Polar Targeting and Endocytic Recycling in Auxin-Dependent Plant Development

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Annu. Rev. Cell Dev. Biol. 2008. 24:447–73

The *Annual Review of Cell and Developmental Biology* is online at cellbio.annualreviews.org

This article's doi:
10.1146/annurev.cellbio.24.110707.175254

1081-0706/08/1110-0447$20.00

Key Words

trafficking, endocytosis, polar auxin transport, PIN proteins

Abstract

Plant development is characterized by a profound phenotypic plasticity that often involves redefining of the developmental fate and polarity of cells within differentiated tissues. The plant hormone auxin and its directional intercellular transport play a major role in these processes because they provide positional information and link cell polarity with tissue patterning. This plant-specific mechanism of transport-dependent auxin gradients depends on subcellular dynamics of auxin transport components, in particular on endocytic recycling and polar targeting. Recent insights into these cellular processes in plants have revealed important parallels to yeast and animal systems, including clathrin-dependent endocytosis, retromer function, and transcytosis, but have also emphasized unique features of plant cells such as diversity of polar targeting pathways; integration of environmental signals into subcellular trafficking; and the link between endocytosis, cell polarity, and cell fate specification. We review these advances and focus on the translation of the subcellular dynamics to the regulation of whole-plant development.

Contents

DEVELOPMENTAL INTRODUCTION

Polar auxin transport: the directional transport of the plant hormone auxin from cell to cell

Animals and plants evolved basic biological differences that characterize their survival strategies. Animals developed elaborate sensory and locomotory capacities that enable complex behavioral responses, such as the fight-or-flight response, to overcome environmental stress. In contrast, during their evolution plants emphasized increased physiological tolerance and phenotypic plasticity. These different life strategies are also adequately reflected in the various ways in which animals and plants establish their body architecture. Whereas during embryogenesis animals are already defining their adult shape to a large extent, in plants this early developmental phase just sketches a basic body plan, and the final shape of a plant will be largely defined by an elaborate postembryonic development (Weigel & Jürgens 2002). To achieve this developmental plasticity, plants maintain permanent populations of stem cells (meristems) at the growing root and shoot apices and are able to redefine the developmental programs as well as the polarity of already specified tissues. Thus, plants can sustain and regulate their growth rate, can postembryonically form new organs, and possess a high capacity for tissue regeneration (Steeves & Sussex 1989, Weigel & Jürgens 2002). Different animal species also retain these capabilities to some extent; however, plants are far superior in utilizing these mechanisms for individually shaping their body according to the demands of the environment. The plant signaling molecule auxin determines many aspects of this flexible plant development. Auxin acts as a prominent signal, providing, by its local accumulation in selected cells, a spatial and temporal reference for changes in the developmental program (Reinhardt et al. 2000, Friml 2003, Leyser 2006, Esmon et al. 2006, Tanaka et al. 2006, Dubrovsky et al. 2008). Auxin is distributed through tissues by a directional cell-to-cell transport system, termed polar auxin transport, that depends on specific auxin carrier proteins (**Figure 1**) (Benjamins et al. 2005, Blakeslee et al. 2005, Kramer & Bennett 2006, Vieten et al. 2007). Auxin efflux carriers of the PIN-FORMED (PIN) family (Gälweiler et al. 1998, Luschnig et al. 1998, Chen et al. 1998, Utsono et al. 1998, Petrášek et al. 2006) show a polar subcellular localization that correlates with and determines the

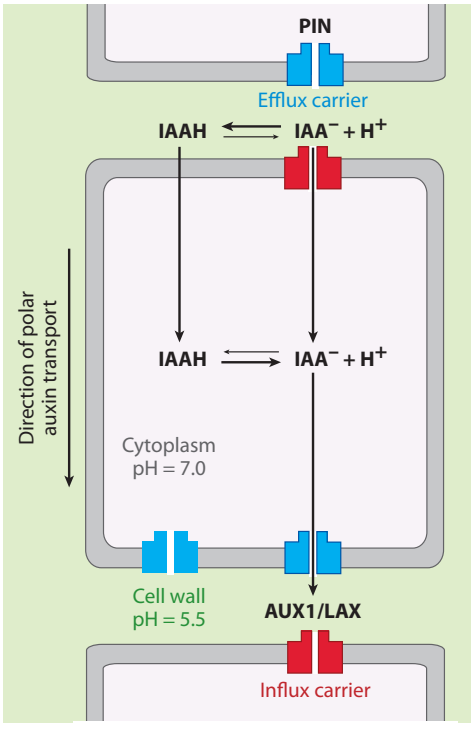

Figure 1

The chemiosmotic hypothesis: far ahead of its time! Rubery & Sheldrake postulated in the mid-1970s the so-called chemiosmotic hypothesis for directional intercellular auxin movement (Rubery & Sheldrake 1974 and, independently, Raven 1975). Accordingly, the auxin indole acetic acid (IAAH) is largely protonated at the lower pH of the cell wall and can pass through the plasma membrane into the cell. In the higher-pH cytosol, part of the IAAH is deprotonated, and the resulting charged IAA^- is largely membrane impermeable and requires transporter activity to exit the cell. The localization of the PIN-FORMED (PIN) auxin efflux carrier at the plasma membrane determines the auxin exit site from an individual cell. Coordinated polar localization of PINs in a given tissue hence determines the direction of cell-to-cell auxin transport. AUX1/LAX1 denotes auxin influx carriers AUXIN RESISTANT1/LIKE AUX1.

direction of auxin flow through tissues (Friml et al. 2004, Wiśniewska et al. 2006). In plants, polarities of tissue and of individual cells are closely connected by the flow of auxin (Sauer et al. 2006), and the cell biological processes depending on vesicle trafficking and polar targeting have an immediate developmental output related to auxin-mediated signaling. At the level of polar auxin transport, many developmental and environmental signals are integrated. By rearranging the subcellular localization of PIN auxin efflux carriers, such signals influence auxin-dependent patterning and contribute substantially to the adaptive and flexible nature of plant development.

Our aim is to review recent advances on subcellular trafficking and polar targeting in plants and to highlight links with physiology and development. A special focus is given to auxin-dependent regulation of development because this area is intimately linked to endocytic recycling and polar targeting. Most of these concepts were formulated on the basis of studies in the model plant *Arabidopsis thaliana*; nonetheless, they seem to apply to a large extent to higher plants in general.

Recycling: membranes and other molecules recycle from intracellular endocytic compartments back to the plasma membrane

Tight junctions: anchored protein complexes forming a physical barrier between polar domains; limit lateral diffusion and are involved in polarity establishment and maintenance in animal epithelial cells

POLAR TARGETING

The establishment and maintenance of cell polarity are central themes of developmental and cell biology because individual cell polarities, transmitted by cell divisions, are translated into tissue and organ polarity and, ultimately, shape. In addition, cell polarity plays a key role in directional signaling and intercellular communication.

At the level of individual cells, polarity is typically reflected by the asymmetric distribution of intracellular components that can form functionally and/or morphologically distinct domains (Bonifacino & Lippincott-Schwartz 2003). Mechanisms for generating or maintaining cell polarity have been extensively studied in different model organisms, such as worms, flies, mammals, and yeasts (e.g., Knoblich 2000, Irazoqui & Lew 2004, Margolis & Borg 2005, Nance 2005). Animal epithelial cells are a favorite model system because their plasma membrane harbors two distinct domains that are separated by tight junctions: an apical domain facing the lumen and a basolateral domain (Mostov et al. 2003, Janssens & Chavrier 2004). These protein-based barriers in the membrane prevent lateral diffusion of proteins and lipids

trans-Golgi network (TGN): the main sorting compartment of the secretory pathway in eukaryotic cells; may act as an early-endosomal compartment in plants

between the two distinct polar domains, maintaining the distribution of various polar-competent proteins. Researchers have identified numerous polar cargos that reside in a cell-line-specific manner preferentially at the apical and/or basolateral plasma membranes in polarized epithelial cells. Apical and basolateral components are recruited differentially by the targeted delivery of membrane and secretory proteins to these domains as a result of three processes. (*a*) Newly synthesized proteins are sorted in the trans-Golgi network (TGN) into carrier vesicles that specifically deliver them to the apical surface or the basolateral surface. (*b*) Some proteins are selectively retained at the plasma membrane. (*c*) Proteins that are not retained are rapidly endocytosed and either recycled back through recycling endosomes or, alternatively, delivered to a different, polar plasma membrane domain by a process called transcytosis (Rodriguez-Boulan et al. 2005).

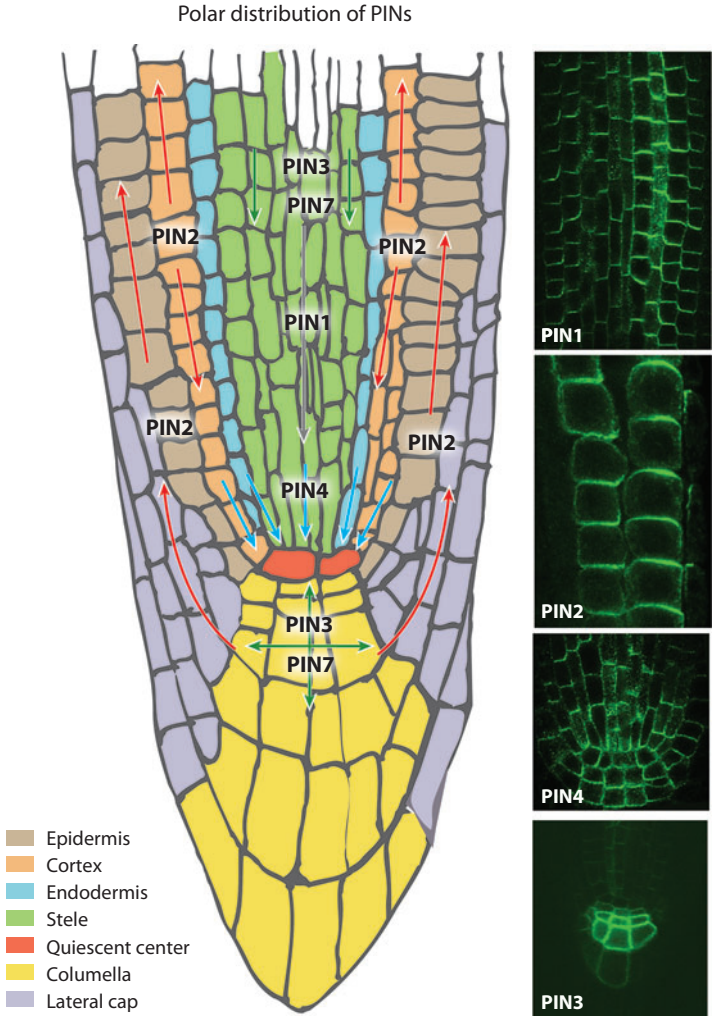

Figure 2

Patterns of PIN protein localization in the *Arabidopsis* root tip. Schematic and immunolocalizations of PIN proteins in the *Arabidopsis* root tip. Arrows indicate polar PIN localization at the plasma membrane, illustrating cell type–dependent decisions in the PIN polar localization. Note the differential PIN2 targeting in the epidermis (apical) and young cortex (basal) cells.

Passengers and Destinations: Polar Cargos and Polar Domains

Even though in no other kingdom is the relation between individual cell polarity and macroscopic patterning as prominent as in plants, knowledge on cell polarity and mechanisms of targeted cargo delivery is still lacking in plants. Most of our understanding on polar targeting has been gained by study of the polar delivery of auxin efflux carriers from the PIN family (**Figure 2**). PIN proteins have emerged in recent years from genetic studies in *A. thaliana* as key regulators of a plethora of auxin-mediated developmental processes, including axis formation in embryogenesis (Friml et al. 2003b), postembryonic organogenesis (Okada et al. 1991, Benková et al. 2003, Reinhardt et al. 2003, Heisler et al. 2005), root meristem maintenance (Friml et al. 2002a, Blilou et al. 2005), vascular tissue differentiation and regeneration (Gälweiler et al. 1998, Sauer et al. 2006, Scarpella et al. 2006), and tropic growth (Luschnig et al. 1998, Friml et al. 2002b).

PIN proteins act as mediators of the auxin efflux from cells (Petrášek et al. 2006) and have different subcellular distributions—including apolar, basal, apical, and lateral plasma membrane localizations—depending on the PIN protein as well as the cell type (Wiśniewska et al. 2006). The most typical are basal (root tip–facing) localization of the PIN1 protein in the inner cells of both shoots and roots, apical (shoot apex–facing) localization of PIN2 in the

root epidermis and lateral root cap cells, and lateral localization of PIN3 at the inner side of shoot endodermis cells (Gälweiler et al. 1998; Müller et al. 1998; Friml et al. 2002b, 2003a).

Other components of auxin transport, such as the auxin influx carrier AUXIN RESISTANT1/LIKE AUX1 (AUX1/LAX) (Bennett et al. 1996, Yang et al. 2006, Swarup et al. 2008) and multiple drug resistance/P-glycoprotein (MDR/PGP) transporters (Geisler et al. 2005, Terasaka et al. 2005), are also localized in a polar manner in some cells while being symmetrically localized in most cells (Mravec et al. 2008). For example, AUX1 localizes to the apical side of protophloem cells opposite to PIN1 or to the same side as PIN1 in the shoot apical meristem (Swarup et al. 2001, Reinhardt et al. 2003). In contrast, PGP4 has a basal or an apical localization in root epidermal cells (Terasaka et al. 2005).

In addition to components of the auxin transport, other polar cargos in plants, including transporters for boron (BOR1 and BOR4) and for silicon in rice (LSI1 and LSI2), have been identified. Such cargos are localized at either the inner or the outer lateral sides of cells, as well as the regulator of anisotropic expansion, COBRA, which is similarly polarly targeted to both longitudinal cell sides (Roudier et al. 2005; Takano et al. 2005; Ma et al. 2006, 2007; Miwa et al. 2007). The PLEIOTROPIC DRUG RESISTENCE (PDR)-type transporter for the auxin-like compounds PIS1/PDR9 resides at the outer lateral side of root epidermis cells. The lateral cargo POLAR AUXIN TRANSPORT INHIBITOR-SENSITIVE1 (PIS1), the basal cargo PIN1, and the apical cargo PIN2 have been simultaneously visualized in the same cells, highlighting that plant cells are able to maintain at least three polar domains within a single cell (Růžička et al. 2008). Future studies will address whether epidermal root cells are potent to maintain, besides the apical, basal, and outer lateral domains, an additional inner lateral polar domain. Nonetheless, although apical-basal targeting in plants and apical-basolateral delivery in animals can reflect a comparable polar competence among the divergent kingdoms, the simultaneous delivery of lateral cargos hints at a more complex situation for cell polarity in plant cells that may once again stress the flexibility and enormous importance of cell polarity regulation in plants (**Figure 3**).

Endocytosis: the uptake of material into a cell by the formation of a membrane-bound vesicle

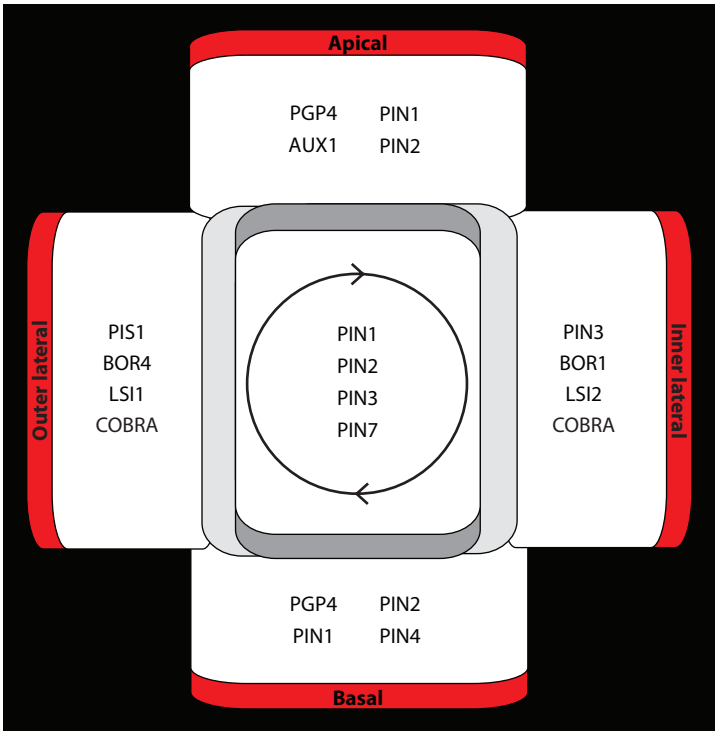

Figure 3

The black box of plant polarity. A schematic representation of various polar cargos in plant cells. Plants are competent to deliver cargos to apical, basal, inner, and outer polar domains. Apical cargos include PGP4 (root epidermis cells), AUX1 (root protophloem cells), PIN1 (epidermal cells of shoot apex and embryonal protoderm cells), and PIN2 (root epidermis and older cortex cells). Basal cargos encompass PGP4 (root epidermis cells), PIN1 (e.g., root stele), PIN2 (young cortex cells), and PIN4 (in the proximal part of the root meristem). Outer lateral cargos are represented by, e.g., PIS1 (root epidermis), BOR4 (root epidermis), LSI1 (root exodermis and endodermis cells), and COBRA (root epidermis). Inner lateral polarity can be defined by PIN3 (shoot endodermis and root pericycle), BOR1 (root pericycle cells), LSI2 (root exodermis and endodermis cells), and COBRA (root epidermis). Moreover, several PIN cargos undergo rapid polarity alterations (depicted in the *middle*), including the establishment of basal localization of PIN1 during embryogenesis or lateral root development, an apical-to-basal polarity shift of PIN7 during embryogenesis in suspensor cells, a basal-to-apical shift in upper cortex cells of PIN2, and dynamic relocation of PIN3 to the bottom sides of root cap cells after gravity stimulation.

Transcytosis: the dynamic translocation of the same molecules from one distinct plasma membrane domain to another via recycling endosomes

Basal polarity: polarity of the lower cell side, the polar plasma membrane domain that faces the root apex

Apical polarity: polarity of the upper cell side, the polar plasma membrane domain that faces the shoot apex

Inner lateral polarity: polarity of the inner periclinal cell side, which points away from the body surface

Outer lateral polarity: polarity of the outer periclinal cell side, which points to the body surface

Tickets to Go or to Stay: Polar Targeting Signals

In animal systems, polar cargo proteins carry signals that determine their residence at different polar domains. These signals may be a combination of plasma membrane retention, internalization, and polar sorting signals (Dugani & Klip 2005, Rodriguez-Boulan et al. 2005). In plants, different polar cargos such as PIN1, PIN2, and PIS1 localize to different polar destinations in the same cell type, suggesting polarity determinants in the protein sequence itself. Moreover, an insertion of green fluorescent protein (GFP) at a specific position within the middle hydrophilic loop causes PIN1 localization to shift to the opposite side of the cell compared with wild-type PIN1 (Wiśniewska et al. 2006). These results demonstrate the presence of polarity signals in the sequence of polar cargos, but detailed insight is still lacking. Polarity signals probably decide to recruit PINs to the distinct apical and basal targeting machineries that are related to phosphorylation sides, because the Ser/Thr protein kinase PINOID (PID) (Friml et al. 2004) as well as the protein phosphatase 2A (PP2A) (Michniewicz et al. 2007) act on PIN phosphorylation and play a decisive role in the apical-versus-basal targeting of PIN proteins. Loss of the PID function causes an apical-to-basal shift in the PIN polarity corresponding with defects in embryo and shoot organogenesis (Christensen et al. 2000, Benjamins et al. 2001, Friml et al. 2004). Accordingly, PID gain of function results in an opposite basal-to-apical PIN polarity shift, leading to auxin depletion from the root meristem and collapse of the root growth (Friml et al. 2004). Similar phenotypes, including the basal-to-apical shift of PIN polarity, can be observed in the loss-of-function mutants of the A regulatory subunits of PP2A (Michniewicz et al. 2007). Importantly, PID directly phosphorylates the hydrophilic loop of PIN proteins, and PP2A antagonizes this action (Michniewicz et al. 2007).

A possible scenario may be that phosphorylated PIN proteins are preferentially recruited into the apical pathway, whereas dephosphorylated PINs become a substrate of the basal targeting pathway (**Figure 4**). This model incorporates important features of mammalian epithelial cells, in which cargos are phosphorylated to influence their polar delivery (Casanova et al. 1990). Importantly, phosphorylation-dependent PIN targeting provides a means for any signaling pathway upstream of PID and PP2A activities to modulate PIN polar targeting and thus directional auxin fluxes. Different relative expression levels of PID and PP2A in various cell types in combination with divergent

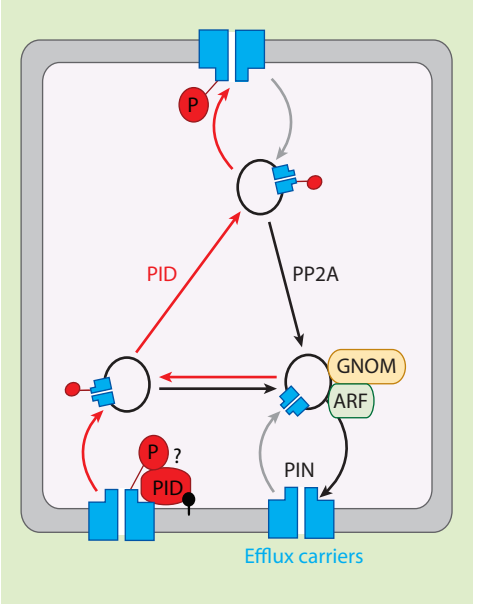

Figure 4

Contribution of PIN-FORMED (PIN) phosphorylation to the decision on the PIN polar distribution. PINOID (PID)-dependent phosphorylation of PIN proteins may affect affinity to distinct apical and basal targeting pathways. An increase in PID kinase or a decrease in protein phosphatase 2A (PP2A) activities leads to a basal-to-apical PIN polarity shift. On the contrary, increased PP2A activity counteracts the PID effect and leads to preferential GNOM-dependent basal PIN targeting. The place of PID and PP2A action is not entirely clear, but PID and PP2A are also partially associated with the plasma membrane. ARF denotes adenosyl ribosylation factor.

phosphorylation sites (some of which would be phosphorylated more or less efficiently) would explain how both PIN-specific and cell type–specific signals are integrated to determine the polar localization of the given PIN protein into a given cell type. The regulation of PID kinase may also be connected with phospholipid signaling. The plant 3-phosphoinositide-dependent kinase 1 (PDK1) binds PID in vitro and increases PID kinase activity (Zegzouti et al. 2006). The involvement of phosphorylation events for the polar delivery of other cargos besides PINs has not been thoroughly addressed. Outer lateral delivery of PIS1 seems to occur independently of the PID activity, but comparable information for other polar cargos is missing.

Extensive work in the coming years is expected to focus on the identification and thorough characterization of polar targeting signals for different polar cargos in plants. The other crucial issues that are completely unknown in plants concern where and how the polar targeting signals are recognized as well as where and how the polar cargos are sorted.

Staying at the Station: Retention at the Polar Domains

Despite the pronounced importance of polar localization of proteins in plant cells for plant development, mechanisms for this phenomenon are still ill defined. So far no indications exist for anything analogical to tight junctions, and we lack even fundamental knowledge of how polar-competent cargos are kept in their polar domains.

Cytoskeleton- and membrane sterol–dependent constitutive endocytosis and targeted recycling may be involved in maintaining the localization of proteins localized in their polar domains. All these cellular components and processes are required for localization of different cargos. Both basal PIN1 localization and even more apical AUX1 localization are sensitive to the disruption of the actin cytoskeleton, leading to internalization and loss of polar localization (Kleine-Vehn et al. 2006). In contrast, disruption of microtubules affects only indirectly the localization of AUX1 and PIN proteins that is observed only when the overall cell morphology is altered (Kleine-Vehn et al. 2006). In contrast, intact microtubules are required to maintain the outer lateral localization of PIS1; following its disruption, PIS1 is found predominantly at apical and basal positions (Růžička et al. 2008).

Polar localization of PIN and AUX1 proteins as well as auxin signaling depend on the sterol composition of plasma membranes (Souter et al. 2002, Grebe et al. 2003, Willemsen et al. 2003, Kleine-Vehn et al. 2006). *Arabidopsis* plantlets defective in the *STEROL METHYLTRANSFERASE1* (*SMT1*) gene, which is involved in sterol biosynthesis and affects membrane sterol composition, have cell polarity defects, including impaired polar localization of PIN proteins and AUX1 (Willemsen et al. 2003, Kleine-Vehn et al. 2006). Furthermore, sterols and PIN proteins have overlapping subcellular trafficking pathways (Grebe et al. 2003). Detergent-resistant, sterol-enriched plasma membrane microdomains, sometimes called lipid rafts, are important for various types of plasma membrane–based signaling processes and are present in higher plants as well (reviewed in Bhat & Panstruga 2005). There are indications that PIN and PGP proteins are directly associated with these structures (Titapiwatanakun et al. 2008), but what the functional relevance could be of such associations remains to be established.

In conclusion, despite the obvious requirements of cytoskeleton and sterol composition for polar localization of various cargos, it is still unclear whether or eventually in which cases they are involved in keeping cargos at their polar positions or whether they play a role in the polar delivery of these proteins to the plasma membrane.

How to Get There: Polar Targeting Pathways

The existence of diverse polar cargos with various polar targeting signals implies a diversified

Sterol: plant sterols are amphiphilic molecules and vital constituents of all membranes, including the plasma membrane

network of distinct polar targeting pathways. Indeed, for example, AUX1 and PIN1 polar delivery occurs by two distinct targeting machineries with different molecular requirements and different sensitivities to inhibitors of cellular processes (Dharmasiri et al. 2006, Kleine-Vehn et al. 2006).

An important factor for the delivery of PIN proteins to the plasma membrane is an endosomal regulator of the vesicle budding, GNOM, which encodes a guanine nucleotide exchange factor for adenosyl ribosylation factors (ARF GEF) (Shevell et al. 1994; Geldner et al. 2001, 2003). In *gnom* (also designated *emb30*) mutant embryos, the coordinated polar localization of PIN1 is impaired (Steinmann et al. 1999), seemingly the result of a failure to establish the initial basal localization of PIN1 at the globular stage (Kleine-Vehn et al. 2008a). Also, in the postembryonic roots, GNOM function seems to be crucial for basal targeting, whereas apical localization of PINs or AUX1 is unaffected in *gnom* mutants (Kleine-Vehn et al. 2006, 2008a). Collectively, these studies demonstrate that apical cargos utilize a targeting pathway that is molecularly distinct from that used by basally localized PIN proteins (Kleine-Vehn et al. 2006, 2008a). In addition, outer lateral PIS1 targeting appears to differ fundamentally from apical and basal pathways because PIS1 polar localization does not involve any known molecular components of apical/basal targeting, such as GNOM or PINOID (Růžička et al. 2008). Although apical and basal PIN targeting appears to be interconnected and, thus, to be used alternatively by PIN proteins, the relation between apical/basal and outer lateral polar targeting needs to be unraveled.

In summary, genetic and pharmacological interference with different cellular processes as well as the simultaneous localization of cargos to the apical, basal, and outer lateral domains in single cells strongly suggest that there are at least three distinct polar targeting mechanisms in plants. However, molecular insight into these pathways remains very limited.

Adenosyl ribosylation factor (ARF): a class of small GTPases; a regulator of clathrin- and COPI-dependent vesicle budding

Guanine nucleotide exchange factor (GEF): induces GDP-to-GTP exchange and hence the activation of small GTPases

Brefeldin A (BFA): a specific inhibitor of some ARF GEFs

ENDOCYTIC RECYCLING IN PLANT CELLS

The Back and Forth: Constitutive Endocytic Recycling of Plasma Membrane Proteins

The internalization of proteins from the plasma membrane is a critical event for all eukaryotic cells. Whereas many internalized molecules are degraded in the lysosomal/vacuolar pathway, other cell surface proteins and molecules undergo sequential rounds of recycling back to the plasma membrane. Eukaryotic cells possess the remarkable ability to turn over the entire plasma membrane on an hourly basis (Tuvim et al. 2001). As such, endocytic recycling is a key for the regulation of the cell surface identity and contributes to rapid cellular responses to intrinsic and extrinsic cues. Regarding the fundamental importance of endocytic recycling, various integral plasma membrane proteins, such as signaling components and transporters, appear to display recycling events to the plasma membrane in plants.

Pharmacological inhibitors have been valuable tools for unraveling the internalization of plant proteins to endosomal compartments and subsequent recycling back to the plasma membrane (Carter et al. 2004). The fungal toxin brefeldin A (BFA) interferes with various vesicle trafficking processes in cells and specifically targets ARF GEFs. Cytosolic GDP-bound ARF proteins are inactive and become recruited to the target membrane by ARF GEF–dependent GDP-to-GTP exchange. ARF proteins play an important role in the formation of vesicle coats required for their budding and cargo selection in different subcellular compartments. BFA is a noncompetitive inhibitor that stabilizes ARF/ARF GEF intermediates and freezes both proteins inactively at the place of action (reviewed by Donaldson & Jackson 2000). In cultured cells of tobacco, BFA interferes with ARF GEF–dependent endoplasmic reticulum (ER)-to-Golgi trafficking, leading to ER-Golgi hybrids (Ritzenthaler et al. 2002). In contrast, in *Arabidopsis*, this process is catalyzed by the

BFA-resistant ARF GEF GNOM-LIKE1 (GNL1) (Richter et al. 2007). The prominent BFA target in *Arabidopsis* is the endosomal ARF GEF GNOM, which mediates mainly the endosomal recycling to the plasma membrane, whereas endocytosis from the plasma membrane seems to remain operational (Geldner et al. 2003). By this differential effect of BFA on exocytosis and endocytosis in *Arabidopsis*, plasma membrane proteins are internally accumulated into so-called BFA compartments (Geldner et al. 2001, 2003).

In *Arabidopsis* seedlings, following BFA treatments PIN1 rapidly disappears from the plasma membrane and simultaneously aggregates in BFA compartments (Steinmann et al. 1999). This process is fully reversible because BFA removal causes PIN proteins to relocalize to their original position at the plasma membrane (Geldner et al. 2001). Both the internalization and the recovery after washout also occur in the presence of protein synthesis inhibitors, indicating that they are not de novo–synthesized proteins but involve continuous endocytosis and recycling of the same PIN molecules (Geldner et al. 2001). The utilization of a green-to-red photoconvertible fluorescent reporter (EosFP) directly visualizes the internalization of PIN proteins and their subsequent recycling to the plasma membrane (Dhonukshe et al. 2007a). These findings indicate an operational constitutive cycling mechanism in plant cells.

BFA-sensitive subcellular dynamics have been demonstrated for a number of plasma membrane proteins, including, for instance, the aquaporin PIP2, the brassinosteroid receptor BRI1, the plasma membrane H^+-ATPase, the stress-responsive plasma membrane protein Lti6a, and the auxin influx carrier AUX1 (Geldner et al. 2001; Grebe et al. 2002, 2003; Russinova et al. 2004; Paciorek et al. 2005). This BFA sensitivity may reflect a PIN-like mechanism of constitutive endocytosis and recycling, as is seemingly the case for many intrinsic plasma membrane proteins, and has been demonstrated for BRI1, whose endocytic recycling rate may be regulated by heterodimerization with the associated kinase BAK1 (Russinova et al. 2004). However, endocytic recycling is not necessarily accompanied with BFA-sensitive trafficking, as exemplified by both polar and nonpolar delivery of AUX1 to the plasma membrane that is largely insensitive to BFA (Kleine-Vehn et al. 2006). Another example for a recycling plasma membrane protein is the inwardly directed K^+ channel KAT1. The hormone abscisic acid, which controls ion transport and transpiration in plants under water stress, may trigger the selective endocytosis of the KAT1 in epidermal and guard cells, leading to changes in K^+ channel activities at the plasma membrane. Abscisic acid treatment sequesters the K^+ channel within an endosomal membrane pool that recycles back to the plasma membrane within hours (Sutter et al. 2007).

Despite the mostly indirect evidence (based mainly on BFA-sensitive targeting), the number of plant proteins that constitutively recycle at different rates from and to the plasma membrane is constantly growing. In fact, it seems rather difficult to find an intrinsic plant plasma membrane protein that would not undergo BFA-sensitive or BFA-insensitive constitutive recycling. However, besides the fact that almost all plant plasma membrane proteins appear to recycle between the plasma membrane and some intracellular compartments, the mechanisms underlying their differential endocytosis and recycling are still not well characterized.

Exocytosis: the process by which materials in the vesicles are secreted from a cell when the vesicle membrane fuses with the plasma membrane

BFA compartment: BFA treatment–induced mixture of aggregated vesicles in *Arabidopsis* that consists of endosomal and TGN-derived structures in the core and that becomes surrounded by aggregating Golgi stacks

Getting Away: Endocytosis in Plant Cells

Endocytosis occurs at the cell surface and is characterized by membrane invagination and pinching off at the plasma membrane, ultimately leading to closed membrane vesicles in the cytoplasm. These mechanisms facilitate the absorption of material from the outside of the plasma membrane and have been studied mainly in animal cells. Several distinct pathways for endocytosis have been unraveled; among these are the relatively well-defined processes of macropinocytosis, clathrin-mediated endocytosis, and caveolae-mediated endocytosis

(Pelkmans & Helenius 2003, Cheng et al. 2006, Benmerah & Lamaze 2007). Macropinocytosis is a less specific invagination of the cell membrane, resulting in the pinching off of vesicles (Pelkmans & Helenius 2003). In contrast, clathrin-dependent endocytosis and caveolae-dependent endocytosis regulate receptor-mediated internalization of specific cargos (Benmerah & Lamaze 2007).

In plants, the existence of endocytosis has been regarded with skepticism, and there have been decades of controversial debates as to whether the high turgor of plant cells renders the plant plasma membrane unsuitable for invagination and subsequent internalization (Saxton & Breidenbach 1988, Gradmann & Robinson 1989). Experimental results have often settled the theoretical discussions: Electrondense as well as lipophilic tracers have been taken up into plant cells (Robinson & Hillmer 1990, Bolte et al. 2004), endocytic (clathrin-coated) vesicles have been detected at the ultrastructure level (reviewed in Holstein 2002, Paul & Frigerio 2007), and numerous proteins have been found to be internalized from the plasma membrane (Geldner et al. 2001; Paciorek et al. 2005; Takano et al. 2005; Dhonukshe et al. 2006, 2007a; Robatzek et al. 2006), even in high-turgor guard cells (Meckel et al. 2004). However, the underlying mechanism of endocytosis in plants has remained unclear until recently (Pérez-Gómez & Moore 2007). There were growing lines of evidence that the endocytosis mechanism involving the coat protein clathrin is operational in plant cells. The deciphering of several plant genomes revealed that homologs to mammalian proteins of the clathrin coat (e.g., clathrin heavy chain, clathrin light chain, adaptor protein (AP)2 subunits, and AP180) and downstream effectors (e.g., epsin, dynamin, auxilin, heat shock cognate 70, and synaptojanin) are encoded in plant genomes (Hirst & Robinson 1998, Holstein 2002, Paul & Frigerio 2007). Additionally, electron microscopy has detected different stages of clathrin-coated vesicle formation at plasma membranes of different plant species (reviewed in Holstein 2002, Paul & Frigerio 2007). Finally, genetic and pharmacological interference with clathrin-dependent processes in plants blocks internalization of PINs and other plasma membrane proteins (Dhonukshe et al. 2007a). Altogether, these studies have demonstrated operational clathrin-dependent endocytosis in plants and have identified the endogenous cargos of this process. Clathrin-dependent endocytosis seems to be remarkably evolutionarily conserved because mammalian cargos of this pathway, such as the transferrin receptor, are internalized by this mechanism in plant cells (Ortiz-Zapater et al. 2006). Moreover, because the internalization of all tested cargos, including general endocytic tracers, requires clathrin, clathrin-dependent endocytosis seems to constitute the predominant pathway for the internalization of numerous plasma membrane–resident proteins in plant cells.

It remains to be seen whether clathrin-independent pathways are operational in plant cells. Pathways for sterol-dependent, caveolae-mediated endocytosis are unlikely to exist because caveolae-like components have not been identified in plants. However, there are indications suggesting the involvement of sterols in endocytosis or endocytic recycling in plants. Polar PIN protein localization is affected in sterol biosynthesis mutants, and sterols notably share a common early-endocytic trafficking pathway with the PIN2 protein (Grebe et al. 2003, Willemsen et al. 2003). Moreover, the depletion of sterols from plant membranes leads to reduced endocytosis in plants (Kleine-Vehn et al. 2006).

During cytokinesis, plants construct cell plates for the separation of a binucleated cell. PIN proteins are inserted into both sides of the plate, resulting in apical-basal localization following plate fusion with the plasma membrane. Sterols also seem to play a crucial role in the endocytosis-dependent reestablishment of apical PIN2 polarity after the division of epidermis cells (Men et al. 2008). Collectively, these results suggest that endocytic sterol trafficking and endocytosis or polar sorting events in plant cells are linked. It remains to be seen how sterols contribute to endocytosis in plants, but

sterols may define microdomains in plant membranes that regulate recruitment or retrieval from clathrin-coated pits.

Besides sterols, other lipids, such as phosphatidylinositol-related signals, are well established to affect vesicle trafficking in animal cells (McMaster 2001, Davletov et al. 2007). In contrast, only little is known on the role of these compounds in plant cells. One of the few reports shows that phospholipase D and its product, phosphatidic acid, appear to regulate the endocytosis rate and vesicle trafficking in general and the PIN2 protein in particular (Li & Xue 2007). In plant cells, as in animals, phosphatidylinositol-dependent signals may regulate endocytosis and vesicle trafficking.

Recent research has demonstrated the importance of endocytosis in a multitude of developmental and physiological processes in plants. Consequently, this field is finally receiving deserved attention and will rapidly progress in coming years.

Getting Back: Recycling in Plant Cells

Following the internalization of material from the plasma membrane, the regulation of the transfer of internalized receptors, transporters, or other molecules back to the plasma membrane is of tremendous importance for cell membrane integrity. In animal cells, various proteins are competent for recycling from distinct endosomal compartments to the plasma membrane. Furthermore, their endocytic routes are relatively well described by distinct molecular markers (Saraste & Goud 2007). In contrast, mechanisms and pathways that guide recycling in plants are still poorly characterized. Endocytic compartments in plants are often defined solely by their ability to incorporate lipophilic endocytic tracers (Bolte et al. 2004), making unambiguous designation of various early- and late-endocytic compartments difficult owing to differences in experimental conditions and possible compartment maturations.

The best-characterized cargo that exhibits constitutive recycling in plants is PIN1. The *Arabidopsis* BFA-sensitive endosomal ARF GEF GNOM, a vesicle transport regulator, is required for the polar localization and recycling of PIN1 (Steinmann et al. 1999, Geldner et al. 2001). Moreover, the utilization of an engineered BFA-resistant version of GNOM proved that the inhibitory effect of BFA on PIN1 cycling is due to the specific inhibition of GNOM (Geldner et al. 2003), indicating that GNOM defines the recycling rate of PIN1 to the plasma membrane. GNOM localizes to intracellular structures that are labeled by the endocytic tracer FM4-64 within 10 min and may define a recycling, but not an early, endosome (Geldner et al. 2003, Chow et al. 2008). GNOM does not exclusively mediate endosomal recycling of PIN proteins. Also, other, nonpolar plasma membrane cargos and cell wall components show BFA-sensitive, GNOM-dependent recycling and are affected in *gnom* loss-of-function mutants (Shevell et al. 2000, Geldner et al. 2003). Notably, the involvement of GNOM in basal-versus-apical targeting differs substantially. GNOM preferentially regulates recycling of PIN proteins to the basal plasma membrane, whereas apical localization of proteins at the apical plasma membrane is largely BFA insensitive and may be controlled by one or more BFA-resistant ARF GEFs (Kleine-Vehn et al. 2008a). Hence, apical and basal PIN targeting pathways are molecularly distinct by means of the ARF GEF vesicle trafficking regulators (Kleine-Vehn et al. 2008a), enabling simultaneous apical and basal polar PIN delivery in a single plant cell (**Figure 5**).

Basal cargos, such as PIN1, rapidly internalize in response to ARF GEF inhibition by BFA, implying that only recycling, but not internalization, of basal cargos is sensitive to BFA treatment (Geldner et al. 2001). This finding illustrates a possible employment of a BFA-resistant ARF GEF in cargo internalization from the basal plasma membrane. The GNL1, which is a BFA-resistant ARF GEF, may be involved in selective endocytosis of PIN proteins (Teh & Moore 2007). However, GNL1 is very important in ER-Golgi trafficking (Richter et al. 2007), and it remains to be seen whether GNL1

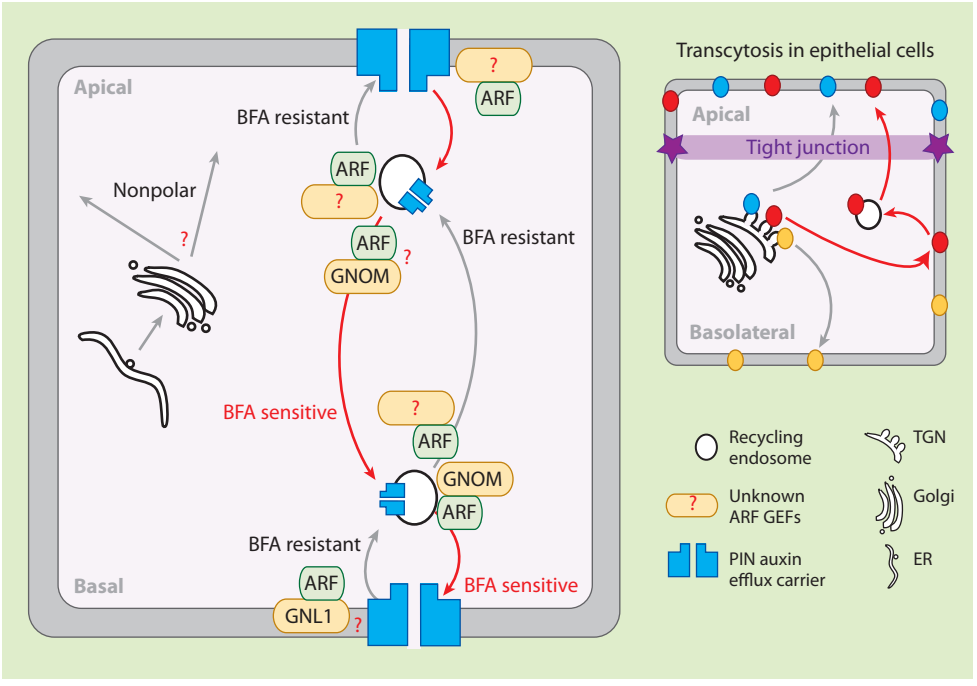

Figure 5

Transcytosis and apical and basal targeting of PIN-FORMED (PIN) proteins. Distinct ARF GEF–dependent apical and basal targeting pathways regulate polar PIN distribution. Alternative utilization of both pathways by the same PIN molecules enables dynamic translocation of PIN cargos between different cell sides. Inhibition of the GNOM component of the basal targeting pathway genetically or by BFA leads to the preferential recruitment of cargos by the apical pathway and to a reversible basal-to-apical PIN polarity shift. The top right panel illustrates that a similar process occurs in animal epithelial cells, in which several polar-competent proteins (depicted in *red*) are initially targeted to the basolateral cell side and subsequently transcytosed to their final destination (the apical cell side). However, other polar cargos (depicted in *yellow* and *blue*) do not require transcytosis for polar localization. Moreover, transcytosis in epithelial cells is also sensitive to BFA. Abbreviations used: ARF GEF, GDP/GTP exchange factor for adenosyl ribosylation factors; BFA, brefeldin A; ER, endoplasmic reticulum; GNL1, GNOM-LIKE1; TGN, trans-Golgi network.

directly or indirectly regulates the PIN endocytosis at the plasma membrane.

Besides the ARF GEF contribution in PIN recycling, SORTING NEXIN1 (SNX1) may define an endosome specific for PIN2, but not PIN1, trafficking (Jaillais et al. 2006) because PIN2 accumulates in SNX1 compartments that are distinct from the GNOM endosomes, after treatment with the phosphatidylinositol-3-kinase (PI3K) inhibitor wortmannin (Jaillais et al. 2006). However, pharmacological or genetic interference with the SNX1 compartment does not affect apical-basal polarity of PIN proteins but preferentially affects vacuolar sorting of plasma membrane proteins, including PIN2 (**Figure 6**) (Kleine-Vehn et al. 2008b). Enhanced PIN2 localization in the SNX1 compartment following gravity stimulation (Jaillais et al. 2006) coincides with enhanced vacuolar targeting and degradation of PIN2 (Abas et al. 2006, Kleine-Vehn et al. 2008b). Interestingly, SNX1 orthologs in yeast and animals are components of the retromer complex that assures the retrieval of vacuolar receptors back from the prevacuolar compartment (PVC) to the TGN (Seaman 2005). In plants, putative retromer components localize to the PVC and may interact with vacuolar sorting receptors (Oliviusson

Retromer: is important in recycling transmembrane receptors from PVCs/multivesicular bodies to the TGN

et al. 2006), which may also be a preferential role for SNX proteins in plants because SNX1 colocalizes with the putative plant retromer component VPS29 at the PVC (Oliviusson et al. 2006, Jaillais et al. 2008). VPS29 is required for storage vacuole formation during embryogenesis (Shimada et al. 2006), indicating that the putative plant retromer complex may be involved in general processes for the formation of multiple vacuole types (Bassham & Raikhel 2000). Furthermore, VPS29 is needed for endosome homeostasis, PIN protein cycling, and dynamic PIN1 repolarization during development (Jaillais et al. 2007). In one possible model, PIN1 first internalizes into GNOM-based endosomes and subsequently is recycled back via VPS29/SNX1-positive endosomes (Jaillais et al. 2007). However, inactivation of retromer-dependent receptor retrieval at the PVC may inhibit anterograde traffic from the PVC to the TGN. Because the TGN may act in plants as an early endosome (Dettmer et al. 2006), recycling of endocytosed cargo would be impaired indirectly (Jürgens & Geldner 2007). Alternatively, the retromer complex may have a gating function for endocytic vacuolar targeting of plasma membrane–localized proteins. Hence, the observed defects in *snx1* and *vps29* mutants (Jaillais et al. 2007, Kleine-Vehn et al. 2008b) may be explained by enhanced vacuolar targeting of PIN proteins (Kleine-Vehn et al. 2008b).

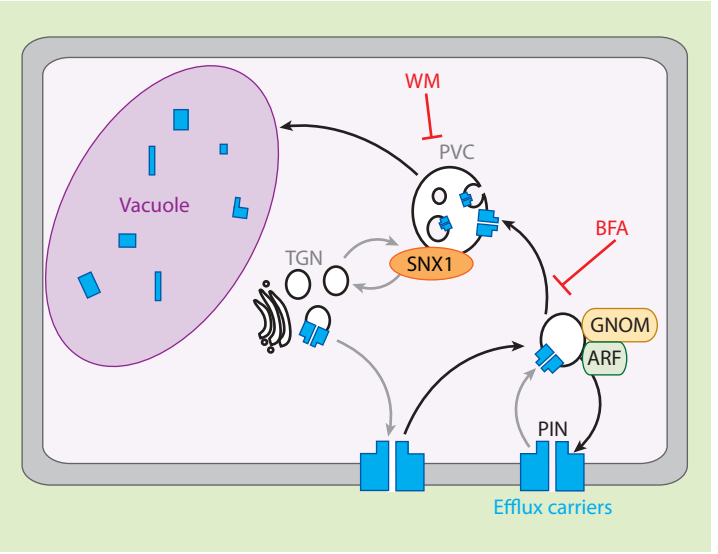

Figure 6

Vacuolar targeting of PIN-FORMED (PIN) proteins. Developmentally important posttranslational downregulation of PIN2 is realized by regulated targeting to the vacuole. PIN2 degradation is controlled by multiple sorting events at the plasma membrane, endosomes, and prevacuolar compartments (PVCs). The putative retromer complex component SORTING NEXIN1 (SNX1) is required for PVC identity. SNX1/VPS29-labeled PVCs appear to have a gating function for endocytic targeting to the vacuole. Other abbreviations used: ARF, adenosyl ribosylation factor; BFA, brefeldin A; TGN, trans-Golgi network; WM, wortmannin.

Going to the Other Side: Transcytosis Linking Endocytic Recycling and Polar Targeting

In animal epithelial cells, endocytic recycling is important for the establishment and maintenance of cell polarity (Rodriguez-Boulan et al. 2005, Leibfried & Bellaïche 2007). The endocytosis and subsequent retargeting to the other cell side by the process of transcytosis illustrate the tight linkage of endocytic recycling and polar targeting in animal cells (**Figure 5**).

In plants, apical and basal PIN targeting is realized by an alternative use of distinct polar targeting pathways by the same cargos (Kleine-Vehn et al. 2008a). Analysis of the GNOM contribution to apical-basal PIN targeting has yielded important mechanistic insights into dynamic polar PIN targeting. When GNOM-dependent, basal targeting is manipulated, for example, by pharmalogical or genetic inhibition of the GNOM function, basal PIN cargos internalize from the basal domain, first accumulate in the BFA compartments, and gradually appear at the apical cell side. This process is independent of de novo protein synthesis and, therefore, hints at a dynamic PIN translocation between distinct polar plasma membrane domains. Live-cell imaging with photoconvertible PIN2 versions visualizes the directional BFA-induced translocation from the basal plasma membrane, through endosomes, to the apical plasma membrane. After BFA removal, the basal localization of PINs is restored by a translocation in an

Prevacuolar compartment (PVC): a multivesicular body/membrane-bound organelle that sorts proteins from the Golgi apparatus to vacuoles, sending missorted proteins back to the Golgi and receiving endocytosed proteins from the plasma membrane

opposite direction from the apical-to-basal cell side (Kleine-Vehn et al. 2008a). Thus, PIN proteins move between the apical and basal sides of cells in a manner similar to that of the transcytosis mechanism known in animal cells, illustrating that endocytic recycling and polar targeting in plants are linked as well (**Figure 5**).

Sorting nexins have been implicated not only in receptor recycling at the TGN/PVC but also in transcytotic events in animal cells. In contrast, genetic or pharmacological interference with SNX1/VPS29-positive endocytic compartments does not seem to interfere directly with GNOM-dependent transcytosis in plant cells (Kleine-Vehn et al. 2008a). In agreement with these findings, SNX-dependent pathways seem to differ substantially between plants and animals. For instance, the human genome encodes for 47 PHOX domain proteins, of which approximately 30 are tentatively referred to as sorting nexins (Seet & Hong 2006). In contrast, *Arabidopsis* encodes only 11 PHOX domain–containing proteins (SMART search at **http://smart.embl-heidelberg.de**), of which 3 (AtSNX1, AtSNX2a, and AtSNX2b) show similarities to the human SNX1/SNX2 and 2 (AtSNX13a and AtSNX13b) show weak similarities to human SNX13. The small number of SNX-like proteins found in plants suggests low evolutionary divergence and, hence, a rather conserved SNX function in the putative plant retromer complex at the PVC/TGN interface.

In animal cells, a prominent example for transcytotic cargo is the transferrin receptor, which resides preferentially at the apical and/or basolateral plasma membranes in polarized epithelial cells. This receptor is able to transcytose from one plasma membrane domain to the other (Cerneus et al. 1993). Madin–Darby canine kidney cells predominantly display a basolateral plasma membrane localization of the transferrin receptor, which is subject to a basolateral-to-apical shift after BFA treatment (Wan et al. 1992, Shitara et al. 1998). The action of BFA on transferrin receptor transcytosis reflects the inhibitory effects of the drug on basolateral recycling, whereas transcytosis to the apical plasma membrane is unaffected (Wang et al. 2001). In an astonishing analogy, apical-to-basal transcytosis of PIN proteins displays a very similar involvement of BFA-sensitive ARF GEFs. Thus, basal targeting in polarized plant cells and basolateral localization in animal cells are remarkably analogous and may follow an evolutionarily conserved principle. However, in plants, the transcytosis mechanism may have acquired unique developmental roles because it may also regulate the dynamic changes in the PIN polarity that accompanies and mediates developmentally important processes such as tropisms and embryonic and postembryonic organ formation (Friml et al. 2002b, 2003b; Benková et al. 2003; Kleine-Vehn et al. 2008a).

Despite obvious analogies between polar targeting mechanisms in plant and animal cells, the overall organization of the polar targeting machinery differs fundamentally. In animal epithelial cells, tight junctions function as barriers to the diffusion of some membrane proteins and lipids between apical and basolateral domains of the plasma membrane (Leibfried & Bellaïche 2007, Niessen 2007). In contrast, such a tight junction–like complex has not been observed in plant cells. Therefore, how plants facilitate the maintenance of distinct membrane compositions remains unclear. PIN proteins display only slow lateral diffusion within the plasma membrane (Dhonukshe et al. 2007a, Men et al. 2008). Thus, a constitutive transcytosis mechanism for polar PIN distribution may be rapid enough to counteract the lateral diffusion of PIN proteins within the plasma membrane and to constantly reestablish the apical-basal localization of cargos. Additionally, this mechanism can mediate the establishment of the polar localization of de novo–synthesized proteins. Polar targeting of de novo–synthesized PIN proteins seems to rely on a three-step mechanism that encompasses nonpolar PIN secretion, clathrin-dependent endocytosis, and subsequent polar endocytic recycling (Dhonukshe et al. 2008b). Therefore, it is tempting to speculate that a transcytosis mechanism regulates dynamic PIN polarity alterations during plant

development as well as establishes and maintains PIN polar localization in polarized cells.

Separating the Daughters: Endocytic Recycling in Cytokinetic Cells

Following mitosis, both animal and plant cells usually split a binucleated cell into two daughter cells. However, animal and plant cells evolved fundamentally different mechanisms of cytokinesis (Barr & Gruneberg 2007). By virtue of the rigid cell wall, plant cells, in contrast to the outside-in constriction of animal cells, construct a cell plate that is formed by intensive delivery and fusion of vesicles containing the components of the future plasma membrane and cell walls. Eventually, the growing cell plate fuses with the lateral sides of the cell, thus completing cytokinesis and separating the two daughter cells.

There is an ongoing debate concerning the origin of the cell plate–forming material. It remains unclear whether cell plate formation depends solely on the secretory pathway or whether endocytosis and, hence, endocytic recycling also contribute. Various endocytic tracers get rapidly incorporated from the extracellular space into the forming cell plate along with multiple plasma membrane proteins and cell wall material (Dhonukshe et al. 2006). Furthermore, endocytosis seems to be upregulated during cytokinesis, and the interference with endocytosis affects cell plate formation and cytokinesis (Dhonukshe et al. 2006). In contrast, the inhibition of secretion dramatically interferes with cytokinesis in plants, illustrating the importance of the secretion of the cytokinesis-specific syntaxin KNOLLE as well as other secreted molecules (Reichardt et al. 2007). In addition, pharmacological reduction of PI3K-dependent endocytosis does not lead to any obvious defects in cell plate formation (Reichardt et al. 2007).

Whether or to what degree endocytic recycling contributes to cell division in plants still remains to be seen. Established molecular tools to satisfactorily tackle this controversial issue in plants are lacking. Unraveling of the contribution of endocytic recycling or secretion is difficult because endocytosed and secreted materials are already merged in early-endosomal/TGN compartments (Dettmer et al. 2006; Lam et al. 2007a,b; Chow et al. 2008). Therefore, targeting of endocytosed material to the cell plate may constitute a default pathway because the endocytosed material may simply follow the bulked secretory flow from the TGN to the cell plate. In the most plausible scenario, which would be consistent with all the data, both the secretory and the endocytic components would contribute to cell plate formation. This model would allow simultaneous arrival of the secretory and endocytosed materials to the forming cell plate, not only to build it with de novo–synthesized material but also to identify it as a future cell surface by incorporating components specific to the mother cell's plasma membrane and cell wall (Dhonukshe et al. 2007b).

The scenario in which the cell plate also incorporates components of the cell surface presents a problem of polarity reestablishment of the polar cargos after completion of cell division. PIN proteins are also targeted to the forming cell plate (Geldner et al. 2001, Kleine-Vehn et al. 2008a); after the plasma membrane is formed, the PIN proteins would be present at both the apical and the basal sides of one of the daughter cells. To maintain the polarity of the mother cell in both daughter cells, there must be a mechanism whereby the polar cargos are stabilized on one side and retrieved from the opposite side of the newly formed cell wall. Very little is known as to which cellular and molecular mechanisms are involved. Sterols seem to play a crucial role in the reestablishment of apical PIN2 polarity (Men et al. 2008). The *cpi1* mutants are defective in endocytosis and deposit PIN2 at both the apical and the basal plasma membranes in post-cytokinetic cells (Men et al. 2008), suggesting a model in which sterol-dependent endocytosis retrieves PIN2 from the "wrong" side of the cell to reestablish uniform polarity in both daughter cells (**Figure 7**). It is possible that the internalized PIN2 is therefore resorted to the opposite,

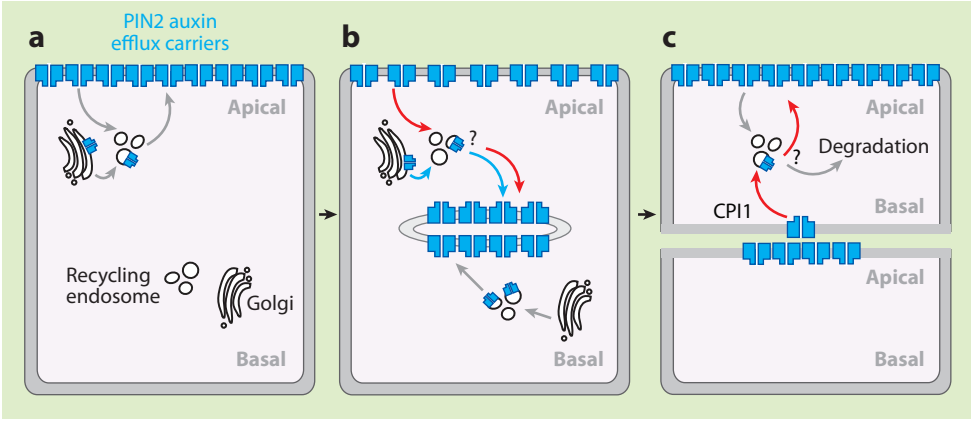

Figure 7

Sterol-dependent endocytosis of PIN2 in postcytokinetic cells. PIN2 displays preferential apical localization in interphase cells (*a*) but is deposited on both sides of the cell plate by transcytosis from the plasma membrane and/or by secretion (*b*). (*c*) PIN2 is retrieved from the newly formed basal plasma membrane by a sterol (CPI1)-dependent mechanism. Following internalization, the PIN2 proteins may translocate to the vacuole for degradation and/or may transcytose to the apical cell side.

"correct" side of the cell, which would be another demonstration of the role of transcytosis in plant cells. However, this scenario and whether similar mechanisms operate for other polar cargos need to be verified experimentally.

EXEMPLIFIED CASES: POLAR TARGETING AND ENDOCYTIC RECYCLING IN PLANT DEVELOPMENT

Induced Endocytosis in Plants

Eukaryotic cells have acquired an enormous adaptative capacity, enabling flexible responses to developmental or environmental cues. In animal cells, ligand- or substrate-induced endocytosis of plasma membrane–resident receptors or transporters has been studied extensively, suggesting a general mechanism for regulated endocytosis in response to external signals (Dugani & Klip 2005). In contrast, in plants, developmental and environmental cues that trigger differential endocytosis are poorly understood.

The first, and so far only, demonstration of ligand-induced receptor endocytosis was in the plant defense response against bacterial pathogens and concerned the bacterial peptide–based signal called flagellin. The *Arabidopsis* flagellin receptor FLS2 localizes to the plasma membrane in various cell types and, upon binding of the flagellin epitope, undergoes internalization in intracellular vesicles, likely leading to subsequent degradation of the receptor/ligand complex (Robatzek et al. 2006).

Interestingly, after binding of the ligand, FLS2 forms a complex with the brassinosteroid receptor–associated kinase BAK1, which seems to be crucial for its internalization (Chinchilla et al. 2007, Heese et al. 2007). Hence, the leucine-rich repeat receptor–like kinase BAK1 not only is instrumental for the brassinosteroid receptor BRI1 (Li et al. 2002, Russinova et al. 2004) but also plays a role in plant immunity by regulating ligand-induced endocytosis. However, although BAK1 may contribute to flagellin-induced FLS2 internalization, BRI1 internalization, recycling, and turnover are seemingly independent of brassinosteroid availability (Geldner et al. 2007). There are indications that brassinosteroids signal through BRI1 at the endosomal level, suggesting that the use of endosomes as signaling compartments is an unexpectedly broad phenomenon in eukaryotes (Geldner et al. 2007). In contrast, treatment

with another inhitor of vesicle trafficking, Endosidin1, leads to intracellular BRI1 accumulation and downregulates BRI1-dependent signaling, suggesting complex regulation of endosomal competence for potential brassinosteroid signaling (Robert et al. 2008). The utilization of a mutual coreceptor for two distinct receptors may influence the availability and/or kinetics of BAK1 binding and, hence, may be involved not only in brassinosteroid signaling or pathogen defense but also in cross talk of these two pathways.

Selective endocytosis has been demonstrated not only for plant receptors but also for some plasma membrane–resident transporters (Takano et al. 2005, Abas et al. 2006, Sutter et al. 2007). Prominent among such transporters is boron exporter BOR1 for xylem loading. Boron availability is crucial for plant development but toxic in high abundance. In the presence of high levels of boron, BOR1 is internalized into ARA7-positive endosomal compartments and is further targeted to the vacuole for degradation, suggesting a control mechanism for boron transporter presence at the cell surface by boron availability (Takano et al. 2005).

The potassium channel KAT1 accumulates in intracellular structures after abscisic acid concentrations are elevated (Sutter et al. 2007). Although the underlying mechanism is unknown, there may be an endocytosis-dependent mechanism for hormone-directed communication between the internal and external environments by the regulation of stomata opening and closure.

During some developmental processes, several PIN auxin efflux carriers also undergo substantial turnover and degradation in addition to constitutive endocytic recycling (Sieberer et al. 2000, Vieten et al. 2005, Abas et al. 2006, Kleine-Vehn et al. 2008b, Laxmi et al. 2008). As shown for PIN2 in **Figure 6**, following endocytosis, ARF GEF– and PI3K/SNX1-dependent sorting events at the endosomal and prevacuolar compartments contribute to the decision of whether to recycle or to translocate to the vacuole (Kleine-Vehn et al. 2008b). Auxin itself regulates PIN abundance at the plasma membrane by inhibiting PIN internalization from the plasma membrane (Paciorek et al. 2005). In addition, prolonged high auxin levels appear to induce PIN2 ubiquitination, internalization, and degradation (Vieten et al. 2005, Abas et al. 2006). Moreover, gravitropic stimulation triggers internalization (Abas et al. 2006) and vacuole-dependent degradation of PIN2 in epidermal cells at the upper side of the root (Kleine-Vehn et al. 2008b). This gravity-induced degradation of PIN2 occurs in cells with low, not high, auxin levels and may indicate posttranslational PIN2 downregulation in response to auxin depletion. It still needs to be seen whether boron, auxin, and possibly substrates for other plasma membrane–based transporters differentially downregulate their transporters by a conserved mechanism. Transient, transport-dependent conformational changes in carrier composition may enable conditional recruitment of machineries mediating, for instance, ubiquitination and subsequent internalization of their substrates.

Although the underlying pathways are largely unknown, the examples of ligand-induced receptor endocytosis, constitutive receptor cycling, and endosome-based signaling as well as the downregulation of receptors and transporters in response to substrate availability show that plant cells use all these endocytosis mechanisms to regulate their physiology. Undoubtedly, other examples of similar regulations will be identified in the coming years.

Integrating Developmental and Environmental Signals through Polarity Modulations

Intercellular auxin transport is the process in plants that makes most apparent the developmental output of subcellular dynamics and cell polarity (Berleth et al. 2007). Polar auxin transport is distinguished by its strictly controlled directionality, which is a crucial feature in auxin-mediated plant development (reviewed by Friml 2003, Zažímalová et al. 2007). The classical chemiosmotic hypothesis proposes that auxin flow polarity is determined by

the polar, subcellular localization of auxin efflux carriers (Rubery & Sheldrake 1974, Raven 1975; **Figure 1**). PIN proteins have been identified as one of the export carriers, and their polar subcellular localization indeed correlates with the direction of the auxin flow. The manipulation of PIN polarity has a clear impact on the ability of auxin to flow in a given direction, thus confirming that cellular PIN positioning is a determining factor in the directionality of polar auxin transport (Friml et al. 2004, Wiśniewska et al. 2006, Boutté et al. 2007).

The finding that PIN proteins undergo permanent subcellular movements (Geldner et al. 2001, Dhonukshe et al. 2007a) was hard to reconcile with the original models of auxin transport. Hence, the important upcoming question concerns the functional role of this constitutive cycling. Besides exotic scenarios, such as neurotransmitter-like release of auxin from cells (Baluška et al. 2003), a plausible assumption is that constitutive trafficking provides the required flexibility for the rapid transcytosis-based PIN polarity changes, allowing rapid redirection of auxin flow in response to various signals, including environmental or developmental cues (Friml 2003). Indeed, rapid PIN relocations have been observed during embryonic development (**Figure 8**) (Friml et al. 2003b), aerial and underground organogenesis (**Figure 9**) (Benková et al. 2003, Reinhardt et al. 2003, Geldner et al. 2004, Heisler et al. 2005), vascular tissue formation (Scarpella et al. 2006), and root gravity responses (**Figure 10**) (Friml et al. 2002b). In all these instances, changes in PIN polarity are followed by the redirection of auxin fluxes and the rearrangement of local patterns of auxin accumulation (auxin gradients) that triggered the changes in the developmental programs (Kramer & Bennett 2006, Leyser 2006, Parry & Estelle 2006). An early PIN polarity switch signals root initiation during embryogenesis. At early stages of *Arabidopsis* embryo development, PIN7 is localized apically (toward the apical cell) in the suspensor, and PIN1 is mostly nonpolar in the proembryo, whereas at a later-defined stage, PIN1 polarizes to the basal side of cells adjacent to the future root meristem, and PIN7 changes its polarity from apical to basal (Friml et al. 2003b). These PIN polarity alterations lead to the rearrangement of auxin gradients and the accumulation of auxin at the presumptive embryo root pole and are among the necessary factors for root specification (Friml et al. 2003b, Weijers et al. 2005).

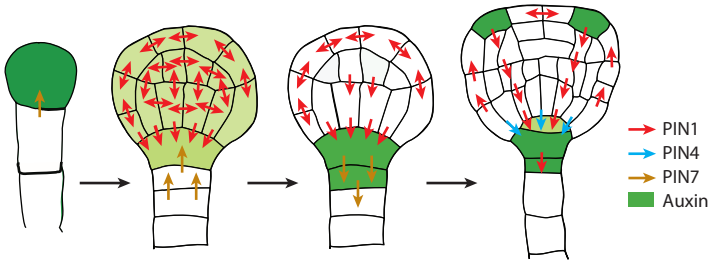

Figure 8

PIN-FORMED (PIN) polarity alterations during embryogenesis. Schematic representation of PIN distribution and polar orientation during *Arabidopsis* embryo development. PIN1 and PIN7 undergo a polarity switch at the globular stage. The GNOM-dependent focus of PIN1 to the basal sides of provascular cells coincides with an apical-to-basal shift of PIN7. These rearrangements of PIN polarity are accompanied by a dramatic change in the apical-basal auxin gradient. A new auxin maximum is established at the position of the future root, contributing to the initiation of the root specification. The analogy to GNOM-dependent transcytosis in polarized root cells may indicate polar transcytosis of PIN proteins during embryogenesis.

Another example of PIN polarity reorganization relates to the perception and response to environmental stimuli. Studies on *Arabidopsis* roots demonstrated that the PIN3 protein relocates in gravity-sensing cells of the root tip in response to gravistimulation (Friml et al. 2002b, Harrison & Masson 2008). When the root is reoriented into a horizontal position, gravity-sensing statoliths in the columella cells sediment, and PIN3 rapidly relocalizes from its originally uniform distribution to the new bottom side of these cells. The asymmetric repositioning of PIN3 is followed by redirection of the auxin flow downward, leading to auxin accumulation at the lower side of the root and, consequently, to downward root bending (**Figure 10**). It is possible that a similar mechanism involving PIN relocations underlies phototropic responses, but the connection between unidirectional light stimulus and PIN relocation has not been demonstrated. The

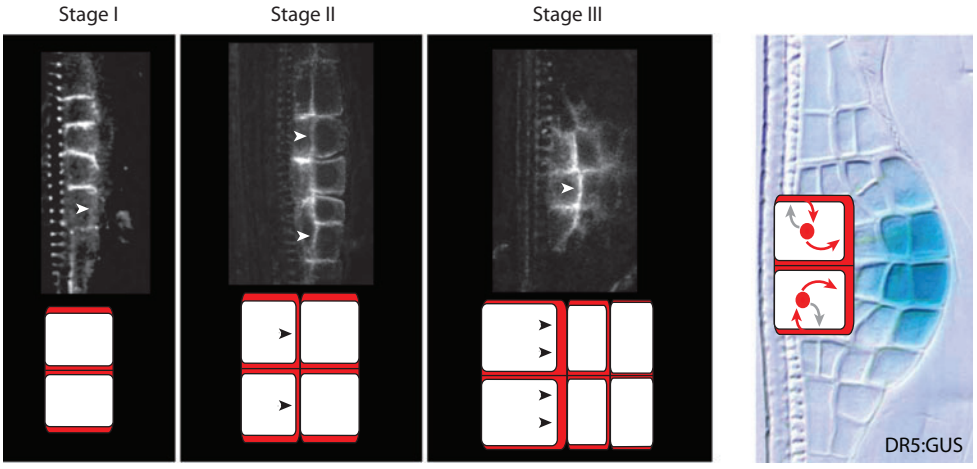

Figure 9

PIN-FORMED (PIN) polarity alterations during postembryonic organ formation. Immunolocalization and model of PIN1 localization during stage I to stage III (*first three panels*) of lateral root primordia development illustrate dynamic PIN1 polarity changes from the anticlinal toward the periclinal cell sides, pointing to the presumptive primordia tip. These changes coincide with the establishment of auxin maxima (visualized here by the DR5 auxin-responsive promoter activity; *fourth panel*) at the primordium tip. GNOM dependency of this event may reveal the involvement of dynamic PIN1 transcytosis between the anticlinal and the periclinal cell sides.

constitutive PIN subcellular dynamics may play a more direct role in the mechanism of auxin efflux because several potent and well-established inhibitors of auxin efflux act as stabilizers of the actin cytoskeleton and also inhibit PIN dynamics (Dhonukshe et al. 2008a). The precise connection between actin stabilization and mechanism of auxin efflux is, however, still unclear. Nonetheless, signal-induced rearrangements of PIN polarity in response to different inputs represent a plant-specific mechanism that integrates various internal and external signals and translates them into different developmental responses.

Canalization Hypothesis and the Effect of Auxin on Its Own Efflux

An important aspect linked to cell polarity and auxin transport relates to a rather fundamental issue in developmental biology: How does the individual cell in polarizing tissues know the polarity of its neighbors and the whole macroscopic context? In plant development, this issue has a pronounced importance because plants possess the remarkable ability to redefine cell and tissue polarities. Outstanding examples of auxin-dependent reorganization of plant tissues are the differentiation of vasculature during leaf venation, the connection of de novo–initiated organs with the preexisting vascular network, and vasculature regeneration after wounding. During these events, plant cells perceive their position within the tissue and can recognize their orientation relative to the rest of the plant body. Insights into underlying mechanisms are widely elusive, but efforts to tackle these processes have led to the formulation of the canalization hypothesis (Sachs 1981), whereby auxin can induce, by a positive-feedback mechanism, the capacity and polarity of its own transport, resulting in the gradual rearrangement of cell polarity and the repolarization of neighboring cells. Ultimately, new auxin conductive channels can be established, determining the position of new vascular strands. This intriguing hypothesis and other auxin-dependent self-organizing models (de Reuille et al. 2006, Jönsson et al. 2006, Smith et al. 2006, Kuhlemeier 2007, Merks et al. 2007)

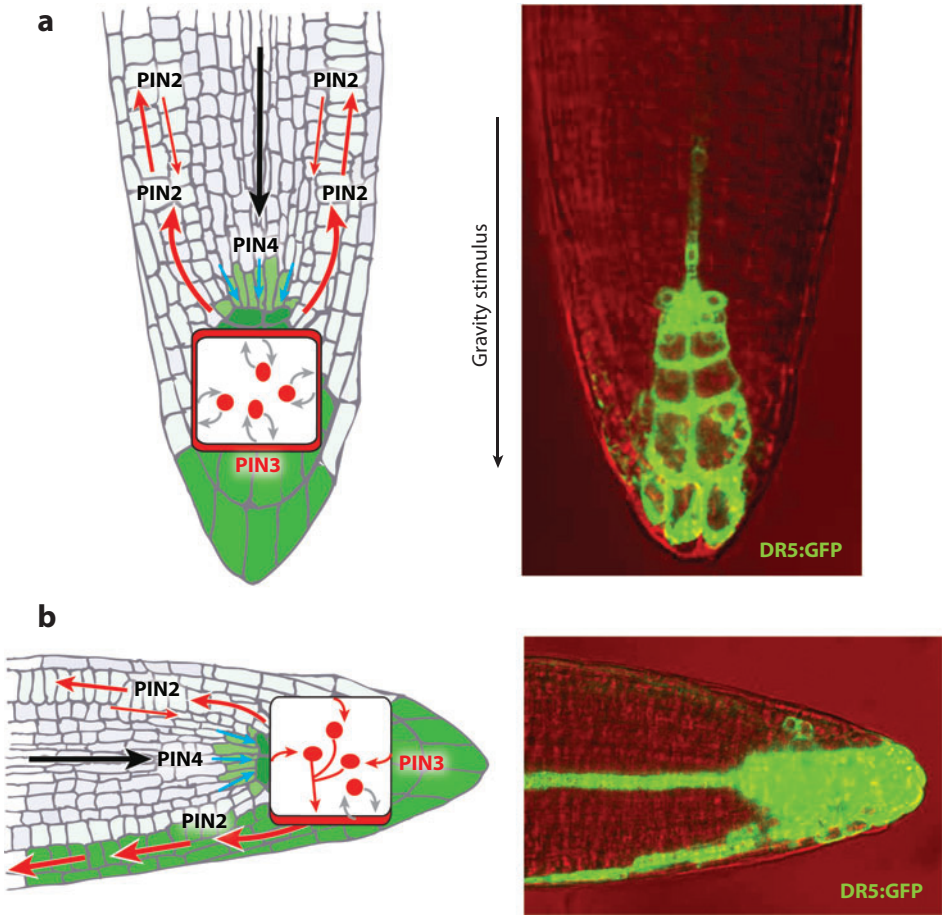

Figure 10

(*a*) PIN-FORMED (PIN)3 translocation during the root gravitropic response. Following gravity stimulation, PIN3 translocates rapidly to the bottom side of root cap cells and thus redirects auxin flow toward the lower side of the root. (*b*) PIN3- and PIN2-dependent asymmetric auxin distribution (visualized by the DR5:GFP-reliant auxin response) leads to differential growth and subsequent downward root bending. The dynamic nature of PIN3 targeting after gravity perception indicates a transcytosis-like mechanism for this polarity switch.

require the existence of positive-feedback regulation between auxin signaling and the capacity and polarity of auxin transport.

In fact, auxin feedback mechanisms regulating PIN activity, involving auxin-dependent regulation of transcription, degradation, and/or subcellular localization of auxin transport components, have been illustrated at multiple levels (Paciorek et al. 2005, Vieten et al. 2005, Sauer et al. 2006, Scarpella et al. 2006, Xu et al. 2006). On the transcriptional level, auxin-dependent cross-regulation of PIN expression may account for the extensive functional redundancy of PIN proteins, in which lack of function of one PIN protein leads to a transient increase in cellular auxin levels and transcriptional upregulation of a functional ortholog (Vieten et al. 2005). Other auxin-dependent feedbacks have been identified at the level of PIN subcellular trafficking: Auxin interferes with endocytosis, including the internalization of PIN proteins, possibly by a mechanism independent of

auxin-induced transcription. This auxin effect leads to elevated PIN levels at the plasma membrane and increased auxin efflux (Paciorek et al. 2005). The underlying mechanism of the auxin effect is unclear but requires BIG, a callosin-like protein with an unclear function (Gil et al. 2001). By this mechanism, auxin regulates the PIN abundance and activity at the cell surface, accomplishing direct feedback regulation of auxin transport (Paciorek & Friml 2006).

Moreover, auxin indeed delegates the polarity of PIN proteins, hence influencing not only its own efflux rate but also its directional output (Sauer et al. 2006). This auxin effect is independent of PIN transcriptional regulation but involves the identified auxin/indole acetic acid (AUX/IAA) and auxin response factor–dependent signaling pathway (Parry & Estelle 2006, Kepinski 2007). Furthermore, auxin-dependent polarization cues are perceived in a cell type–dependent manner, eventually leading to averted polarity between neighboring cells (Sauer et al. 2006). These feedback regulations provide a conceptual framework for polarization during multiple regenerative and patterning processes in plants and are the unavoidable legacy of most models dealing with auxin-dependent patterning (Kramer 2008).

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We are grateful to M. De Cock for help in preparing the manuscript. This work was supported by grants from the Research Foundation-Flanders (Odysseus) and the EMBO Young Investigator Program.

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