., 1998; Müller et al., 1998) and AUX1 (Bennett et al., 1996; Swarup et al., 2001; Swarup et al., 2005) (Fig. 5A). The flow along the lower side of the root is further enhanced by the vacuolar targeting of PIN2 and its degradation on the upper root side (Abas et al., 2006; Kleine-Vehn et al., 2008b). In addition, ABCB-dependent auxin transport might regulate the gravitropic response, considering that *abcb4* and *abcb1 abcb19* mutants show an enhanced gravitropic response (Lewis et al., 2007) and a genetic interaction with *pin2* (Mravec et al., 2008) (Fig. 5A). Moreover, flavonoids, the putative endogenous modulators of auxin transport, might contribute to root bending through their influence on PIN and ABCB4 expression and activity (Santelia et al., 2008; Lewis et al., 2007).

In shoots, gravity is detected in endodermal cells (starch sheath cells), where PIN3 is localized at the inner plasma membrane. The corresponding *pin3* mutants are partially defective in hypocotyl gravitropism (Friml et al., 2002b). It is likely, but has not been conclusively demonstrated, that, similar to the root gravitropic response, the PIN3 relocation to the bottom side of endodermis cells triggers auxin accumulation in the lower side of the shoot, where the auxin response promotes growth and upward bending (Fig. 5B).

The mechanisms that generate auxin asymmetry in response to light remain unclear, but studies with mutants or inhibitors show that phototropism also requires the activity of all auxin transport components, such as PIN3 (Friml et al., 2002b), AUX1 (Stone et al., 2008), ABCB1 (Lin and Wang, 2005) and ABCB19 (Lin and Wang, 2005; Nagashima et al., 2008a; Nagashima et al., 2008b; Noh et al., 2003).

Conclusions

As discussed here, the polarized transport of auxin is crucial for plant development. In addition to the passive diffusion of auxin molecules across plasma membranes, three active and mutually cooperating auxin-transporting systems have been described so far. Whereas the PIN auxin transporters are the primary determinants of directionality, AUX1/LAX and ABCB proteins mainly generate auxin sinks and control auxin levels in the auxin channels. The open questions for future studies include the identification of the core action of the different auxin transporters, how exactly auxin is transported across the plasma membrane, how this process is regulated and how individual transporters cooperate. Furthermore, the analysis of the regulatory sequences in promoters of genes that code for auxin transporters, together with the study of crosstalk with other plant hormones, will be crucial for understanding how this system is controlled by other signaling pathways. The wealth of available genetic tools will significantly contribute to answering these questions; however, more biochemical and structural biology work will also be needed, in particular to address the issues of the precise mechanism of auxin movement across the plasma membrane.

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