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Polar expedition: mechanisms for protein polar localization

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Most cells show asymmetry in their shape or in the organization of their components that results in poles with different properties. This is a fundamental feature that participates in modulating the development of an organism and its responses to external stimuli. In plants, a number of proteins that are important for developmental and physiological processes have been shown to display polar localization. However, how these polarities are established, maintained, or dynamically modulated is still largely unclear for most of these proteins. In this review we report recent updates on the mechanisms of polar protein localization, focusing on a subset of these proteins that are the focus of current research efforts.

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Introduction

Cell polarity can be defined as heterogeneities within a cell, generated by the presence of domains with distinct molecular properties. In plants, cell polarity is often described as apical (shootward), basal (rootward) and lateral (inner or outer). Such a polarity is already established at the first step of embryo development [1] and is continuously used as a reference to direct plant developmental programs.

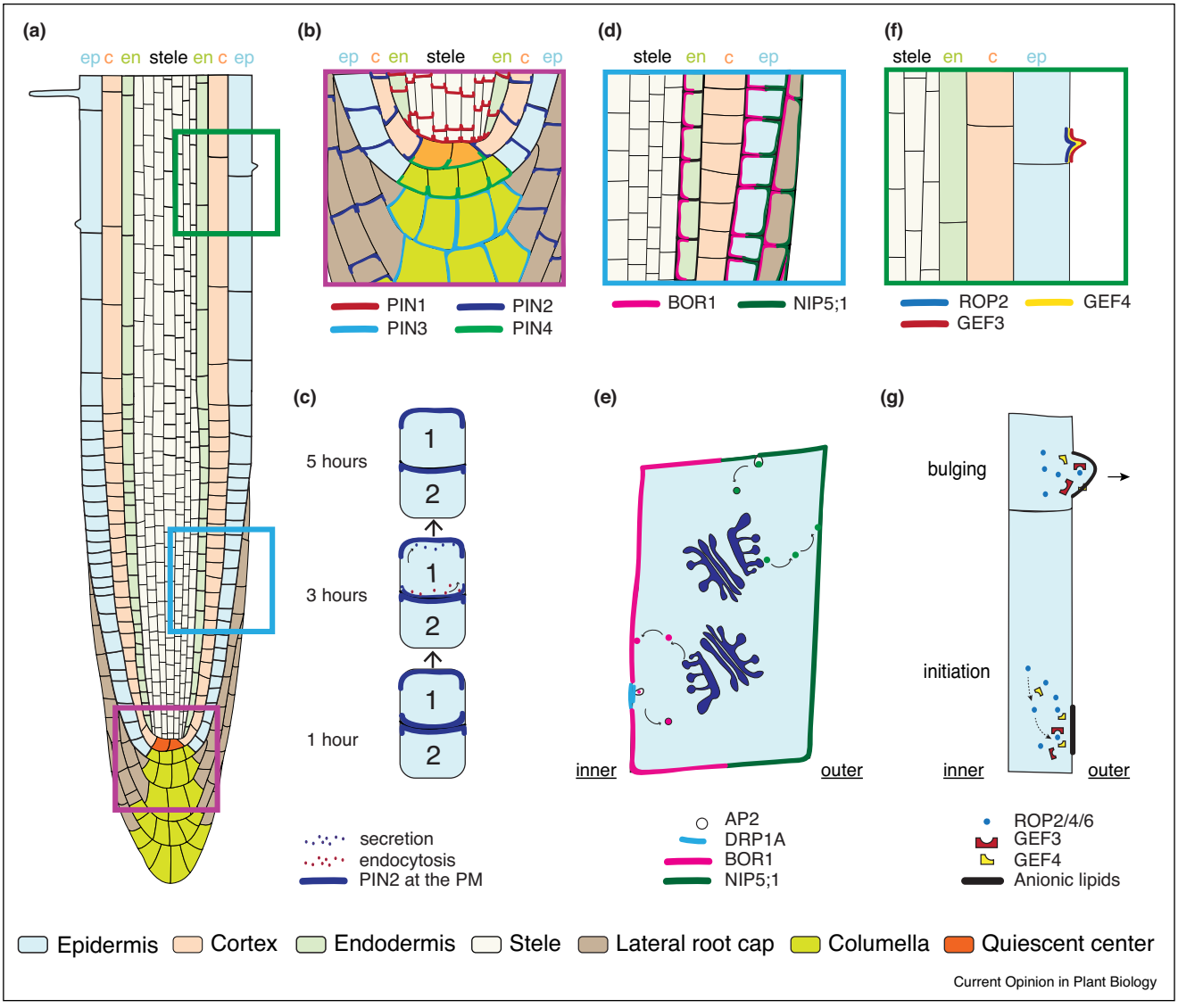
In this review we report recent updates regarding the mechanisms controlling polar localization of plasma membrane (PM) or PM-interacting proteins. We particularly focus on the intracellular components that regulate the precise delivery and maintenance of such proteins at the PM, as well as guiding their polarity.

Regulation of PIN polarity

Plant development and responses to environmental conditions are largely controlled by auxin gradients that are mainly generated by the PM localized auxin efflux facilitators, PIN-FORMED (PIN) proteins [2]. PIN proteins are defined as canonical and noncanonical, depending on whether or not their structure contains conserved domains typically located in a long central loop [3]. *Arabidopsis* canonical PINs are localized at the PM in a polar manner, displaying a tissue-specific distribution pattern (Figure 1a,b) while the noncanonical PINs are present at the endoplasmic reticulum [4].

The endomembrane machinery is a key regulator of PIN polarity establishment and maintenance, within which different trafficking pathways contribute to distinct polar localization [5,6]. This is exemplified by recent work focusing on PIN2 apical polarity establishment after cytokinesis in *Arabidopsis* root epidermis. First, when cell division occurs, PIN2 is localized at both sides of the cell plate, such that after cell division PIN2 is present on both apical and basal sides of the upper daughter cell [7,8]. Then, *de novo* PIN2 is secreted to the apical membrane and the basally localized PIN2 is removed via endocytosis before being targeted to the vacuole (Figure 1c). Interestingly, this occurs independently from the cytoskeleton [9]. Later on, apical PIN2 polarity in the epidermis is maintained by constant recycling between the PM and the *trans*-Golgi network (TGN) and restricted lateral diffusion within the PM [10]. Notably, besides its apical localization in the epidermis, PIN2 displays basal and apical polarity in young and older cortex cells respectively. Thus PIN2 polar localization is not only specific to certain tissues but also to the cell developmental stage [11]. Recently it was shown that the lack of three *Arabidopsis* post-Golgi localized 14-3-3 epsilon proteins, which modulate biological processes by phosphorylation-mediated protein-protein interaction, affects PIN1 and PIN2 polarity and increases the accumulation of PIN2 in intracellular compartments [12]. While no direct binding of these proteins to PIN2 has been detected, co-immunoprecipitation suggested their potential interaction with proteins involved in trafficking and vesicle formation/fusion.

Figure 1



Distribution of polar proteins in *Arabidopsis* primary root. **(a)** *Arabidopsis* primary root. Squares indicate magnifications in **(b)** (purple), **(d)** (blue), and **(f)** (green). (b) Localization of PIN proteins in the root tip. PIN1 is basal in the endodermis and stele. PIN2 is apical in the lateral root cap and epidermis while being basal in the young cortex cells. PIN3 is apolar and lateral in the columella. PIN4 is basal in the upper part of the columella. **(c)** PIN2 apical polarity re-establishment in epidermal cells from the bottom to the top. One hour after cytokinesis PIN2 is apical and basal in cell 1 and apical in cell 2. Three hours after cytokinesis, basal PIN2 in cell 1 is removed by endocytosis and newly synthesized PIN2 is secreted to the apical membrane. Five hours after cytokinesis, PIN2 apical polarity is re-established. (d) Localization of B transporters in the root meristematic zone. NIP5;1 is localized at the outer (soil facing) PM of root cap and epidermal cells and BOR1 at the inner (stele facing) PM of root cap, epidermal and endodermal cells. **(e)** NIP5;1 and BOR1 polarity establishment in the epidermis. NIP5;1 and BOR1 are constantly recycled between the PM and the TGN to maintain their polarity. BOR1 is secreted towards the stele side (left in the image) and recycled via AP2-dependent and DRP1A-dependent CME. NIP5;1 polar localization requires AP2-dependent CME. (f) Proteins which mark the positioning of root hair emergence accumulate at the basal side of the root outer epidermal cell PM. **(g)** Representation of the root hair initiation phase (bottom cell), corresponding to the recruitment of ROP proteins by GEF3 to the initiation domain, which co-localize with anionic lipids and where they interact with GEF4. At the bulging phase (upper cell), ROPs are activated by GEF4, initiating emergence of the root hair, which is regulated at its tip by the presence of anionic lipids. ep—epidermis; c—cortex; en—endodermis.

PIN polar distributions are also regulated by their phosphorylation through the antagonistic activities of the serine-threonine protein kinase PINOID (PID) and the PROTEIN PHOSPHATASE 2A (PP2A) complex [13]. Plants overexpressing PID display apicalization of basally localized PIN1, PIN2 and PIN4 [14] and reduced relocalization of PIN3 upon gravistimulation in the root and shoot [15]. None of these phenotypes are observed in plants overexpressing D6 PROTEIN KINASE (D6PK), which regulates PIN activity [15,16], indicating that the

function of PID, but not D6PK, plays a role in PIN polar targeting. However, both PID and D6PK phosphorylate S1–S4 of the PIN1 canonical loop, indicating the necessity of additional mechanisms to regulate PIN polar distribution [16–18].

Auxin itself regulates PIN localization and polarity [19–21]. For example, upon gravistimulation, PIN3 is relocated to the basal side of the endodermis cells in the upper hypocotyl side. This redirects the flow of auxin towards the lower hypocotyl side, establishing an auxin maximum that stimulates differential growth and consequent bending of the hypocotyl against the gravity vector [22]. This process is terminated by an auxin feedback that re-establishes PIN3 symmetry in the endodermis by actin-mediated and PID-mediated mechanisms [15,23]. Conversely, upon extended auxin treatment, the lateralization of PIN1 (in the root pericycle and endodermis) and PIN2 (in the cortex) do not strictly require PID activity but do require functional auxin signaling downstream of the AUX/IAA17 transcriptional repressor [20]. A microarray-based approach identified WRKY DNA-BINDING PROTEIN 23 [24] and phosphatidylinositol transfer proteins named PATELLINs [25] as important elements for the rearrangement of PIN subcellular localization leading to auxin flux canalization. The newly described molecule pinstatic acid (PISA) inhibits BFA-induced bodies containing PIN1 and PIN2 and induces lateralization of these proteins without triggering the auxin co-receptor complex $SCF^{TIR1/AFB}$. Understanding this molecule's mode of action will bring new insight regarding the effect of auxin on PIN trafficking [26].

Different models aim to explain the auxin-induced mechanism for the coordination of PIN polarity in neighboring cells. Among others, the activity of the AUXIN RESPONSE FACTOR, MONOPTEROS, has been shown to determine PIN1 polarity change in the shoot apical meristem for phyllotaxis establishment [27]. Alternatively and non-exclusively, mechanical signals have also been shown to be involved in orienting PIN polarity [28–30].

Coordination of root cell polarity generates unidirectional transport

Boron (B) is a microelement that crosslinks rhamnogalaturonan II in the cell wall [31]. B uptake requires PM importers and exporters such as NODULIN INTRINSIC PROTEIN 5;1 (NIP5;1) and BORON TRANSPORTER 1 (BOR1) respectively. These proteins display lateral polarity in the root epidermis, endodermis and root cap, orienting the B flux towards the stele, where NIP5;1 localizes at the outer cell side facing the soil, while BOR1 localizes at the inner cell side toward the stele [32,33] (Figure 1d). In mature root endodermis, their polarity domains are restricted by the presence of the Casparian strip [32]. As for PINs, vesicular trafficking determines their polarity. NIP5;1 polar localization requires recycling via clathrin-mediated endocytosis (CME) through ADAPTOR PROTEIN COMPLEX 2 (AP2) [34] (Figure 1e). This process is accelerated upon phosphorylation of threonine residues in the N-terminus of NIP5;1 without affecting either NIP5;1 activity or its lateral membrane diffusion [34]. Similarly, BOR1 polarity is established by CME via AP2 and DYNAMIN-RELATED PROTEIN 1A (DRP1A) [35,36] (Figure 1e). This is mediated by the interaction between AP2 and the C-terminus domain of BOR1 [35]. The BOR1 protein also contains three tyrosine-based motifs in its big cytosolic loop that are involved in regulating BOR1 polar localization, likely through secretion rather than endocytosis [33]. Interestingly, AP2-dependent endocytosis and the tyrosine-based signals are not necessary for the maintenance of BOR1 polarity in mature endodermal cells where instead, the Casparian strip is thought to play a role [32,35]. These mechanisms take place when the levels of B are low to ensure its absorption and unidirectional transport. Such nutrient transport processes show how, for plants, the awareness of "outer" and "inner" is fundamental to correctly distribute proteins at a cellular level.

Growth polarity through ROP GTPase signaling

The Rho of plants (ROP) small GTPases are master regulators of cell polarity and morphogenesis in plants [37]. They act as polarly localized molecular switches. Their activity is regulated by guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs), and GDP dissociation inhibitors (GDIs), which control conformational changes from inactive GDP-bound to active GTP-bound states [37]. Upstream, ROP activity and polar localization are regulated by hormones, cell wall sensing and receptor-like kinase signaling [37–39]. Downstream, ROPs act on cytoskeleton organization, reactive oxygen species production, kinase activities and many other physiological mechanisms, which ultimately regulate developmental processes such as root hair, pollen tube and leaf pavement cell lobe formation [37–41]. In each case, they adopt a particular subcellular localization at the PM that is crucial for their function (Figure 1f).

Recent work on root hair and pollen tube growth has brought new insights into the mechanisms regulating ROP polarity. In root hairs, the polar localization and activation of ROPs appear to require proper trafficking to the PM, mediated by proteins called YPT/RAB GTPase INTERACTING PROTEIN 4a (YIP4a) and YIP4b [42]. Following polar trafficking, ROP polarity must be maintained against its diffusion gradient at the PM. A recently revealed mechanism for this in pollen tubes involves localization of the newly identified protein ROP ENHANCER 4 (REN4) at the periphery of the ROP polar domain [38]. REN4 interacts with the active forms of ROPs, leading to removal of both proteins from the PM by clathrin-mediated endocytosis and thus restricting the diffusion of ROPs to their polar domain [38]. Another

mechanism recently shown to be involved in ROP localization involves the lipid composition of the PM [43]. ROPs co-localize and may interact with anionic phospholipids at the PM. Interestingly, active ROPs can interact with and contribute to the polar localization of lipid modifying enzymes involved in the generation of such anionic phospholipids, forming a feedback loop in which ROP activity and membrane composition influence each other to stabilize ROP polarity [44,45].

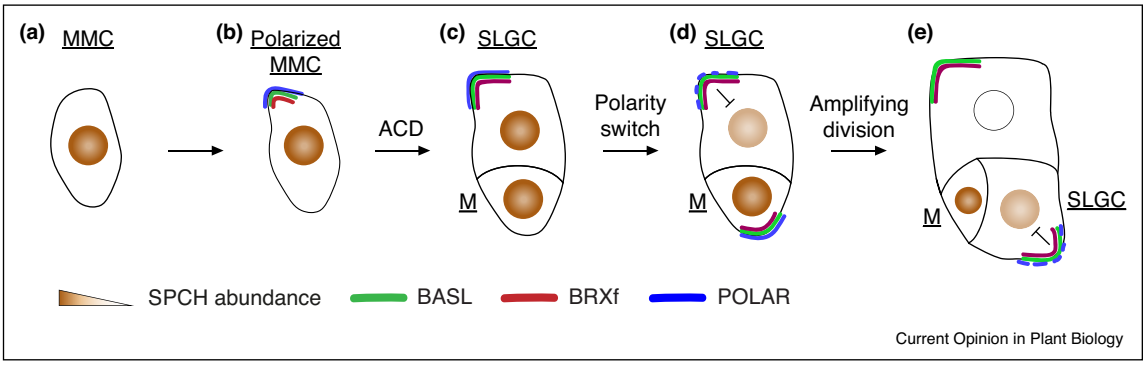
Beyond these mechanisms, how the polarity of ROPs is determined remains largely unknown. Extracellular signals could influence their polarization through the cell surface receptor kinase FERONIA (FER), which can sense cell wall pectins and respond by activating a signaling pathway involving ROPs and influencing pavement cell lobe formation. Local activation of ROPs by extracellular signals could initiate a feedback loop, leading to their stable polarization. However, this may only be an amplifying mechanism. Recent investigation of the successive events taking place during root hair initiation revealed that ROP, phospholipid and FER polarization events were preceded by the polarization of a GEF (GEF3) (Figure 1g), leaving open the question of the initial cue leading to this polarity.

Control of asymmetric cell division in the leaf epidermis by BASL, BRXf and POLAR

BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE (BASL), POLAR LOCALIZATION DURING ASYMMETRIC DIVISION AND REDISTRIBUTION (POLAR), and BREVIS RADIX family proteins (BRXf) display specific cellular polarization in the leaf epidermis stomatal cell lineage and are required for asymmetric cell division (ACD) [46]. These proteins accumulate in a single polarized crescent at the cell cortex before ACD [46–48] (Figure 2a,b), which determines the asymmetry of the division and fate of the daughter cells. The smaller cell will retain its meristemoid identity conferred by the transcription factor SPEECHLESS (SPCH) and complete additional ACDs before differentiating into guard cells to form a stoma [49,50] (Figure 2c–e). In the larger cell, the polarity module acts as a scaffold retaining negative regulators of SPCH expression. After ACD, these negative regulators are released from the polarity module, leading to the repression of SPCH expression only in the larger cell, which will later differentiate into a pavement cell [49,50] (Figure 2c–e). Interestingly, after each ACD, BASL, BRXf and POLAR undergo a polarity switch in the new meristemoid daughter cell, generating a specific pattern of cell division in the leaf epidermis [51] (Figure 2c–e).

Phosphorylation of BASL by kinases (YDA-MPK3/6) and the scaffolding of such kinases by BASL have been shown to act in a positive feedback loop generating a polarity module [50,52] (Figure 2a,b). Recent work notably revealed that BRXf and POLAR also act in this feedback loop [46,48], interacting with BASL and thus also contributing to the stability of this polarity module [53,54]. Other recent research has started to uncover the mechanisms involved in orienting the polarity of these proteins. The localization of BRXL2, a member of BRXf, is influenced by mechanical signals as well as peptide signaling [48]. Furthermore, the ectopic expression of BASL throughout the leaves has revealed a coordinated proximodistal polarity field independent of the stomatal lineage [53]. Interestingly, since BASL, BRXf and POLAR

Figure 2



Polarity switch, and meristemoid identity maintenance during asymmetric cell division (ACD) in the leaf epidermis stomatal cell lineage. **(a)** The meristemoid mother cell (MMC) displays high levels of SPCH in the nucleus. **(b)** Before ACD, BASL, POLAR and BRXf polarize at one cell side. **(c)** ACD leads to the formation of a larger cell containing the polarity module, and a small cell with originally no polarity module. **(d)** After ACD POLAR is released from the plasma membrane (dashed blue line) consequently leading to the release of negative regulators of SPCH expression from the polarity module. These negative regulators of SPCH start to suppress the expression of SPCH in the larger cell (stomatal lineage ground cell, SLGC) that will differentiate into a pavement cell, while the small meristematic cell (meristemoid, M) retains high levels of SPCH. At the same time a new polarity module is formed in the meristemoid cell with a different orientation compared to the polarity module that was present in the mother cell (polarity switch). **(e)** The new polarity module then guides the next ACD, and each new division leads to the formation of a new polarity module with a polarity switch in further amplifying ACDs.

physically interact, this may represent the general mechanism for the localization of this polarity module.

Compared to PINs, BOR1, NIP5;1 and ROPs, little is known about the mechanisms that establish this polarity. While affecting microtubules did not affect BASL polarity [53], the potential roles of actin, trafficking, endocytosis and diffusion, remain to be characterized.

Conclusions

In this review we have reported major updates on the mechanisms underlying protein polarity in plants. The various mechanisms described seem to share many components: phosphorylation, cytoskeleton, endomembrane trafficking including endocytosis, recycling, and polarized delivery of *de novo* synthesized proteins, as well as control of lateral diffusion and the presence of the cell wall [55,56]. Furthermore, in some cases these components exhibit a degree of functional interaction. For instance, ROPGEF1 has recently been shown to be involved in PIN2 distribution [57]. On the other hand, these different proteins often harbor different polarities, which appear to be independent from each other. For instance, PIN1 has been shown to be located either together with, or opposite to BASL within a single cell [53]. Recent work identifying a new family of polarized SOSEKI proteins showed that each member displays discrete polarities in the embryo and root, suggesting that they somehow define different polar coordinates [58]. How this is achieved remains a major question, but one may speculate that particular combinations of molecular, chemical and physical cues can contribute to the establishment of such polar coordinates along the cell cortex.

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Conflict of interest statement

Nothing declared.

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