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Focus Review

The march of the PINs: developmental plasticity by dynamic polar targeting in plant cells

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Development of plants and their adaptive capacity towards ever-changing environmental conditions largely depend on the spatial distribution of the plant hormone auxin. At the cellular level, various internal and external signals are translated into specific changes in the polar, subcellular localization of auxin transporters from the PIN family thereby directing and redirecting the intercellular fluxes of auxin. The current model of polar targeting of PIN proteins towards different plasma membrane domains encompasses apolar secretion of newly synthesized PINs followed by endocytosis and recycling back to the plasma membrane in a polarized manner. In this review, we follow the subcellular march of the PINs and highlight the cellular and molecular mechanisms behind polar foraging and subcellular trafficking pathways. Also, the entry points for different signals and regulations including by auxin itself will be discussed within the context of morphological and developmental consequences of polar targeting and subcellular trafficking.

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Introduction

Auxin as a versatile trigger for developmental programming

In all multi-cellular organisms, different kinds of cells are specialized for different tasks such as reproduction, nutrient uptake or light perception. The proper functioning of these organisms requires that the distinct cell types are positioned correctly relative to one another. Therefore, the formation of axes along the body plan is crucial and provides the initial sketch for different cell types to arise at their proper destinations. In plants, the hormone auxin and especially its spatial distribution has been shown to have a fundamental function in embryonic and post-embryonic axis formation (Berleth *et al*, 2007; Benjamins and Scheres, 2008; Mockaitis and Estelle, 2008; Moller and Weijers, 2009; Vanneste and Friml, 2009). At the very onset of a plant's life, during embryogenesis, auxin already orchestrates all of its developmental programs, guiding dividing cells towards a basic body plan. After the initial division of the zygote, auxin accumulates in the upper apical cell (Friml *et al*, 2003b), where upon it initiates the strictly regulated divisions that lead to the formation of the globular pro-embryo in contrast to the slowly proliferating divisions of the auxin-deficient suspensor cells (Figure 1A). Later on, auxin accumulates in the uppermost suspensor cell (Friml *et al*, 2003b) thereby reprogramming it as the hypophysis or future root pole (Figure 1B; Weijers *et al*, 2006; Schlereth *et al*, 2010). Inhibition of auxin accumulation during embryogenesis disturbs apical–basal axis formation and subsequently, the conserved cell division program (Friml *et al*, 2003b; Blilou *et al*, 2005; Vieten *et al*, 2005). After germination, auxin continues to contribute to polarity establishment and organ initiation (Figure 1C–E), for example during lateral root initiation and outgrowth (Benkova *et al*, 2003; Dubrovsky *et al*, 2008; Swarup *et al*, 2008; Peret *et al*, 2009), initiation of leaves and inflorescences (Benkova *et al*, 2003; Reinhardt *et al*, 2003; Heisler *et al*, 2005), formation of vasculature (Scarpella *et al*, 2006), development of ovules (Benkova *et al*, 2003) and gamete specification in the female gametophyte (Pagnussat *et al*, 2009). Interestingly spatial auxin depletion can also trigger developmental pathways, as auxin minima are indispensable for the establishment of the valve margin separation layer needed for fruit formation and seed dispersal (Sorefan *et al*, 2009).

Alongside their strictly regulated developmental processes, plants have evolved a remarkable plasticity to adjust their individual growth according to changes in environmental conditions; in many cases by adapting auxin-regulated developmental programs. Stems elongate to cope with shading, bend to recover from lodging or to optimize light perception, whereas roots redirect their growth upon hitting hard objects in the soil (Whippo and Hangarter, 2006; Richter *et al*, 2009). Developmental and adaptive programs are interconnected and the rudimentary body plan, which is laid down during plant embryogenesis, is probably one of the strategies to anticipate putative unfavourable conditions from germination onwards.

Auxin transport for auxin distribution patterns

Supported by numerous genetic and pharmacologic studies, it is now commonly accepted that in addition to auxin biosynthesis (Ljung *et al*, 2005; Cheng *et al*, 2006, 2007; Stepanova *et al*, 2008; Tao *et al*, 2008; Ikeda *et al*, 2009; Petersson *et al*, 2009; Zhao, 2010), directional auxin transport from one cell to another is crucial in building up the spatial

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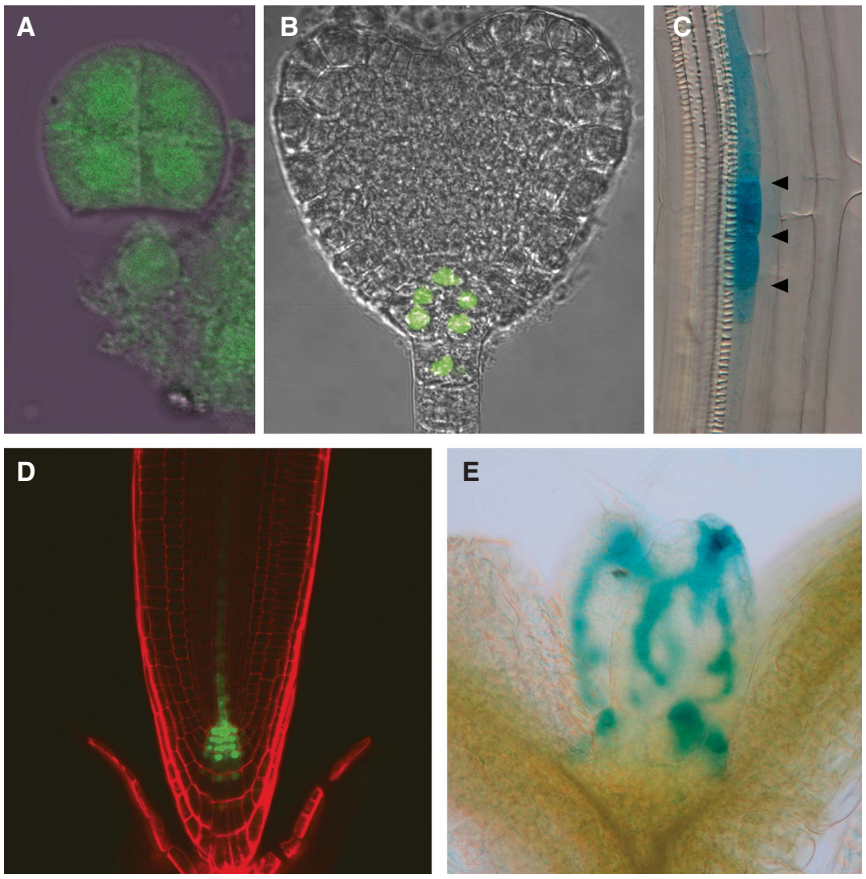

Figure 1 Auxin gradients during plant development. (**A**, **B**) DR5 promoter activity during embryogenesis, visualizes the auxin response first in all apical cells of octant embryos (**A**) and then only in the root stem cell niche of early heart (**B**) embryos. (**C**) During lateral root initiation DR5::GUS activity increases in the lateral root founder cells. Arrowheads indicate the asymmetric divided pericycle cells. (**D**) A restricted auxin gradient in the tip of root meristems maintains the stem cell niche. (**E**) DR5::GUS staining precedes the initiation of vascular strands in young leaves.

auxin maxima and minima, also called auxin gradients, that are required for development and adaptation (Galweiler *et al*, 1998; Friml *et al*, 2002a, 2003b, 2004; Benkova *et al*, 2003; Weijers *et al*, 2005; Grieneisen *et al*, 2007; Sorefan *et al*, 2009). The model plant *Arabidopsis thaliana* and in general all higher plants have several specific auxin influx and efflux carriers, which act at the plasma membrane and are indispensable for intercellular auxin flow.

Members of the family of PIN-formed (PIN) proteins have been shown to facilitate cellular auxin efflux in both plant and heterologous systems (Petrasek *et al*, 2006; Yang and Murphy, 2009). Interestingly, their specific polar subcellular localizations modulate and correlate with the spatial pattern of activities of auxin-responsive reporters, strongly suggesting that PINs determine the direction of the auxin flow (Wisniewska *et al*, 2006). Consequently, genetic interference with PIN proteins disturbs multiple developmental processes and establishes PIN auxin exporters as key players in auxin-mediated development (Vieten *et al*, 2007). PIN proteins can be targeted towards the apical, basal or lateral plasma membrane depending on the type of protein as well as the cell type and developmental context. In wild-type, PIN1 proteins are basally localized (towards the root tip) in the vascular tissues of embryos, leaves and roots whereas they are apically localized (towards the shoot apex) in the epidermis of shoot apices, embryos and gynoecia (Galweiler *et al*, 1998; Benkova *et al*, 2003; Reinhardt *et al*, 2003; Friml *et al*, 2003b; Sorefan *et al*, 2009). PIN2 on the other hand is apically localized in the lateral root cap and root epidermis cells and basally targeted in the root cortex cells (Muller *et al*, 1998; Friml *et al*, 2003a). Lateral localization is mainly reserved for PIN3 at the inner side of shoot endodermis cells (Friml *et al*, 2002b; Zadnikova *et al*, 2010). The other plasma membrane localized PIN proteins in Arabidopsis also show polar localizations in different tissues such as PIN4 in the central root meristem (Friml *et al*, 2002a) and PIN7 in the embryonic suspensor (Friml *et al*, 2003b). The remaining members of the eight-protein Arabidopsis PIN family are likely to localize to the endoplasmic reticulum (ER) and to facilitate intracellular auxin transport as suggested for PIN5 (Mravec *et al*, 2009).

The P-glycoproteins of the ABCB transporter family (ABCB/PGP) are another group of auxin transporters. The best-characterized members are ABCB1/PGP1, ABCB4/PGP4 and ABCB19/PGP19, which all mediate auxin efflux both in plant and non-plant systems (Geisler *et al*, 2005; Petrasek *et al*, 2006; Cho *et al*, 2007). Intriguingly, PGP4 mediates a reversible active transport mechanism and contributes to auxin uptake in some cells (Yang and Murphy, 2009). The PGP auxin transporters are localized more symmetrically than PIN export transporters and presumably function in non-polar auxin efflux thereby controlling the amount of auxin available for PIN-mediated auxin transport (Mravec *et al*, 2008). Nevertheless, more direct physical and

Box 1 The endomembrane system in plants

Newly synthesized proteins enter the endomembrane system through the endoplasmatic reticulum (ER). These proteins are folded and glycosylated before being transported to the Golgi apparatus. In plant cells, the Golgi apparatus consists of numerous Golgi stacks (Jürgens, 2004). Each Golgi stack consists of several morphological distinct cisternae arranged from the *cis* to the *trans* side. The proteins are further modified as they move from the *cis* to the *trans* cisternae and ultimately they end up in the *trans*-Golgi network (TGN). At the TGN, critical sorting events take place to target proteins either to the cell surface or to the vacuole (Viotti *et al*, 2010).

Endocytic trafficking begins at the plasma membrane with the internalization of membrane and extracellular cargos into vesicles, typically coated by clathrin. These vesicles fuse with endosomes, organelles that function in sorting, recycling and further transport of the cargo. Endosomes can be roughly divided in early endosomes and late endosomes. However, both types of endosomes should be seen as a continuum of compartments that continuously evolve rather than as distinct organelles. The early endosomes (100–300 nm) are involved in sorting and recycling of plasma membrane proteins back to the plasma membrane and in plant cells seem to be equivalent to the TGN (Dettmer *et al*, 2006; Lam *et al*, 2007). Thus, in this complex compartment, the secretory and endocytic trafficking pathway meet. Late endosomes (200–500 nm) are multi-vesiculate bodies (MVB, also referred to as the prevacuolar compartment (PVC)) that are on their way to the lytic vacuole (Robinson *et al*, 2008). Plant vacuoles can be divided in lytic vacuoles and protein-storage vacuoles. Lytic vacuoles are equivalent to animal lysosomes and function as compartments for degradation and waste storage. In contrast to the destructive character of the lytic vacuoles, protein-storage vacuoles stimulate growth by accumulating proteins that are used as nutrients during seed germination.

functional interactions with PIN proteins may also contribute to the concerted action of these two transporter families in regulating intercellular auxin flow (Blakeslee *et al*, 2007; Titapiwatanakun *et al*, 2009).

In the apoplast, indole-3-acetic acid (IAA), the major form of auxin, is present in its protonated form and as such it can diffuse through the plasma membrane. However, in specific developmental situations, as for example in root gravitropic responses, lateral root outgrowth and root hair development, passive auxin uptake is supported by the amino acid permease-like proteins of the AUX1/LAX family (Bennett *et al*, 1996; Swarup *et al*, 2008; Jones *et al*, 2009). These auxin influx carriers are symmetrically or asymmetrically localized depending on the cell type and are assumed to act as H^+/IAA^- symporters as was demonstrated for AUX1 (Yang *et al*, 2006). AUX1, the most-studied member of the AUX1/LAX family, mainly antagonizes PIN localization, being localized to the apical side of protophloem cells in the vasculature and basally targeted in the lateral root cap cells (Bennett *et al*, 1996; Swarup *et al*, 2001).

Although polar subcellular localizations have been shown for all of the auxin transporters described above in certain cases, it is only in the case of the PIN proteins that polar targeting is a typical feature. Manipulations of PIN polarity, either by random (Wisniewska *et al*, 2006) or targeted (Huang *et al*, 2010; Zhang *et al*, 2010) interference with polarity signals within the PIN sequence or through genetic manipulation of PIN polarity regulators (Friml *et al*, 2004; Michniewicz *et al*, 2007), consistently show that changes in PIN polarity are always associated with predictable rearrangements of auxin-response distribution patterns and changes in plant development. Thus, polarity of PIN localization is a crucial factor in controlling the directionality of auxin flow between cells and thus in controlling auxin distribution-mediated plant development. Moreover, in response to different developmental or environmental signals, polar PIN localizations can dynamically change to deviate the auxin flow, and enable a suitable plant response (Friml *et al*, 2002b, 2003b, 2004; Benkova *et al*, 2003; Reinhardt *et al*, 2003; Heisler *et al*, 2005). These dynamic, rapid switches of PIN polarity are presumably enabled by permanently, repeating cycles of endocytosis and exocytosis of PIN proteins, allowing polar retargeting after each internalization event. In this way, the polar subcellular localization of PIN proteins often underlies tissue polarity and perfectly illustrates how polar targeting of plasma membrane proteins is fundamental in translating a signal perceived at the cellular level into a response on plant level. In this review, we will focus on this intracellular movement of PINs and we will discuss the molecular and cellular mechanisms behind polar targeting of PIN proteins.

Always on the road: constitutive intracellular movement of PIN proteins

Endocytosis is defined as the uptake of molecules from the extracellular milieu or as the internalization of plasma membrane proteins and receptor–ligand complexes. The initial internalization process at the plasma membrane is followed by a series of transfer steps, which carry the cargo molecules through different pathways to and through several endosomal compartments (background information on the endomembrane system of plants is given in Box 1 and illustrated in Figure 2). The early endosome (EE) is the first compartment to be reached by endocytosed plasma membrane proteins. It seems that despite being for the main part evolutionarily conserved, the plant endocytic mechanism shows specific features as the *trans*-Golgi network (TGN) itself or an immediate TGN derivative acts as an EE (Dettmer *et al*, 2006; Lam *et al*, 2007). From the TGN/EE on, the cargos can be directed to recycling endosomes (RE), which deliver the cargo back to the plasma membrane, or to the prevacuolar compartment (PVC, also called the multi-vesicular body (MVB) or late endosomes) if they are destined for vacuolar degradation (Viotti *et al*, 2010).

The endocytic movement from the plasma membrane towards the vacuole can be visualized using the lipid binding, endocytic tracer FM4-64 (Vida and Emr, 1995). The observation that PIN proteins colocalize with FM4-64 (Paciorek *et al*, 2005) demonstrated that PINs, in addition to their plasma membrane localization, are continuously internalized. Next to this, it could be shown that PIN proteins are being foraged from the RE back to the plasma membrane. The first evidence for the recycling of plasma membrane proteins in plants was provided for PIN1 using Brefeldin A (BFA) (Geldner *et al*, 2001). The fungal toxin BFA blocks trafficking from RE to the plasma membrane thereby causing an aggregation of endosomes and internalized endocytic cargos into so-called BFA compartments. Following BFA treatment, PIN proteins localize to these BFA bodies but their plasma membrane localization can be restored by washing out the BFA. Performing the wash out in the presence of the protein

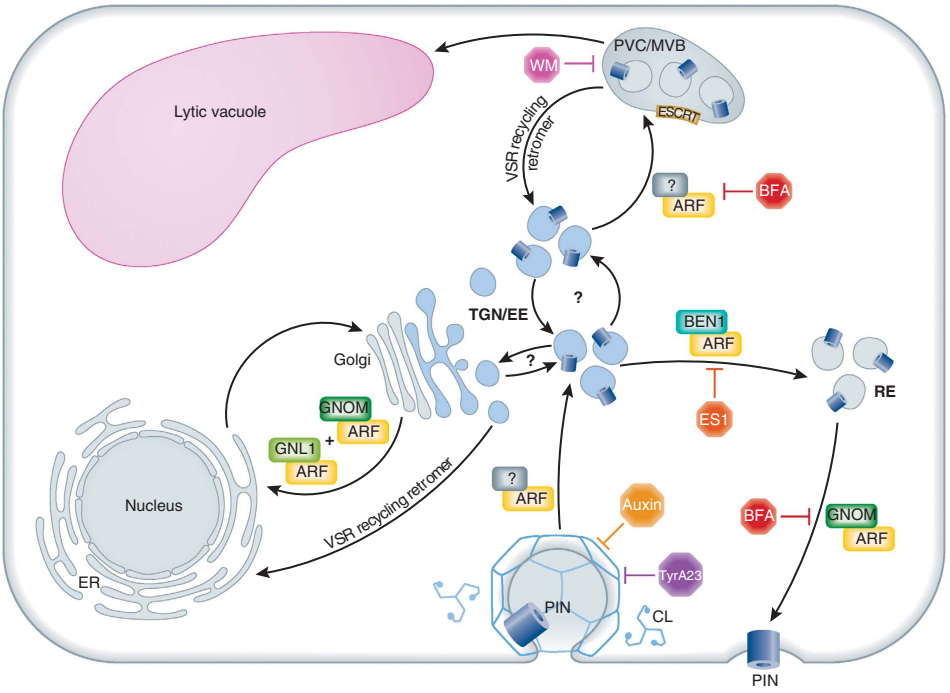

Figure 2 Subcellular trafficking mechanisms controlling PIN polarity and degradation. BFA, brefeldinA; EE, early endosomes; ER, endoplasmatic reticulum; MVB, multi-vesiculate bodies; PVC, pre-vacuolar compartments; RE, recycling endosomes; TGN, trans-golgi network; WM, wortmannin.

synthesis inhibitor cycloheximide also restored the PIN signal at the plasma membrane (Geldner *et al*, 2001), providing indirect evidence for PIN recycling. Later on, constitutive PIN cycling was demonstrated more directly by making use of the green-to-red photo-convertible EosFP fluorescent reporter (Dhonukshe *et al*, 2007). After photo-conversion in the presence of BFA (to inhibit the recycling to the plasma membrane), PIN2-EosFP could be tracked from the plasma membrane to the endosomes. Vice versa, when BFA was used first and the BFA-body-localized PIN2-EosFP was converted, PIN proteins could be followed back to the plasma membrane. This set of results provided compelling evidence that PIN proteins undergo constant cycles of endocytosis and exocytosis.

Intracellular trafficking pathways

Setting off: internalization and clathrin-dependent endocytosis

The study of endocytosis in plants is lagging behind that of mammalian cells, mainly because of the longstanding notion that endocytosis would not work against the high turgor pressure in plant cells (for a history on endocytosis in plants see Robinson *et al*, 2008). Nowadays, unequivocal evidence is provided for endocytosis of several plant plasma membrane proteins such as the PIN proteins (Dhonukshe *et al*, 2007), BOR1, which acts as transporter for the nutrient boron (Takano *et al*, 2005, 2010) and receptors such as BRI1 for the brassinosteroid plant hormones (Russinova *et al*, 2004; Geldner *et al*, 2007) and FLS2 for pathogen recognition (Robatzek *et al*, 2006). In contrast to animal cells that make use of multiple endocytotic pathways (Soldati and Schliwa, 2006), so far only a clathrin-dependent mechanism of endocytosis has been demonstrated in plants (Dhonukshe *et al*, 2007).

Clathrin-mediated endocytosis requires the coordinated interaction of a plethora of cytosolic and membrane proteins. The clathrin coat, which drives the invagination of the endocytic vesicle, consists of three heavy and three light chains. These chains assemble into triskeleon units that interact with each other and with adaptin proteins to form a coat around internalizing vesicles. The adaptins, together with a collection of accessory proteins, recruit membrane proteins into the forming clathrin-coated vesicles (Perez-Gomez and Moore, 2007). In Arabidopsis root cells, coated vesicles and coated pit structures could be visualized at the plasma membrane using an antibody against the plant clathrin heavy chain (Dhonukshe *et al*, 2007), indicating that clathrin is present at different stages of coated-vesicle formation at the plasma membrane. Moreover, interfering with clathrin coat formation in protoplasts by overexpressing the HUB domain (truncated C-terminal part) of the clathrin heavy chain, inhibits endocytosis of the FM4-64 endocytic tracer as well as internalization of a selection of plasma membrane proteins, such as PIN1, PIN2 and the aquaporin PIP2 (Dhonukshe *et al*, 2007). In intact Arabidopsis root tip cells, pharmacological interference with clathrin function using Tyrphostin A23 also inhibits PIN internalization and endocytosis in general (Dhonukshe *et al*, 2007). In animal cells, the cargos of clathrin-dependent endocytosis have tyrosine-containing Yxxϕ motifs in their sequence, which mediate their recruitment into clathrin-coated vesicles by binding to the μ-subunit of the adaptin complex. Tyrphostin A23 is a well-characterized inhibitor of these adaptin–cargo interactions (Banbury *et al*, 2003).

The general effect of Tyrphostin A23 as well as the impact of a defective clathrin pathway on overall endocytic processes (Dhonukshe *et al*, 2007), suggest that clathrin-mediated endocytosis is the major mechanism for endocytosis in plant cells. However, the existence of other endocytotic

mechanisms in plant cells cannot be excluded, for example sterol-mediated endocytotic mechanisms have been proposed for re-establishment of cell polarity during cell division (Men *et al*, 2008). Moreover, in plants, as in other eukaryotic cells, clathrin has been detected at the TGN, where it also mediates vesicle budding (Staehelin and Moore, 1995) and thus should not be regarded as specific for endocytosis.

There and back again, a PIN's tale: constitutive recycling

Once the plasma membrane proteins are internalized by endocytosis, the vesicles containing them are sorted and translocated to their new destinations (Figure 2). Although our knowledge of these processes in plants is rapidly increasing, our current view of endocytic pathways in plants is still mainly based on the data and concepts from animal and yeast studies.

ADP-ribosylation factors (ARFs) are small guanine nucleotide-binding proteins that have important functions in endocytic trafficking in all eukaryotic kingdoms by mediating the formation, targeting and fusion of vesicles that shuttle cargoes between intracellular compartments. In general, the inactive GDP-bound form of ARFs is soluble, although it can associate weakly with membranes, whereas the active GTP-bound form binds tightly to the membrane. ARFs function at the membrane surfaces where they encounter their activators, the guanine nucleotide exchange factors (ARF-GEFs) and functional inhibitors, the GTPase-activating proteins (ARF-GAPs) (Donaldson and Jackson, 2000). From the eight ARF-GEF families found in animals, only the BIG and GBF classes are found in plants (Cox *et al*, 2004; Mouratou *et al*, 2005). All ARF-GEF proteins have their own specificity, localization and regulation thereby controlling specific ARF-mediated vesicular transport steps.

The most-studied ARF-GEF in intracellular trafficking in plants is GNOM, a member of the GBF subclass. GNOM mainly acts at the late endosome and has been shown to be involved in the recycling of PIN and others proteins to the plasma membrane (Geldner *et al*, 2003). The study of GNOM is facilitated by the finding that BFA (see above) is an inhibitor of GNOM (Steinmann *et al*, 1999). Loss-of-function of GNOM as well as BFA treatments result in severe embryo defects because of disturbance of coordinated PIN1 polarity establishment during embryogenesis (Steinmann *et al*, 1999; Friml *et al*, 2003b). GNOM function seems to be crucial for basal PIN targeting in particular as the apical delivery of PINs is less affected in *gnom* mutants (Kleine-Vehn *et al*, 2008a). In line with this, the apical delivery of the auxin influx carrier AUX1 is also unaffected in *gnom* mutants (Dharmasiri *et al*, 2006; Fischer *et al*, 2006; Kleine-Vehn *et al*, 2006). However, AUX1 does accumulate in BFA compartments upon BFA treatment (Grebe *et al*, 2002), demonstrating that AUX1 requires a BFA-sensitive, but GNOM-independent, ARF-GEF pathway to be targeted from BFA bodies to the plasma membrane (Fischer *et al*, 2006; Kleine-Vehn *et al*, 2006). The trafficking pathways of AUX1 are still not fully clarified and it is not even been unequivocally demonstrated whether AUX1 proteins cycle continuously. On the other hand, the secretion of *de novo* synthesized AUX1 has been discovered and was shown to depend on the ER-localized protein AUXIN-RESISTANT4 (AXR4) (Dharmasiri *et al*, 2006). Loss of AXR4 resulted in abnormal accumulation of AUX1 in the ER of epidermal cells, whereas both PIN1 and PIN2 localizations were not affected.

In addition to GNOM, Arabidopsis contains two other GBF ARF-GEF, GNOM-like (GNL) proteins (Geldner *et al*, 2003). Although the cellular function of GNL2 is unresolved so far, it seems to be specific for pollen germination (Jia *et al*, 2009). In contrast, GNL1 is involved in membrane trafficking between the Golgi and ER, the ancestral function of GBF class proteins conserved among eukaryotes (Richter *et al*, 2007; Teh and Moore, 2007). GNL1 acts primarily at Golgi stacks, regulating COPI-coated vesicle formation. However, GNL1 also has a selective function in the internalization of PIN2 (Teh and Moore, 2007). In BFA-treated *gnl1* roots, the internalization of PIN2 was inhibited. As GNL1 is BFA-resistant, this implies that GNL1 acts together with a BFA-sensitive ARF-GEF. Interestingly, although GNOM is involved in endosomal recycling (see above), GNOM can take over the Golgi function of GNL1 in its absence, whereas GNL1 has no obvious function in endosomal recycling (Richter *et al*, 2007).

In contrast to PIN1, in plants expressing BFA-insensitive versions of GNOM, PIN2 and H^+-ATPase were still found to be partially sensitive to BFA (Geldner *et al*, 2003), implying that there must be other ARF-GEFs or mechanisms involved in intracellular trafficking of PINs and other plasma membrane proteins. In line with this, apical targeting of PINs is not primarily regulated by GNOM. To test the hypothesis that the ARF pathway is involved in apical targeting, a dominant-negative GTP-bound version of ARF1 was conditionally overexpressed to genetically interfere with ARF-dependent pathways overall (Kleine-Vehn *et al*, 2008b). This approach resulted in intracellular PIN2 accumulation in both epidermal and cortical cells, suggesting that in addition to the well-known GNOM pathway, other so far uncharacterized ARF-GEF pathways are required for apical PIN targeting. Consistent with its function in Golgi-to-ER trafficking, GNL1 is not involved in this step (Kleine-Vehn *et al*, 2008b).

In contrast to GBF ARF-GEFs, the BIG class has hardly been functionally characterized at all. Recently, BIG5/BEN1 (previously known as MIN7) was identified by a forward genetic screen as a regulator of BFA-induced PIN internalization (Tanaka *et al*, 2009). In BFA-treated *ben1* mutants, PIN1-GFP does not accumulate efficiently in intracellular BFA compartments. BEN1 localizes to the TGN/EE, distinct from GNOM-positive RE, and was shown to mediate the trafficking of constitutively cycling plasma membrane proteins such as PIN1, PIN2 and H^+-ATPase from the EE to the RE (Tanaka *et al*, 2009).

Whereas BFA paved the way to study GNOM-dependent recycling of plasma membrane proteins, the chemical compound endosidin1 (ES1) might do the same in the future for endocytosis studies. ES1 selectively interferes with endocytosis in seedlings and induces the agglomeration of PIN2, AUX1 and BRI1 into distinct endomembrane compartments called 'endosidin bodies' (Robert *et al*, 2008). However, other plasma membrane proteins including PIN1 and PIN7 are not affected, hence supporting the existence of at least two distinct endocytosis pathways. This is consistent with the previously mentioned difference in AUX1 and PIN recycling pathways. The endosidin bodies were labelled with TGN markers, suggesting that ES1 blocks the BEN1-mediated step from TGN/EEs to RE (Robert *et al*, 2008).

Sorting into vacuoles: the final journey

In the endosomes, cargos can be sorted either to the BFA-sensitive GNOM-dependent recycling pathway back to the plasma membrane or towards the PVC/MVB (Figure 2). The PVC/MVB, also known as the late endosomes, functions as a sorting station for receptors and ligands on their way to the vacuole. The elucidation of trafficking pathways towards the vacuole was assisted by chemical inhibitors. Wortmannin (WM), an inhibitor of phosphatidylinositol 3-kinase (Emans *et al*, 2002), predominantly targets PVC-based trafficking as manifested in the strong effect on vacuolar trafficking of PIN2 as well as additional plasma membrane proteins (Jaillais *et al*, 2006; Kleine-Vehn *et al*, 2008c). In addition to WM, BFA also affects vacuolar targeting as both BFA-treated wild-type and BFA-resistant versions of GNOM reduced the PIN2 targeting to the lytic vacuole (Kleine-Vehn *et al*, 2008c). Similar results were obtained using a constitutively active version of an ARF-GEF substrate ARF1. All these results argue for the existence of BFA-sensitive, ARF-GEF-mediated trafficking from endosomes to the vacuole (Kleine-Vehn *et al*, 2008c).

As was shown for PIN2, BFA also inhibited the arrival of BRI1 and BOR1-GFP to the vacuole (Geldner *et al*, 2007; Takano *et al*, 2010), suggesting that vacuolar trafficking of BOR1 and BRI1 involves BFA-sensitive endosomes in a pathway shared with PIN2. Tyrosine-based signals in BOR1 control the turnover of the protein from the plasma membrane to the vacuoles via endocytic trafficking (Takano *et al*, 2010). It will be interesting to see whether similar tyrosine motifs are present in PIN2 and BRI1 and to what extent they are needed for sorting to different cellular destinations. Alternatively, phosphorylation, monoubiquitination, or polyubiquitinations could act as the tags for different sorting events but their exact function in different PIN trafficking events is still unclear.

In addition to ARF-GEF proteins, which are probably involved in specific vacuolar targeting pathways, transport of cargo molecules to the lytic vacuole requires vacuolar-sorting receptors (VSRs). In order to save energy and reduce waste, plant cells recycle VSRs after dissociation from their ligands (Seaman, 2005). This recycling is mediated by the retromer, a heteropentameric complex consisting of a dimer of sorting nexin proteins (SNX) and a trimer composed of vacuolar protein sorting (VPS) 26, VPS29 and VPS35 proteins. The SNX dimer recruits the complex to the endosomes, whereas the VPS triple subcomplex is proposed to bind the cargo (Seaman, 2005; Bonifacino and Hurley, 2008; Collins, 2008).

The retromer components SNX1 and VPS29 are important for plant growth and development as their mutants show pronounced auxin-transport-related phenotypes (Jaillais *et al*, 2006, 2007). SNX1 and VPS29 had originally been proposed to function directly in the recycling of PIN2 (Jaillais *et al*, 2006) and PIN1 (Jaillais *et al*, 2007) at the plasma membrane; however, an alternative interpretation of their function in PIN protein trafficking had been provided (Jürgens and Geldner, 2007). Consistent with this alternative view, a subsequent study revealed that SNX1 and VPS29 may rather act in a pathway that retrieves PIN proteins from the PVC back to the recycling pathways and thus regulates the balance between vacuolar targeting and recycling of PIN proteins (Kleine-Vehn *et al*, 2008c). This is in agreement with studies in yeast and animals systems where the retromer is required for recycling of VSRs back to the TGN (Seaman, 2005; Bonifacino and Hurley, 2008). This view also fits with the observation that SNX1, VPS26, VPS29 and VPS35 proteins all localize to the PVC membranes (Jaillais *et al*, 2008). However, recent immunofluorescence and immunogold electron microscopy experiments indicate that both subunits of the retromer localize to the TGN (Niemes *et al*, 2010b). Moreover, new data show that genetic interference with SNXs inhibits ER export of VSRs to the TGN, suggesting that the retromer functions in recycling the VSRs from the TGN to the ER (Niemes *et al*, 2010a).

It still needs to be seen whether all the reported observations in plants involve the same mechanism or whether the putative plant retromer complex regulates several trafficking pathways (e.g. PVC to TGN or/and TGN to ER). The latter hypothesis has already been demonstrated in animals where the retromer mediates both recycling of the mannose 6-phosphate receptors after release of their ligands from late endosomes towards the TGN (Arighi *et al*, 2004; Carlton *et al*, 2004), and of the Wntless receptor from the plasma membrane to the Golgi cisternae after secretion of its ligand Wingless (Franch-Marro *et al*, 2008). Nevertheless, steadily increasing evidence suggests that the multi-faceted TGN is the main sorting hub for the designation of vacuolar cargos.

Having reached the PVC/MVB, plasma membrane proteins have to be incorporated into the lumen of their transporting endosomes to allow subsequent lytic degradation in the lumen of the vacuole. This internalization is mediated by the endosomal-sorting complexes required for transport (ESCRT) machinery (Piper and Katzmann, 2007). The ESCRT device thus counteracts the function of the retromer. Although the retromer rescues transmembrane proteins from the endosomal membrane and returns them to the TGN, ESCRT proteins sort them from the membrane into the lumen of the endosomes. Genetic interference with the ESCRT-related CHMP1A and CHMP1B proteins comprises the sorting of PIN1, PIN2 and AUX1 into the lumenal vesicles of the PVC, leading to a retention of the proteins in the PVC membrane and their accumulation in the tonoplast upon PVC vacuole fusion (Spitzer *et al*, 2009). As a result, *chmp1a chmp1b* mutant embryos fail to establish normal auxin gradients, resulting in severe embryonic and post-embryonic developmental defects (Spitzer *et al*, 2009).

Cytoskeleton: the Tom Thumb cherrystones for vesicles

As in other eukaryotes, the plant cytoskeleton consists of actin filaments and microtubules. It provides the structure and the shape of the cell and has an important function in intracellular trafficking as it guides trafficking vesicles towards their destinations as well as mediating the movement and positioning of whole organelles (Petrasek and Schwarzerova, 2009; Szymanski, 2009). Because of this general function in cellular processes, both genetic and pharmacologic experiments with cytoskeleton interference should be interpreted with care. Nevertheless, we comment here on the reported findings regarding the function of the cytoskeleton in subcellular trafficking.

It has been shown that cytochalasin D and latrunculin B (LatB), which both depolymerize actin filaments, inhibit BFA-induced intracellular PIN1 accumulation as well as its

recycling back to the plasma membrane following BFA wash out, suggesting that PIN1 cycling is actin dependent (Geldner *et al*, 2001). The same holds true for AUX1 and PIN2: upon LatB treatment both proteins can be detected in intracellular agglomerations (Kleine-Vehn *et al*, 2006, 2008b). PIN2 vacuolar trafficking also depends on actin-mediated vesicle transport (Kleine-Vehn *et al*, 2008c). There is a tendency for the localization of apical cargos, including AUX1 and apically localized PIN proteins to be more sensitive to interference with actin as compared with basal PIN1 targeting (Kleine-Vehn *et al*, 2006, 2008b). On the other hand, microtubules do not seem to have a direct function in trafficking of AUX1 or PIN proteins in interphase but are required to deliver these cargos to the forming cell plate during cell division (Geldner *et al*, 2001). Thus, the observed polarity defects after interference with microtubules (Boutte *et al*, 2006) are presumably the indirect result of general cell shape and cell division defects but the exact function of microtubules in different trafficking processes still needs to be addressed.

Thus, though our information in plant cells is still limited, it appears that the actin cytoskeleton has a major function in different subcellular trafficking pathways, whereas the microtubules are required more during cell division and for structural cellular functions.

Phosphorylation-related signals for polar targeting

Polar targeting of PIN proteins is an ideal study system to elucidate the mechanisms behind distinct polar localizations, as PIN proteins have different localizations depending on the protein and the cell type. PIN proteins travel by BFA-sensitive and -insensitive cellular pathways towards their transient destinations; however, these mechanisms involve general cellular trafficking pathways that are not specific to PIN proteins or polar cargos. A landmark observation in understanding the mechanisms underlying the polar delivery of proteins was that a sequence disruption within the middle hydrophilic loop of PIN1 caused a basal-to-apical shift in its localization (Wisniewska *et al*, 2006). This strongly indicated that it is not only the trafficking pathways *per se* but rather the specific polarity signals within the protein sequence that determine the localization of the protein at distinct polar domains. In line with this, an increasing number of findings suggest that the PIN polarity signals are related to the phosphorylation sites found in the PIN sequences (Friml *et al*, 2004; Michniewicz *et al*, 2007; Kleine-Vehn *et al*, 2009; Huang *et al*, 2010; Zhang *et al*, 2010).

The Ser/Thr protein kinase PINOID (PID) has been shown to directly phosphorylate the hydrophilic loop of PIN proteins *in vivo* and *in vitro*, whereas the protein phosphatase 2A (PP2A) antagonizes this action (Michniewicz *et al*, 2007). High levels of PIN phosphorylation, as achieved by overexpression of PID (Benjamins *et al*, 2001; Friml *et al*, 2004) or inhibition of PP2A activity (Michniewicz *et al*, 2007), lead to preferentially apical PIN targeting. Consequently, the characteristic polarization of PIN1 to the basal sides of embryonic cells and resulting switch in auxin-transport direction at the globular stage does not occur, resulting in a defective auxin distribution that leads to the misspecification of the hypophysis and rootless seedlings (Friml *et al*, 2004). On the other hand, low phosphorylation levels in the *pid* mutants result in a preferentially basal PIN targeting as shown for example at the shoot apical meristem, which ultimately yields *pin1*-like inflorescences (Friml *et al*, 2004; Treml *et al*, 2005). Hence, the balance of phosphorylation and dephosphorylation activities appears to determine if the PIN proteins will be recruited in the apical or in basal polar targeting pathway (Figure 3).

Recently, three evolutionary conserved TPRXS(S/N) motifs within the PIN1 hydrophilic loop were identified, and it was demonstrated that the central serine residues (S231, S252 and S290) are directly phosphorylated by PID (Huang *et al*, 2010). Moreover, the motifs are highly conserved among the Arabidopsis plasma membrane PIN proteins and among PIN1 homologues from other plant species. Inactivation of these Ser residues, by converting them to Ala, strongly compromised the capacity of these PIN variants to be phosphorylated by PID *in vitro* and resulted in a predominantly basal PIN1 targeting. Consequently, these mutations increased the auxin flow to the root tip and could rescue the early root meristem collapse characteristic of *PID* overexpressor roots. Vice versa, phosphorylation-mimicking PIN variants, produced by replacing Ser residues with Glu residues, led to a dramatic disturbance of the basal-localized PIN1 signal and reduced auxin flow towards the root tip (Huang *et al*, 2010). Neither the loss-of-phosphorylation nor phosphomimicking variants could rescue the defects of the *pin1* mutants, indicating that reversible phosphorylation of the conserved Ser residues by PID is required for proper PIN1 localization and thus auxin-mediated plant development (Friml *et al*, 2004). In addition to the phosphorylation targets of PID, two other phosphorylation targets (Ser337 and Thr 340) in the PIN1 hydrophylic loop were shown to be functionally important for polar PIN localization and resulting auxin flow *in planta* (Zhang *et al*, 2010). However, it is unclear whether these residues are directly phosphorylated by PID and therefore indicates that other protein kinases could coordinately regulate PIN polarity with PID. Indeed in addition to PID, several other protein kinases have been shown to be involved in auxin-transport-regulated plant development (Zourelidou *et al*, 2009) and the complete loss of PIN phosphorylation led not only to PID-related morphological and cellular defects, but also to defects in processes that are not regulated by PID (Huang *et al*, 2010).

To date, it is unclear where in the cell the (de)phosporylation and polar sorting of cargos occurs. PID colocalizes with PINs at the plasma membrane (Kleine-Vehn *et al*, 2009) but the polar sorting of cargos presumably takes place at the TGN/EE (Viotti *et al*, 2010; discussed above). This suggests that polar PIN distribution is regulated by PID at another subcellular destination than the actual endosomal-sorting event and implicates that PID might not affect the polar targeting of *de novo* synthesized PIN proteins. In line with this, it was shown that newly formed PIN proteins are secreted in an apolar manner and subsequently undergo endocytic-recycling-dependent polarization (Dhonukshe *et al*, 2008; Men *et al*, 2008). In contrast to PID, PP2A is broadly distributed in the cell (Michniewicz *et al*, 2007) and its place of action is still unclear.

As the ARF-GEF GNOM mediates preferentially basal polar targeting, genetic or pharmacological interference with GNOM function leads to a basal-to-apical PIN polarity shift similar to that observed in *pp2a* loss-of-function and *PID* gain-of-function lines. These polarity changes also correlate

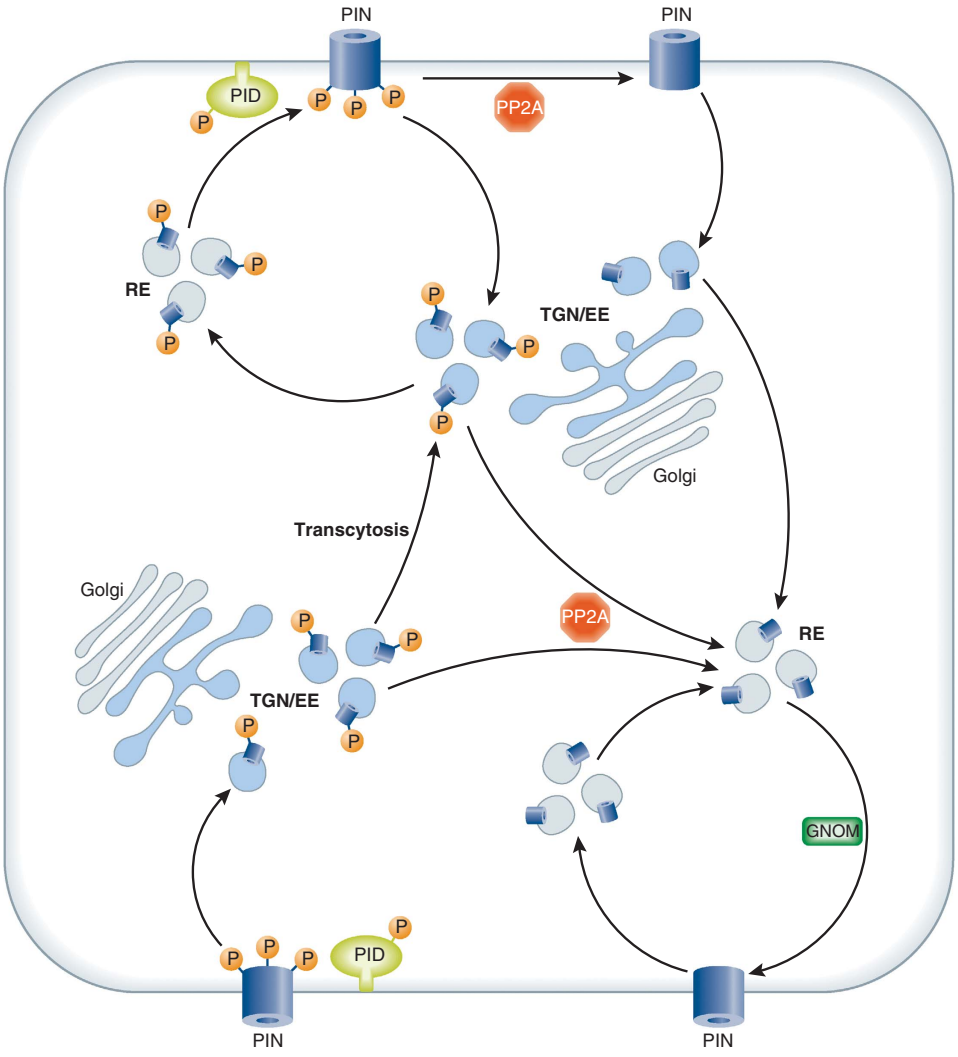

Figure 3 Schematic representation of transcytosis and phosphorylation-dependent polarity changes. EE, early endosomes; P indicates phosphorylated protein; PID, PINOID; RE, recycling endosomes; TGN, trans-golgi network.

with the auxin distribution and developmental defects seen in these mutants (Benjamins *et al*, 2001; Friml *et al*, 2004; Michniewicz *et al*, 2007). These observations suggest that both GNOM and PID/PP2A act antagonistically at the cellular and plant level and are part of the same mechanism of polar PIN delivery (Kleine-Vehn *et al*, 2009). PID does not appear to alter either GNOM localization or its activity and PID is still active in BFA-treated cells. The current working model to explain the interaction between these regulators is that phosphorylated PIN proteins have a decreased affinity for the GNOM-dependent basal recycling pathway and increased affinity for a distinct apical targeting pathway. This scenario allows for different signals to modulate, via regulation of PID activity, the trafficking and thus polar distribution of PIN proteins thereby regulating direction of auxin fluxes and auxin distribution-dependent development.

Lateral targeting of non-PIN polar cargos

Besides auxin transporters, other plasma membrane proteins such as NIP5;1, BOR1/4, ABCB37/PIS1 and ABCB36/PEN3 also show polar localization (Takano *et al*, 2005, 2010; Langowski *et al*, 2010). However, these proteins do not show the apical/basal polarity typical of PIN proteins but are instead localized at the lateral sides of the cell. The concomitant localization of apical, basal and lateral cargos in the same cells (Langowski *et al*, 2010) demonstrates the complexity of polar targeting pathways in plant cells. Although laterally localized proteins can be involved in certain developmental processes, for example root hair initiation as exemplified by Rho-of-plant GTPases (Molendijk *et al*, 2001; Jones *et al*, 2002), the more recently identified, lateral targeted proteins appear to be crucial for other physiological processes, including nutrient uptake from soil and interaction with pathogens (Langowski *et al*, 2010).

NIP5;1 and BOR1/4 are, respectively, import and export proteins of boron, an essential plant nutrient that is taken up from the soil and transported to the shoot (Takano *et al*, 2002, 2010; Miwa *et al*, 2007). Although NIP5;1 and BOR4 are expressed in and localized at the outer lateral side of root epidermal cells, BOR1 is expressed in the inner root tissues and acts as a boron exporter for xylem loading (Takano *et al*, 2005, 2010; Langowski *et al*, 2010). ABCG37 exports the plant hormone IBA (Ito and Gray, 2006), and was also detected at the outer sides of epidermal cells similar to PEN3/ABCG36, whose function is related to pathogen-defence (Stein *et al*, 2006).

Although studies on the outer and inner lateral targeting pathways have only just been initiated, it already appears to be well established that these lateral targeting pathways differ mechanistically from the apical–basal targeting machinery (Langowski *et al*, 2010). For example, BFA treatment leads only to a weak internalization of BOR4, PIS1 and PEN3 into BFA bodies and these accumulations disappear when protein synthesis is inhibited. This suggests BFA-sensitive secretion of these proteins but not necessarily constitutive recycling. Furthermore, the secretion seems to establish the outer lateral polarity directly (Langowski *et al*, 2010) without subsequent need of internalization and polarized recycling as in the case of PIN proteins (Dhonukshe *et al*, 2008). In line with this, interference with the actin cytoskeleton affects secretion but not the lateral localization of BOR4, PIS1 or PEN3 that has already been secreted to the plasma membrane. Finally, delivery to the lateral outer domain does not require the currently identified molecular components of polar trafficking such as AXR4, GNOM or PID. These finding thus argue for the existence of distinct trafficking pathways involving distinct molecular components.

In contrast to the outer lateral cargos, inner lateral localization of BOR1 is strongly sensitive to BFA treatment, indicating constitutive cycling of inner lateral cargos (Takano *et al*, 2005). Moreover, specific tyrosine motifs (Y398 and Y405) contribute to the inner polar localization of BOR1. Mutating these tyrosine residues in BOR1 inhibited not only its vacuolar targeting but also its polar localization, suggesting that these tyrosine residues are important for the post-endocytic polar trafficking of BOR1. It remains to be seen if tyrosine motifs are present and also needed for the polar localization of outer, apical and basal cargos.

In contrast to apical–basal targeting mechanisms, where we have obtained important insights in the last decade, our knowledge of inner–outer polar targeting is still limited. Nonetheless, these studies (Alassimone *et al*, 2010; Langowski *et al*, 2010; Takano *et al*, 2010) clearly establish the high complexity of polar targeting mechanisms in plant cells as compared with other eukaryotes and demonstrates the importance of inner–outer targeting mechanisms for multiple, plant-specific physiological and developmental functions.

Retention at polar domains: staying put without tight junctions

The apical, basal, inner and outer polar localizations of different proteins show that plant polar targeting machineries are more complex than in animal cells. However, there is also another fundamental difference between animal and plant cell polarity that is related to the retention of cargoes at polar domains. The plasma membrane is not a static environment but consists of a fluid lipid bilayer enabling efficient intramembrane diffusion of plasma membrane proteins (Simons and Vaz, 2004) that makes a stable polar plasma membrane domain difficult to realize. In animal epithelial cells, plasma membrane domains are separated by tight junctions (Shin *et al*, 2006). In addition to their function in forming tight seals between epithelial cells and creating a selectively permeable barrier for diffusion through the intercellular space, tight junctions demarcate the boundary between the apical and baso-lateral membrane domains and thus serve as a barrier to intramembrane diffusion of proteins and macromolecules between the apical and baso-lateral membrane domains (Shin *et al*, 2006). In plant cells, with exception of the so-called casparian strip that forms a diffusion barrier between inner and outer polar domain of differentiated root endodermis cells (Alassimone *et al*, 2010), no obvious tight junctions-like barriers have been detected. To cope with the absence of diffusion barriers in the plasma membrane and at the same time to allow more complex polar fragmentation of the plasma membrane, plants have probably evolved extra-regulatory mechanisms.

One mechanism that maintains polarity in the absence of tight junctions might be the constitutive recycling of polar-localized plasma membrane proteins such as apical PIN2, basal PIN1 or inner lateral BOR1. The constitutive cycling of PIN proteins has been hypothesized to be needed for fast polarity changes in response to developmental or environmental signals (Kleine-Vehn and Friml, 2008). However, without taking anything away from this hypothesis, another and possibly more original function of this process could be to maintain the PIN polar localization without tight junctions: laterally diffused PINs can re-establish their initial polar localization upon internalization followed by polar recycling. In line with this, *Saccharomyces cerevisiae* cells also lack diffusion barriers during mating, yet the localization of plasma membrane proteins to the tips of polarized mating intermediates is maintained because endocytic recycling occurs faster that protein diffusion in the plasma membrane (Valdez-Taubas and Pelham, 2003).

Another possible way to maintain polarity is to limit diffusion of proteins within the plasma membrane by providing the plasma membrane itself with a more rigid structure. Indeed, multiple observations confirm that polar localization of plasma membrane proteins also depends on the composition of the plasma membrane. Disturbing PAS1 function, required for very long-chain fatty acids, led to mislocalization of PIN proteins and consequential developmental defects (Roudier *et al*, 2010). Polar localization of PIN proteins is also impaired in several sterol biosynthesis mutants in Arabidopsis including *sterol methyltransferase1/2/3* and *cyclopropylsterol isomerase1-1* (*cpi1-1*) (Willemsen *et al*, 2003; Men *et al*, 2008; Carland *et al*, 2010). The function of plasma membrane sterol composition has been nicely demonstrated in the (re)establishment of PIN polarity following cell division (Men *et al*, 2008). During cytokinesis, PIN proteins localize to the newly formed cell plate (Geldner *et al*, 2001; Dhonukshe *et al*, 2006) and after completion of cytokinesis they need to be removed from one side to re-establish the asymmetric localization. It has been shown that PIN2 frequently remains at both daughter membranes in the sterol biosynthesis mutant *cpi1-1* (Men *et al*, 2008). Similarly, filipin, which binds sterols, affects endocytosis of plasma membrane proteins including PIN2 (Grebe *et al*, 2003; Kleine-Vehn *et al*, 2006; Men *et al*, 2008). Interestingly, sterols also undergo BFA-sensitive endocytic trafficking and the observation that they colocalize with PIN2 in endosomal vesicles, indicates that both sterol and PIN2 trafficking follow a common BFA-sensitive endocytic pathway (Grebe *et al*, 2003). It furthermore strengthens the notion that the polarity of PIN and other plasma membrane proteins at least partly depend on sterol function. Indeed detergent-resistant, specific membrane microdomains, so-called 'lipid rafts', are enriched in sterols and are important for various

types of plasma membrane based signalling processes in higher plants including auxin transport (Titapiwatanakun and Murphy, 2009).

Thus, although detailed, mechanistic knowledge is still missing, there are multiple trafficking- and membrane-composition-related conceptual possibilities regarding how polar cargos can be retained at the complex multitude of polar domains in plant cells.

Dynamic polarity switches: being flexible when it comes to changes

Transcytosis

Transcytosis can be defined as the dynamic translocation of polar cargoes between separated plasma membrane domains via RE and has been studied intensively in animal cells (Tuma and Hubbard, 2003).

A similar phenomenon can be induced in plant root cells during prolonged BFA treatments. As mentioned above, BFA preferentially interferes with basal PIN recycling by inhibiting the action of the ARF-GEF GNOM without affecting endocytosis, thereby inducing the accumulation of PIN proteins in so-called BFA bodies (Geldner *et al*, 2001). However, prolonged BFA treatments lead to an artificial recruitment of the basal PIN cargoes trapped in BFA bodies into the apical pathway. This was shown both by using protein synthesis inhibitors and by observing PIN movement in real-time using a photo-convertible PIN2-fluoresent-protein fusion (Kleine-Vehn *et al*, 2008a). Similarly, partial loss-of-function alleles of GNOM lead to a gradual appearance of the basal PIN cargoes at the apical side. These results demonstrate that the apical and basal trafficking pathways are interconnected and can be used by PIN proteins to change their polarity.

Interestingly, although phosphorylated PINs have a higher affinity to the apical pathway and will be transported accordingly (Kleine-Vehn *et al*, 2009), the basal-to-apical transcytosis is independent of PID-mediated PIN phosphorylation, as long BFA treatments still induce the basal to apical shift in *pid* mutants (Kleine-Vehn *et al*, 2009). These experiments suggest that there is only one trafficking pathway for basal targeting, namely the BFA-sensitive GNOM-dependent pathway, which is used by non-phosphorylated PIN1 proteins in the root stele. Shutting the basal trafficking pathway down by pharmacological or genetic interference with GNOM, leads to a recruitment of the basal cargoes in the non-basal trafficking pathways. Indeed after prolonged BFA incubation or in *gnom* mutants, cell files in the stele showed a basal-to-apical/lateral shift rather than the strict basal-to-apical switch observed in *PID* overexpression lines. This might indicate the existence of a BFA-insensitive, GNOM-independent trafficking pathway that enables targeting to the lateral membrane domains. Unfortunately, in order to provide evidence for this assumption, the apical and lateral trafficking pathways will need to be manipulated separately and this will depend upon first identifying these mechanisms.

Transcytosis induced by treatments with inhibitors, such as BFA, represents an artificial situation that solely shows the capacity of cargoes to move between the different polar trafficking pathways and does not address the endogenous function of transcytosis. In this respect, dividing cells are an ideal study system. During cell division, PIN proteins are targeted to the forming cell plate (Geldner *et al*, 2001) but after the fusion of the cell plate to the plasma membrane, one of the daughter cells will have the same PIN proteins at both the apical and basal side of the cell. In order to maintain the polarity of the mother cell in both daughter cells, the polar cargo at one side of the newly formed cell wall should be stabilized, whereas the cargo at the opposite side should be retrieved. Experiments using photo-convertible EosFP showed that following the completion of cell division, basal PIN2-EosFP translocates to the apical side of the cell thus re-establishing the apical PIN2 apical polarity of the mother in the daughter cells (Kleine-Vehn *et al*, 2008a).

Integration of developmental and environmental signals into PIN polarity switches

Although the function of transcytosis beyond the re-establishment of polarity after cell division remains to be documented, apparent translocations of PIN proteins from one side of the cell to another has been observed during the course of several developmental processes including adaptation responses to environmental signals such as gravity (Friml *et al*, 2002b, 2003b; Benkova *et al*, 2003; Reinhardt *et al*, 2003; Heisler *et al*, 2005). Below we will discuss how dynamic plasticity in plants utilizes relocation of the PIN protein polarity.

As mentioned earlier, PIN2 is localized to the apical side in root epidermal cells and to the basal side in young cortex cells contributing to the reflux of auxin towards to root tip, which is crucial in the control of root meristem size (Blilou *et al*, 2005). However, in older cortex cells that have differentiated and moved out of the meristem, PIN2 is targeted to the apical side in both epidermal and cortex cells (Kleine-Vehn *et al*, 2008b), arguing for the existence of a developmental trigger that induces the transcytosis of PIN2 from the basal to apical cell side. A similar event takes place during early embryogenesis. PIN7 is originally localized to the apical plasma membranes of the suspensor cells, transporting auxin towards the forming pro-embryo (Friml *et al*, 2003b). In the pro-embryo itself, auxin is evenly distributed by non-polar-localized PIN1. Later, from the globular stage on, the embryo is producing auxin autonomously (Cheng *et al*, 2006; Stepanova *et al*, 2008) and PIN1 and PIN7 polarize to the basal side of cells to redirect the auxin flow towards the future root pole (Friml *et al*, 2003b). Specifically, PIN1 polarizes to the basal side and PIN7 flips from the apical to the basal side (Figure 4A and B). These events presumably occur by the recruitment of both polar cargos into the GNOM-dependent basal targeting pathway. Accordingly, in strong *gnom* mutants this coordinated PIN polarization is perturbed (Steinmann *et al*, 1999) and *gnom* embryos show strong embryonic defects (Mayer *et al*, 1993; Shevell *et al*, 1994) reminiscent of quadruple *pin* mutants or embryos treated with auxin-transport inhibitors (Friml *et al*, 2003b).

In astonishing similarity to embryogenesis, root pericycle cells undergo a strict and organized division and patterning program in order to initiate and produce lateral roots (Peret *et al*, 2009). Given that the lateral root axis is at a right angle with that of the primary root, polarity has to switch at the lateral root initiation site. Indeed, during stage II of lateral root initiation, PIN1 polarity has already changed from the anticlinal to the periclinal cell sides (Figure 4C and D) providing a new axis for further growth and development.

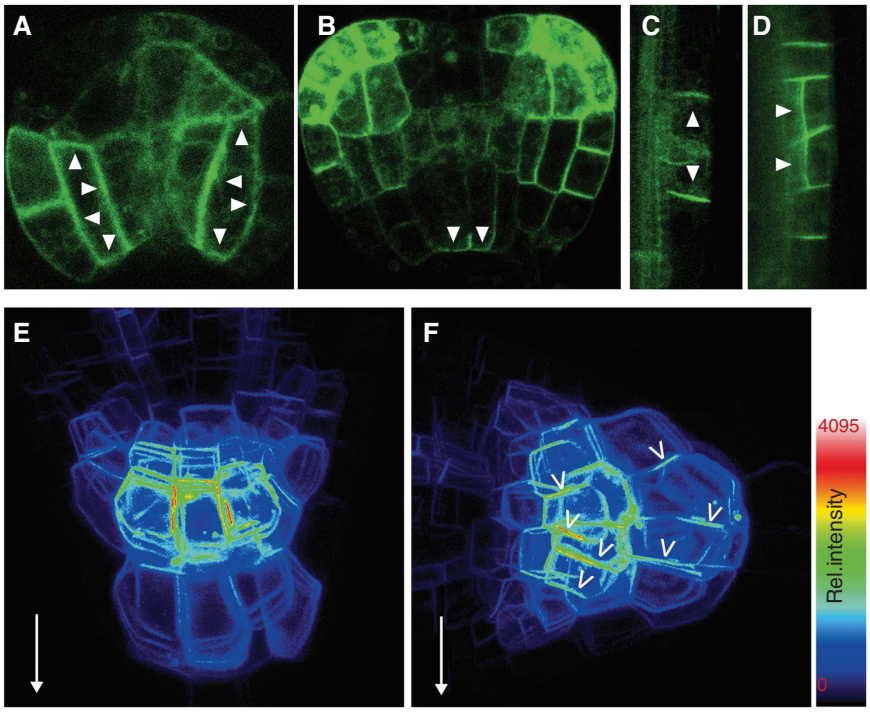

Figure 4 PIN polarity changes during plant development. (**A**, **B**) Apolar PIN1 localization in apical cells of a 16-cell stage embryo (**A**), changes to basal PIN polar localization in early heart stage embryos (**B**). (**C**, **D**) PIN1 polarity changes from the anticlinal (**C**) to the periclinal cell sides (**D**) during lateral root initiation. (**E**, **F**) PIN3 polarization in response to gravity. Arrowheads indicate polar localization; arrows illustrate the gravity vector.

As plants are sessile, they have evolved to be experts in adapting to changing environmental conditions by switching on auxin-regulated programs. The gravitropic response exemplifies in a beautiful way that polar targeting and subcellular trafficking are fundamental for the plant's plasticity. To a large extent, gravity sensing takes place within specialized cells called statocytes located in the root cap and the endodermis of hypocotyls and shoots. Statocytes contain dense, starch-filled amyloplasts, whose position or movement within the cell provide information about the organ's orientation relative to the gravity vector. Although the exact mechanism by which the movement of the amyloplasts is perceived is not yet known, upon changing the direction of the gravity vector and repositioning of the amyloplasts or statoliths, PIN3 localization polarizes towards the new bottom cell sides (Figure 4E and F) (Friml *et al*, 2002b; Harrison and Masson, 2008). This PIN3 translocation redirects the auxin flow leading to auxin accumulation at the lower side of the root, inhibition of growth and results in the root bending towards the gravity vector. It remains to be demonstrated, but it is plausible, that light can also regulate auxin-mediated phototropic bending via repolarization of PIN proteins.

Besides environmental signals, PIN polarity can also be modulated in response to endogenous signals. Classical theories such as the canalization hypothesis (Sachs, 1981) had already proposed that auxin acts as a polarizing signal that regulates both the rate and polarity of its own polar transport. Recent observations support the notion that this feedback regulation of auxin transport might occur at the level of PIN trafficking modulation. For example, accumulation of auxin after wounding induces PIN polarity changes and subsequent vasculature regeneration (Sauer *et al*, 2006), whereas rearrangements of PIN polarity have been observed during leaf vasculature formation (Scarpella *et al*, 2006). The underlying mechanism is unknown, but feedback regulation of PIN polarity by auxin itself is necessary in the numerous mathematical models describing different auxin-dependent patterning processes (Rolland-Lagan and Prusinkiewicz, 2005; Jonsson *et al*, 2006; Smith *et al*, 2006; Merks *et al*, 2007; Stoma *et al*, 2008; Bayer *et al*, 2009; Kramer, 2009; Prusinkiewicz *et al*, 2009). Furthermore, it has been shown that auxin inhibits internalization of PIN proteins, which leads to elevated PIN levels at the plasma membrane and thus to increased auxin efflux (Paciorek *et al*, 2005). By this mechanism, auxin regulates PIN abundance and activity at the cell surface, creating a direct feedback regulation on its own transport. It will be a challenge to elucidate the molecular mechanisms by which auxin regulates the subcellular trafficking of its transporters.

Although we still grope in the dark, it seems that soil microorganisms with both good and less good intentions have already deciphered the auxin-mediated code for plasticity of root development (Grunewald *et al*, 2009b). Consider plant-parasitic nematodes, which rely for their life cycle on the establishment of a nematode feeding site within the root vasculature of their host plants. During infection, the feeding site expands radially by incorporating its neighbouring cells. This process requires effective transport of auxin as both genetic and pharmacological interference with the auxin-transport machinery inhibit this lateral expansion (Goverse *et al*, 2000; Grunewald *et al*, 2009a). Interestingly, it could be shown that nematodes induce a basal-to-lateral PIN3 polarity switch, suggesting that they are able to hijack the mechanisms of PIN polar targeting and consequently the flow of auxin (Grunewald *et al*, 2009a).

All of these examples show that despite the lack of final proof for the involvement of transcytosis, many endogenous as well as external biotic and abiotic signals regulate plant development via modulation of PIN polar localization.

Conclusion

Many aspects of plasticity of adaptive plant development can be attributed to dynamic changes in polar localization of the PIN transporters of the plant hormone auxin in response to environmental changes. The polar, plasma membrane localization of PIN proteins determines the direction of auxin flow within tissues and thus can modulate different aspects of auxin distribution-mediated development, including gravitropism, phototropism, embryogenesis, organogenesis, vascular tissue formation and regeneration as well as others (Vanneste and Friml, 2009). A fine-tuned regulation of cellular polarity is, however, not only involved in auxin-transport-mediated development but also in the exchange of compounds between roots and the soil as well as in the interaction with pathogens as suggested by strict outer lateral localization of components of these processes (Alassimone *et al*, 2010; Langowski *et al*, 2010; Takano *et al*, 2010).

The simultaneous localization of apical, basal and lateral cargos in single plant cells (Langowski *et al*, 2010) demonstrates the complexity of polar trafficking pathways in plant cells as compared with other eukaryotes. Another intriguing plant-specific polarity issue concerns how plant cells retain the localized distribution of cargos at polar domains without the tight junctions that limit the lateral spreading of polar cargos in mammalian cells. It appears that the polarized distribution of proteins in plant cells depends on the coordinated contribution of at least three regulatory levels: (1) intrinsic protein-sorting signals and cellular decoding machineries determine to which plasma membrane domain the proteins are trafficked; (2) distinct intracellular trafficking pathways control the direction of membrane traffic to a given polar domain and (3) the membrane composition of distinct polar domains regulates which proteins can come or go.

Slowly, but progressively, more information is being gathered on all the different regulatory levels. However, the otherwise so successful forward genetic approaches in plants are limited when it comes to investigating dynamic endomembrane processes, because of extensive functional redundancy or lethality of the corresponding mutants. Valuable tools for unravelling these cell biological processes have come from chemical genomic approaches that have provided a battery of inhibitors that interfere with various vesicle trafficking processes (De Rybel *et al*, 2009; Hicks and Raikhel, 2009). These compounds have shown their utility in the past and will continue to do so in the future.

The major current challenges for our understanding of how subcellular dynamics including polar trafficking contribute to the regulation of development and physiology, concern the plant-specific modifications of the evolutionarily conserved subcellular trafficking machineries and the entry points for environmental and endogenous signals into this system.

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Conflict of interest

The authors declare that they have no conflict of interest.

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