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Outer, inner and planar polarity in the Arabidopsis root

Moritaka Nakamura[1] and Markus Grebe[1,2]

Plant roots control uptake of water and nutrients and cope with environmental challenges. The root epidermis provides the first selective interface for nutrient absorption, while the endodermis produces the main apoplastic diffusion barrier in the form of a structure called the Casparian strip. The positioning of root hairs on epidermal cells, and of the Casparian strip around endodermal cells, requires asymmetries along cellular axes (cell polarity). Cell polarity is termed planar polarity, when coordinated within the plane of a given tissue layer. Here, we review recent molecular advances towards understanding both the polar positioning of the proteo-lipid membrane domain instructing root hair initiation, and the cytoskeletal, trafficking and polar tethering requirements of proteins at outer or inner plasma membrane domains. Finally, we highlight progress towards understanding mechanisms of Casparian strip formation and underlying endodermal cell polarity.

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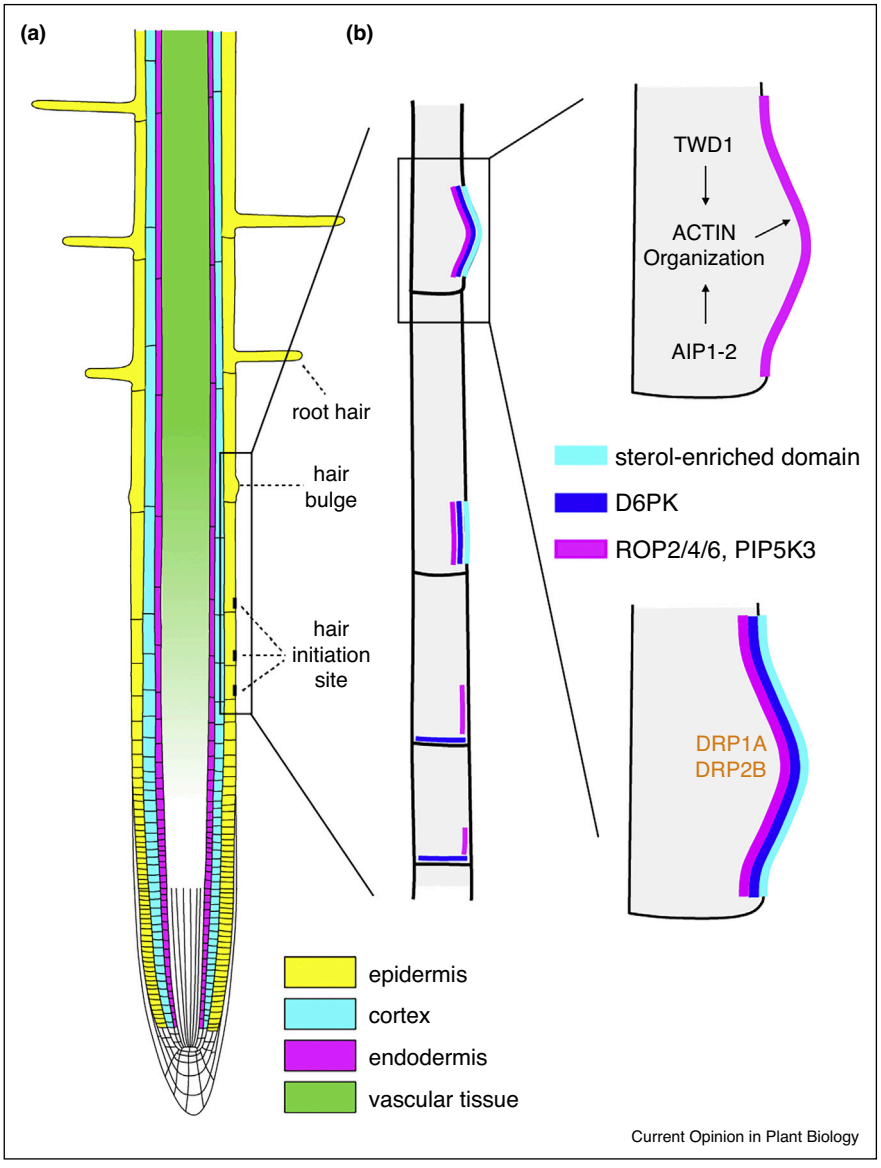
Introduction

Cells of diverse organisms display asymmetric distributions of molecular components along one or more axes [1]. This essential feature, termed cell polarity, contributes to the acquisition and segregation of cell fates as well as to the functional specialization of cells during cell differentiation [1–3]. When the polarity of multiple cells is coordinated within the plane of a single tissue layer this simple tissue polarity is referred to as planar polarity [4]. In *Arabidopsis thaliana* (Arabidopsis), the terms inner polarity and outer polarity describe the polar localization of molecules along plasma membranes aligned parallel to the surface of the organism. Outer membranes are oriented towards the surface of the organ and inner membranes towards the innermost tissues [5]. Cells of the outermost cell layer of the root, the root epidermis, form long protuberances named root hairs [6]. These provide surface extensions facilitating, for example, the uptake of water and nutrients [7]. A number of recent advances have been made towards the identification of components contributing to the polar placement of root hairs along root epidermal cells of Arabidopsis, which provides an example for planar polarity. In addition, understanding of the cytoskeletal, the trafficking and the polar tethering requirements of proteins at outer and inner root plasma membrane domains has significantly advanced. Finally, outstanding progress has been made towards the elucidation of molecules and mechanisms underlying the formation of the major endodermal root diffusion barrier, the Casparian strip, including insight into the underlying outer endodermal cell polarity. We have focused our review on these recent advances in understanding the establishment of cell polarity in the Arabidopsis root and refer readers interested in related topics highly relevant to the expanding field of plant cell polarity to several recently published relevant review articles [2,3,8–10].

Planar polarity — cytoskeletal and lipid domain contributions

The hair forming cells (trichoblasts) of the Arabidopsis root epidermis display a coordinated polarization of emerging root hairs within the plane of the tissue layer (planar polarity). Root hairs emerge from the outer plasma membrane close to the root tip-oriented (basal) ends of cells [11] (Figure 1a). Site-specific accumulation of Rho-of-plant (ROP) proteins marks the hair initiation site prior to the emergence of a hair bulge [12,13] (Figure 1b). The polar placement of this ROP mark is determined by a concentration gradient of the plant hormone auxin in the root tip [4,14,15]. Although short-term pharmacological disruption of the cytoskeleton did not reveal an effect on ROP placement [12], recent genetic studies demonstrate that the function and organization of both actin filaments and microtubules are required for ROP placement during planar polarity establishment [16,17••,18,19••]. In particular, the ACTIN7 (ACT7) and ACT2 isoforms contribute to polar ROP and root hair positioning [17••,19••,20]. The negative actin modulator ACTIN-INERACTING PROTEIN1-2 (AIP1-2), which interacts both physically (*in vitro*) and genetically with actins including ACT7 and ACT2, modulates polar positioning of ROP proteins during planar polarity establishment [17••] (Figure 1b). Moreover, ACT7 has recently been identified as an indirect interactor of the ABCB chaperone TWISTED

Figure 1



A proteo-lipid microdomain and D6PK signalling in planar polarity. **(a)** Schematic structure of the Arabidopsis root tip. **(b)** Site-specific accumulation of ROP proteins and PIP5K3 marks the hair initiation site prior to emergence of a root hair bulge, and D6PK switches its localization from the basal plasma membrane domain to the hair initiation site just prior to hair bulge formation (left). AIP1-2-dependent and TWD1-dependent actin organization contributes to planar polarity of root hair positioning (right, top). DRP1A, DRP1B, ROP2, ROP4, ROP6, PIP5K3 and D6PK are enriched at the hair initiation site and the root hair bulge. Sterol enrichment at the hair initiation site contributes to polar positioning of ROPs and D6PK (right, bottom) that also rely on PIP5K3 function, with D6PK directly binding to phospholipids including PtdIns(4,5)P_2 *in vitro* [24••,25••].

DWARF1 (TWD1), which impacts actin organization [19••]. Strikingly, the *twd1-1* mutant revealed an alteration in root hair positioning, suggesting TWD1-dependent actin organization contributes to planar polarity [19••] (Figure 1b). At the hair initiation site, cortical microtubules form a distinctive radial star-like pattern [16], which correlates with radial stress patterns suggested by mathematical modelling [21]. Intriguingly, MICROTUBULE-ASSOCIATED PROTEIN18 (MAP18), which controls root hair tip growth, has recently been shown to physically interact with ROP2 *in vitro* and *in vivo* [22•], raising the question as to whether MAP18 may accumulate at the hair initiation site and contribute to planar polarity. A dependence of polar ROP positioning on the CLASP microtubule regulatory protein and its genetic interactor SABRE has previously been shown, but this interaction appears to occur indirectly [16].

The hair initiation site displays enrichment in sterols [23,24••] and in phosphatidylinositol-4-phosphate5-kinase 3 (PIP5K3), the enzyme that catalyzes production of the signalling phospholipid PtdIns(4,5)P_2 [16,24••] (Figure 1b). Analyses of the *cyclopropylsterol isomerase1* sterol biosynthesis mutant as well as pharmacological interference with sterol biosynthesis revealed that polar ROP placement at the hair initiation site relies on correct sterol composition [24••] (Figure 1b). Furthermore, PIP5K3, DYNAMIN-RELATED PROTEIN (DRP) 1A, DRP2B and the AGCVIII kinase D6 PROTEIN KINASE (D6PK) all accumulate at the sterol-enriched domain and contribute to the regulation of polar ROP placement [24••] (Figure 1b). Strikingly, D6PK directly binds to phospholipids including PtdIns(4,5)P_2 [24••,25••], suggesting a lipid-dependent recruitment of molecular players to the hair initiation site, which subsequently signal to mediate polar ROP placement during planar polarity establishment.

Outer and inner root epidermal cell polarity

In addition to the root hair initiation site, the root epidermal cell membrane displays inner or outer polar localization of several proteins. These include the ABCG ATP-binding cassette (ABC) transporter PENETRATION3 (PEN3)/ABCG36/PDR8 [26,27], originally identified as a pre-invasive defense component against fungal non-host pathogens [28], its homologue ABCG37/PDR9/PIS1 [27] and the distantly related ABCG34/PDR6 protein recently found to contribute to defense against necrotrophic pathogens [29•] (Figure 2b). While these examples may highlight the importance of polar outer domain proteins in defense against biotic challenges, their function in this context remains to be investigated in roots.

Polarly localized proteins exemplifying outer domain functions in uptake of inorganic compounds or in response to abiotic stresses include the BOR4 boron exporter [30], the NIP5;1 boric acid uptake channel [31,32] and the IRON-REGULATED TRANSPORTER1 [33,34] (Figure 2b). The *trans*-Golgi network (TGN) trafficking of PEN3 and NIP5;1 requires ACT7 function [35••] (Figure 2c,d), a generic TGN trafficking requirement shared by apically, basally and non-polarly localized cargos [19••,35••]. The importance of TGN trafficking of NIP5;1 and other membrane proteins is further highlighted by the NIP5;1 misplacement to TGN-derived vesicle aggregates observed in mutants defective in the gene encoding UDP-D-glucose-4-epimerase 4 (UGE4) [36,37•] (Figure 2d). However, polar outer domain tethering of PEN3 and NIP5;1 is mediated by the EXO84b exocyst complex subunit [35••], which like other exocyst subunits is polarly localized at the outer domain [38] (Figure 2c,d). Interestingly, precise EXO84b localization itself relies on actin [38], and specifically ACT7, function in dividing and elongating root epidermal cells [35••] (Figure 2c,d). This suggests a second distinct role for actin in the correct placement of EXO84b at the outer domain [35••], because the TGN misplacement observerd for PEN3 and NIP5;1 in *act7* mutants, is not displayed by EXO84b [35••]. Instead, the so-called "superpolar" EXO84b localization to the centre of the outer domain is perturbed in *act7* mutants [35••]. "Superpolar" cargo delivery, as reflected by enrichment in the centre of a polar membrane domain, has recently been observed for various polarly localized proteins [39••].

Recently, PEN3 trafficking has been found to involve endocytic recycling [35••,40•] partly based on the application of photoswitchable protein technology [35••]. This highlights the potential utility of photoswitchable proteins for obtaining a more accurate vision of secretory and endocytic trafficking contributions to polar targeting [35••,41•].

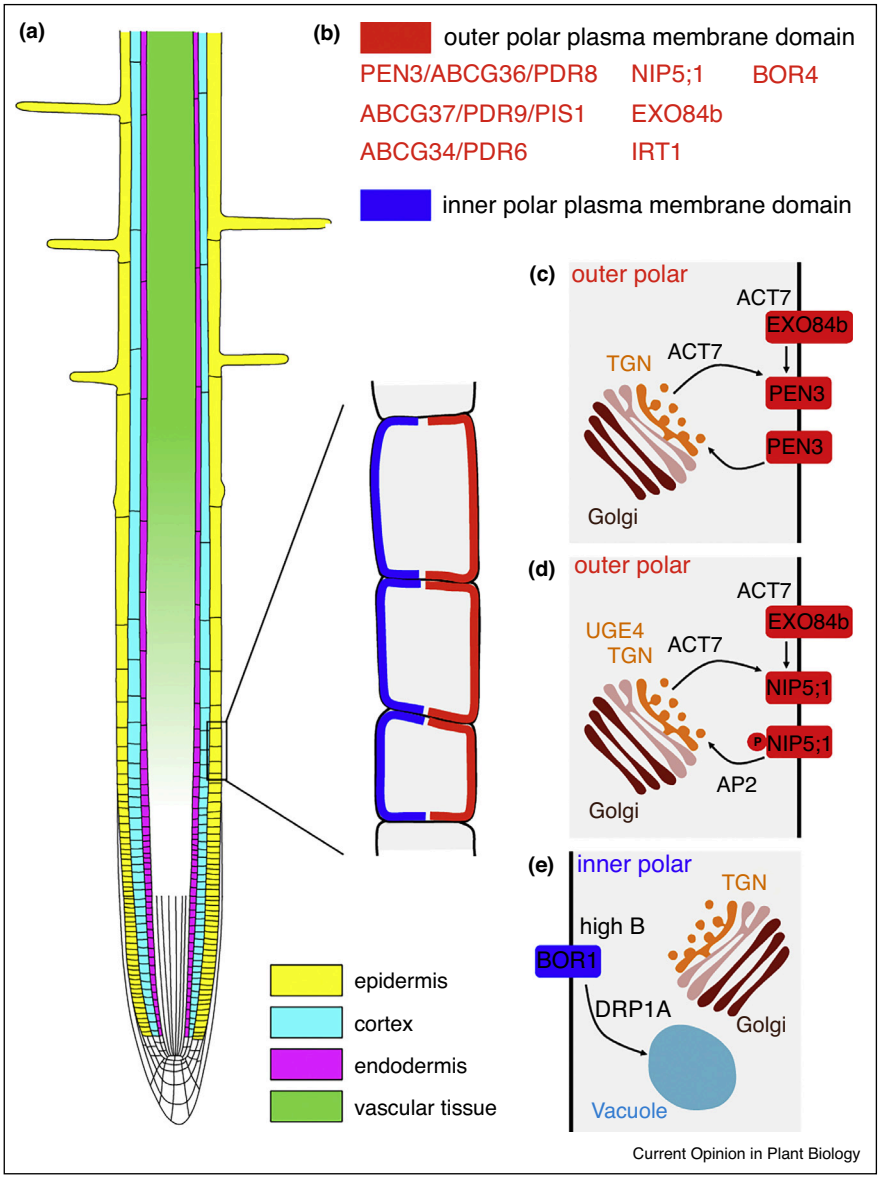
Decisive progress has been made towards identification of specific threonine phosphorylation sites essential for polar NIP5;1 localization and endocytosis [42••]. The N-terminus of NIP5;1 contains three distinctive Thr-Pro-Gly repeats and substitution of the conserved Thr residues inhibits NIP5;1 endocytosis. Moreover, loss of AP2 clathrin adapter function compromises NIP5;1 polar localization, revealing that polar outer localization of NIP5;1 is maintained by threonine phosphorylation-dependent clathrin-mediated endocytosis [42••] (Figure 2d).

Contrary to BOR4, which is enriched at the outer domain [30], the borate exporter BOR1 localizes to the inner membrane domain of root epidermal cells [32] (Figure 2b). BOR1 is required for borate transport into inner root tissues under low-borate conditions, while BOR4 mediates borate export under toxic high-borate conditions [30,32]. An evolutionary conserved di-leucine motif in BOR1-type clade transporters has recently been shown to mediate BOR1 polar localization and vacuolar sorting for degradation under high-boron conditions [43••]. Evolutionary divergence between the two differently polarly localized boron transporter clades occurred in the common ancestor of land plants as revealed by studies including the BOR homologues of the lycophyte *Selaginella moellendorffii* [43••]. Interestingly, BOR1 polarity is established after cytokinesis and relies on DRP1A-dependent, clathrin-mediated endocytosis. Similarly, boron-induced degradation of BOR1 requires DRP1A-dependent endocytosis [44••] (Figure 2e). Together with previous reports on post-cytokinetic functions of endocytosis in PIN2 and PIN1 positioning [45,46], these findings suggest that post-cytokinetic, DRP1A-dependent endocytosis represents a major mechanism contributing to polarity establishment of apical, basal and lateral cargos.

Cell polarity underlying Casparian strip formation in the root endodermis

Outstanding progress has recently been made towards understanding Casparian strip formation. The

Figure 2



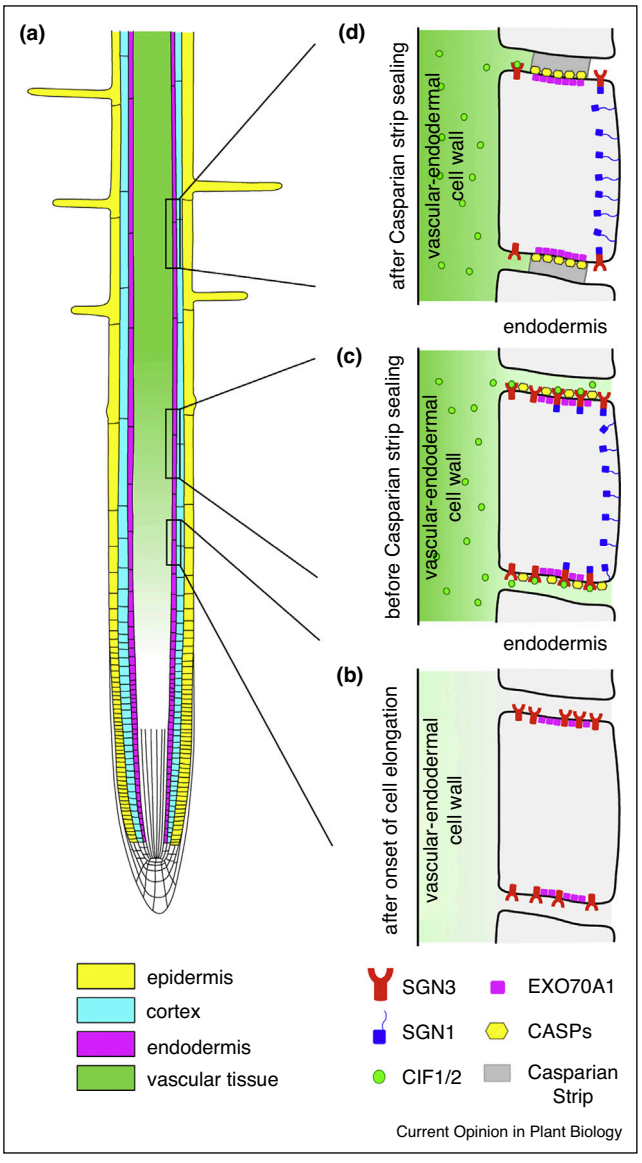
Outer and inner polar domain localization of proteins in root epidermal cells. **(a)** Schematic structure of the Arabidopsis root tip. **(b)** The outer lateral plasma membrane domain displays polar localization of PEN3/ABCG36/PDR8, ABCG37/PDR9/PIS1, ABCG34/PDR6, NIP5;1, BOR4, IRT1 and EXO84b (as well as other exocyst components), while the inner lateral plasma membrane domain displays polar BOR1 localization in root epidermal cells. **(c)** ACT7 function is required for TGN trafficking of PEN3 and correct positioning of EXO84b at the plasma membrane. EXO84b mediates outer polar localization of PEN3. **(d)** ACT7 function and TGN integrity provided by UGE4 are required for TGN trafficking of NIP5;1 and EXO84b mediates outer polar localization of NIP5;1. Outer polar localization of NIP5;1 is maintained by AP2-dependent and phosphorylation-dependent endocytosis. **(e)** DRP1A dependent-endocytosis is required for vacuolar sorting of BOR1 under high-boron (B) conditions.

GRAS-domain transcription factor SCARECROW (SCR), which has long been known to specify cortex/endodermis initials as well as cortical progenitors and is expressed in endodermal cells, has recently been shown to regulate (either directly or indirectly) transcription of the MYB DOMAIN 36 (MYB36) transcription factor [47•]. MYB36 drives expression of the *CASPARIAN STRIP MEMBRANE DOMAIN PROTEIN1* (*CASP1*) gene [47•,48•], encoding a key scaffolding factor contributing to Casparian strip formation [49]. MYB36 is necessary and sufficient for Casparian strip formation [48•] and activates expression of several Casparian strip genes including *CASP1* likely by direct binding to their promoters, as supported by chromatin immunoprecipitation-qPCR experiments [48•]. While MYB36 positively regulates transcription of five *CASP* genes, *CASP1* to *CASP5*, six *ENHANCED SUBERIN* (*ESB*) genes, *ESB1* to *ESB6*, *the PEROXIDASE64* gene, and the *SCHENGEN1* (*SGN1*)

gene [47•,48•], all of which are necessary for Casparian strip formation, it causes downregulation of the *SGN3* gene [47•]. This currently remains difficult to understand, because *SGN3* (also named *GASSHO1*, *GSO1*) encodes a receptor-like plasma membrane kinase expressed during early endodermal cell differentiation [50]. The SGN3 protein is symmetrically located in a ring-like plasma membrane domain surrounding the CASP domain and mediates fusion as well as integrity of the CASP domain that is formed in the centre of the SGN3 domain [50] (Figure 3b–d). More recently, *SGN1* has been shown to encode a cytosolic receptor-like kinase required for Casparian strip integrity and positioning [51••]. Intriguingly, the SGN1 protein dynamically and polarly localizes to the plasma membrane via reversible palmityolation. SGN1 is found at the cortical, outer domain of the endodermal plasma membrane, where its localization overlaps with that of SGN3 just at the cortical (outer) side of its domain [51••] (Figure 3c). Genetic analyses suggest *SGN3* and *SGN1* action in one pathway with respect to central CASP domain positioning. Strikingly, polar SGN1 localization requires neither *SGN3* function nor the activity of other MYB36-dependent factors, but relies on still unknown tissue-specific polarity cues. While SGN3 and SGN1 appear to act in the same pathway directing CASP domain positioning, this may not involve their direct interaction, for which positive evidence is currently lacking [51••]. The question of which signalling cue might activate SGN3 has been answered by the identification of the SGN2 tyrosylprotein sulfotransferase, which mediates sulfation of the CASPARIAN STRIP INTEGRITY FACTORS1 and 2 (CIF1/2) [52••]. These small sulfated peptide ligands bind to the extracellular leucine-rich repeat domain of SGN3 [52••,53••] (Figure 3c). Application of sulfated CIF1/2 peptides complements the *sgn2* Casparian strip phenotypes but does not complement the *sgn3* and *sgn1* mutants, suggesting that SGN3 and SGN1 act in CIF1/CIF2 signal perception or downstream signalling [52••]. The *CIF1/2* genes are expressed in the stele, from where the peptides are thought to move to the endodermis [52••,53••] (Figure 3c). The establishment of an intact Casparian strip is proposed to restrict peptide movement from the outer cortical membrane domain of the endodermal cells [52••] (Figure 3b). Hence, Casparian strip integrity may be controlled by a SGN2-dependent CIF1/CIF2 peptide-mediated diffusion barrier surveillance system that signals asymmetrically from the stele to activate the SGN3-SGN1 pathway [52••] (Figure 3b,c). In this scenario, the reliance of SGN3 signalling on polar, cortical domain localization of SGN1 would lead to a signalling shut down, once the diffusion barrier has been established. How polar SGN1 localization and early SGN3 placement are established remain intriguing open questions.

Further insight into factors involved in CASP placement comes from the discovery of the specific localization of

Figure 3



SGN3 signalling and SGN1 polarity in endodermal cells are required for Casparian strip positioning and sealing. **(a)** Schematic structure of the Arabidopsis root tip. **(b)** After the onset of cell elongation, SGN3 is expressed just prior to the onset of CASP1 expression [50]. Accumulation of EXO70A1 in the central plasma membrane domain precedes the onset of CASP1 expression [54••]. SGN3 symmetrically accumulates in the transversal and anticlinal sides of the plasma membrane [50]. **(c)** Prior to Casparian strip sealing, SGN1 and CASP1 expression occurs with similar timing [51••]. SGN3 maintains a symmetrical localization at the transversal and anticlinal sides of the plasma membrane and SGN1 locates to the outer plasma membrane domain [50,51••]. CIF1/2 bind to SGN3, and the CIF1/2-SGN3-SGN1 signalling module generates a signalling cascade supporting Casparian strip positioning and sealing [52••]. EXO70A1 strictly accumulates at the future site of the CASP domain [54••], and CASP proteins subsequently accumulate at this site in an EXO70A1-dependent manner [54••], facilitating completion of Casparian strip sealing. **(d)** During and after Casparian strip sealing, CASP proteins strictly accumulate at the Casparian strip membrane domain. CIF1/2 movement to the endodermal/cortical apoplastic space and outer membrane domain of the endodermis is prevented by Casparian strip sealing [52••].

the EXO70A1 exocyst subunit at the incipient CASP domain and the requirement for EXO70A1 for CASP1 localization [54••] (Figure 3c,d). Strikingly, early EXO70A1 localization is accompanied by a PtdIns(4,5)P_2 signature preceding CASP1 localization and positioning of this signature relies on EXO70A1 activity [54••]. Future studies may clarify the potential requirement of PtdIns(4,5)P_2 for EXO70A1 localization as well as the relationship between EXO70A1 and SGN3 early during Casparian strip formation.

Conclusions

During the last two to three years, genetic, cell biological and biochemical approaches have allowed progress towards identification of new players contributing to the execution of planar polarity including a polar proteo-lipid microdomain required for signalling during polar root hair initiation. A late signalling component regulating placement of the domain and depending on lipid interaction for its own polar localization has been identified, as well as cytoskeletal requirements for polar domain placement. The interdependence of the polar localization of multiple players at this site suggests the involvement of positive feedback, but a full understanding of underlying mechanisms may require the application of mathematical modelling approaches. Such approaches have been initiated to explore the relationship between microtubule localization and stress patterns at the root hair initiation site [21], but now require extension to give a deeper understanding of the connection between the molecular and mechanical properties of the site. Similarly, tools for exploring cell autonomous and non-autonomous functions as well as the necessity for subcellular restriction of the identified players will be helpful in future studies. Generic trafficking requirements of outer and inner polar cargoes have been elucidated, and specific amino acids required for polar localization and endocytosis of some cargos have been mapped. Nonetheless, how specificity of polarity establishment through endocytosis after cytokinesis is achieved remains to be understood. Considerable progress has been made towards our understanding of mechanisms signalling Casparian strip positioning and sealing, and insight into the underlying endodermal cell polarity has been gained. How the combination of symmetric SGN3 localization and polar SGN1 outer domain placement establishes CASP domain positioning and sealing, as well as how the symmetric and polar localizations of these early signalling components are established can be addressed in future studies, as can the potential interplay between SGN3 signalling and phospholipid-exocyst interactions during early CASP domain positioning.

Conflict of interest

The authors declare that they have no competing interests.

Acknowledgements

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This study reveals a sterol-enriched polar domain accumulating PIP5K3, ROP2, ROP6, DRP1A, DRP2B and D6PK during hair initiation, and the requirement of the functions of all these players for polar ROP positioning. It identifies D6PK as a late signalling component switching polarity from basal to the hair initiation site, just prior to hair initiation. D6PK directly binds to phospholipids including PtdIns(4,5)P_2 and requires PIP5K3 function for polar localization at the hair initiation site.

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This study identifies the amino acids within a polybasic motif essential for direct binding of D6PK to phospholipids including PtdIns(4,5)P_2. It demonstrates that those amino acids determine D6PK basal polar localization, reveal the *in vivo* requirement of PIP5K1 and PIP5K2 for D6PK basal localization, and reveals the requirement of the polybasic motif for tropic responses mediated by D6PK. Together with ref. [24••] this study suggests that phospholipids including PtdIns(4,5)P_2 instruct D6PK recruitment to different polar domains.

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