HHS Public Access

Author manuscript

Curr Opin Plant Biol. Author manuscript; available in PMC 2020 April 26.

Published in final edited form as:

Curr Opin Plant Biol. 2018 October ; 45(Pt A): 127–135. doi:10.1016/j.pbi.2018.06.003.

Cell polarity: compassing cell division and differentiation in plants

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Abstract

Protein polarization underlies directional cell growth, cell morphogenesis, cell division, fate specification and differentiation in plant development. Analysis of *in vivo* protein dynamics reveals differential mobility of polarized proteins in plant cells, which may arise from lateral diffusion, local protein–protein interactions, and is restricted by protein–membrane–cell wall connections. The asymmetric protein dynamics may provide a mechanism for the regulation of asymmetric cell division and cell differentiation. In light of recent evidence for preprophase band (PPB)-independent mechanisms for orienting division planes, polarity proteins and their dynamics might provide regulation on the PPB at the cell cortex to directly influence phragmoplast positioning or alternatively, impinge on cytoplasmic microtubule-organizing centers (MTOCs) for spindle alignment. Differentiation of specialized cell types is often associated with the spatial regulation of cell wall architecture. Here we discuss the mechanisms of polarized signaling underlying regional cell wall biosynthesis, degradation, and modification during the differentiation of root endodermal cells and leaf epidermal guard cells.

Introduction

Cell polarity is fundamental to cell functions in all living organisms. How proteins are asymmetrically distributed in a cell and how polarized proteins regulate a diverse array of cellular events have been fascinating questions for biologists.

In animal systems, many polarized proteins, for example, Ste5 [1] and Bem1 [2] in yeast and PAR3/6 in *Caenorhabditis elegans* [3], are scaffold proteins that convene multiple components to ensure concerted interaction for signaling specificity and fidelity. Homologs of these conserved polarity proteins are not encoded in plant genomes. The plant-specific protein BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE (BASL) represents a polarity factor [4] that scaffolds a mitogen-activated protein kinase (MAPK) signaling cassette in Arabidopsis stomatal lineage cells [5]. How BASL is associated with the plasma membrane (PM) and how BASL polarity is maintained remain unknown.

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In plants, protein polarization provides mechanisms for polar cell expansion (thus morphogenesis), asymmetric cell division and functional differentiation. A number of cell types produce daughter cells with unequal sizes and distinct cell fates. Such asymmetric cell divisions are often preceded by protein polarization that presumably leads to the specification of division plane orientation and/or differential cell fates [4,6,7]. What the polarity cue is and how this cue controls subsequent divisional asymmetries (size and fate) are fundamental questions in biology. Using stomatal lineage divisions in maize and Arabidopsis as model systems, we discuss how cell polarity might be instructive to division plane orientation in plants.

In multicellular organisms, cell differentiation typically manifests unique cell morphology and structures for specific physiological roles. One prominent example is that plant cells utilize localized cell wall modification to diversify cell form and function. The differentiation of root endodermal cells and leaf guard cells exemplifies the elaborate spatial regulation of cell wall architecture. Exciting progress has been made towards elucidating the formation of Casparian strip, a lignin band structure in Arabidopsis roots (reviewed in [8]), at a polarized cel signaling level [9]. The differentiation of stomatal guard cells requires spatiotemporally dynamic cell wall deformation and modification that may also require polarized cell signaling.

Protein dynamics and polarity regulation at the plasma membrane

Protein dynamics are critical for their cellular functions. Characterization of protein dynamics and how these dynamics relate to a protein's physiological roles *in vivo* is enabled by advanced microscopy techniques, for example, FRAP (fluorescence recovery after photobleaching) and photoactivation [10], which have greatly enlightened our understanding of the mechanisms underlying the regulation of cell polarity.

In the establishment and maintenance of cell polarity in eukaryotic organisms, scaffold proteins were commonly found to assemble various signaling components in the cytoplasm and at the PM to a polar site [11]. For example, Ste5 scaffolds a MAPK cascade in budding yeast to activate the mating-specific MAPK Fus3p [11] (Figure 1a). Yeast Bem1 organizes a feedback loop to generate localized activation of the small GTPase Cdc42 to ensure bud polarity axis establishment [12]. In *C. elegans* embryos, the PAR3 (partitioning defective 3) and PAR6 scaffolds organize the anterior and posterior polarity complexes to regulate asymmetric cell division [13].

Scaffold proteins, due to their functions in the assembly of protein complexes, were thought to be stable, but emerging evidence suggests that they are often surprisingly dynamic. Based on FRAP data, the recovery rates, t_{half} or $t_{1/2}$ (the time point when half of the final recovered intensity is reached), suggested rapid movement of polarity factors: Ste5 $t_{1/2}$ = 8 s at the mating tip [14], Bem1 $t_{1/2}$ = 2.37 s at the bud site [15], and cortical PAR6 reached nearly full fluorescence recovery in 1 min [16]. The dynamic features of scaffold proteins suggest that they are under intricate regulation and might confer quick adjustment of the cell polarity machinery in response to external cues and to ensure signaling specificity [17,18].

FRAP analyses were also performed in plant cells to characterize polarity protein dynamics. The available studies examined the membrane-embedded PIN-FORMED (PIN) auxin efflux carriers [19••], ABCG transporters [20], and boron transporters in root cells [21]. In addition, the membrane-associated small GTPase ROPs [22,23] and the MAPK scaffold protein BASL have also been analyzed by FRAP. Interestingly, except for ROP2 ($t_{1/2}$ = 25 s), all other polarity proteins were found to be relatively immobile, for example, PIN3 ($t_{1/2}$ = 120 s) and BASL ($t_{1/2}$ = 125 s), and neither of them reached plateau in 5 min in leaf epidermal cells [24••] (Figure 1b).

The PIN auxin effluxers are fundamental players in the regulation of directional auxin flow. The maintenance of PIN polarity domains mainly depends on three membrane-based mechanisms: polar secretion, endocytosis and endocytic recycling, and lateral diffusion (reviewed in [25,26]). The stabilization of PINs in the PM is regulated by auxin via ROP-dependent inhibition of endocytosis [27,28]. Additionally, the extracellular matrix, cell walls, seemed to play a critical role in restricting protein lateral diffusion at the membrane ([19••] and Figure 1b). Detaching the PM from the cell wall by plasmolysis released the cell wall constraints so that the membrane proteins became more mobile, and interestingly, polarization was also abolished in these cells [20]. These studies provided exemplary models for membrane-centered protein polarization dynamics and highlighted both signaling feedback on trafficking and the unique function of cell walls in polarity maintenance in plant cells.

Rho GTPases from plants (ROPs) breaks cellular symmetry at the PM to regulate many plant developmental processes [29]. The ROP proteins are attached to the PM via lipid modifications [30]. The fast FRAP recovery rates of ROPs were comparable to that of their analog Cdc42 in yeast polarity site [31•], both of which switch between GTP-bound and GDP-bound forms and undergo recycling on and off the PM. The polarity maintenance of these G-proteins involves positive feedback loops via cytoskeleton-dependent cytoplasmic exchange and cytoskeleton-independent lateral diffusion (Figure 1b) [29,32,33].

BASL is another highly polarized protein that is expressed in the stomatal lineage cells to control asymmetric cell division in Arabidopsis [[24••]]. The BASL protein does not contain recognizable functional motifs for membrane localization and was therefore proposed to associate with membranes by interacting with proteins or lipids [34]. Based on *in vivo* FRAP, BASL showed surprisingly slow mobility at the polarity site, strikingly contrasting to that of the membrane-attached ROPs, but comparable to that of the membrane-embedded PIN3 (Figure 1b) [24••]. Then, why was BASL so immobile at the polarity site? We hypothesize that BASL may bind to a physical partner that is embedded in or tightly associated with the PM (Figure 1b). Because of minimal lateral diffusion detected for BASL [24••], its recovery at the polarity site might mainly arise from cytoplasmic replenishment, either through vesicle delivery of the membranes it tightly binds to or through its diffusion from the cytosol to associate with the membrane partner that is delivered to the PM. This hypothesis is supported by the recent discovery that, after tagged with an artificial transmembrane domain, Cdc42 remains polarized, but is significantly immobilized in yeast cells [31•].

When BASL was engineered with an N-terminal myristoylation site, this covalent modification fixed the protein at the PM but did not disturb the polarity formation [24••]. The FRAP data showed that unequal protein dynamics of myr-BASL along the PM highly resembled that of the membrane-fixed Cdc42 in yeast [24••,31•]. Also, the active forms of both molecules (GTP-bound Cdc42 and a phospho-mimicking form of BASL) exhibited lower mobility [31•]. It was thus proposed that the distinctly lowered mobility of active BASL and Cdc42 at the polar site is due to their association with large protein complexes or membrane microdomains [24••,31•]. Another polarized protein in plants, the AGCVIII D6 protein kinase (D6PK), a PIN activator colocalized with PIN1 at the basal side of root cells [35], directly binds to PtdIns(4,5)P_2 and is recruited to the sterol-enriched microdomains at the root hair initiation sites [36]. The D6PK mobility has not been determined by FRAP yet; it would be interesting to investigate how the D6PK dynamics is regulated by its interaction with PIN1 and lipid microdomains.

Altogether, it appears that protein dynamics at the PM is dependent on the modes of their interaction with the membrane, either embedded in the PM, anchored to the PM by lipids, or associated to the PM through protein–protein or protein–lipid interactions. The identification of their binding components at the PM and investigation of their regulatory relationship will shed new light on the mechanisms underlying protein polarity formation and maintenance in plant cells.

Cell polarity and division plane orientation: mechanistic connections

During mitosis, the pre-mitotic microtubule (MT) structure, the preprophase band (PPB), marks the future cortical division zone and is accepted as the causal determinant of division plane placement in plant cells [37]. Mutations in key regulators of PPB lead to defects in the formation, orientation and maintenance of the cell division plane (Figure 2, for details, see comprehensive reviews [38-40]).

However, mutants lacking PPBs usually also exhibit significant defects in cortical microtubule organization [41,42], thus hampering an explicit evaluation of the mitotic defects from the interphase misregulation. Mutants lacking apparent PPBs while maintaining mostly normal interphase cortical MT arrays were only reported very recently [43,44••]. Mutations in the MT-associated TONNEAU1a (TON1a) caused the failure of PPB formation in Arabidopsis, but interestingly, different cell types seemed to respond differently: cell divisions in the epidermis were severely misorientated, but normally maintained in the cortical layer, suggesting that the root cortical cell divisions can be controlled by a PPB-independent mechanism [43]. Schaefer *et al.* identified an important Arabidopsis triple mutant *trm6;7;8* that failed to produce discernable PPBs, but displayed nearly normal growth and development. The primary defect of the *trm* mutant is the reduced precision in division orientation in the roots [44••]. This study reassessed the dominant role of PPB in directing division orientation, and raise the possibility that the PPB might instead provide a correction mechanism. In support of this hypothesis, the moss *Physcomitrella patens* produces gametophore tissues by cell divisions in the absence of PPBs but relying on the presence of cytoplasmic microtubule-organizing centers (MTOCs) [45••]. In the asymmetrically dividing gametophore initial cells, the discrete cytoplasmic MTOC named ‘gametosomes’ and the

MT nucleation factor γ-tubulin were found to control cell division orientation [45••]. Taken together, these new findings support a PPB-independent mechanism that might set the initial spindle axis through cytoplasmic MT nucleation to assist in division plane orientation in seed plants.

These mechanisms, however, are inadequate to explain asymmetric division plane placement. The identification of a few polarly localized proteins at the cell cortex, for example, PAN1/ROP in maize [7,46] and BASL/YDA in Arabidopsis [4,5], provided useful tools towards revealing the regulatory mechanisms connecting cell polarity and division orientation in plants.

In a maize stomatal complex, the asymmetric division of two subsidiary mother cells (SMCs) is controlled by a PAN1 receptor-centered ROP polarity module (see reviews [38,47,48]). The polarized PAN1/ROP signaling was hypothesized to induce the formation of proximal actin patch, thereby nuclear migration, thus asymmetric placement of the division plane [7,46]. Alternatively, polarized PAN1/ROP signaling might influence the PPB positioning indirectly by promoting local cell wall expansion that is achieved by ROP-mediated actin nucleation and vesicle trafficking [38,49]. An uneven mechanical tension in the cell wall imposed by the regional expansion of the SMC may trigger cortical MT reorganization, which guides MT bundling and PPB formation [7,46,50].

In Arabidopsis, the polarity protein BASL controls asymmetric division of the stomatal precursor cells [4]. The orientation of BASL crescent was found to be guided by tissue-level mechanical forces as well as local peptide-receptor chemical signaling [51]. Before the mitotic division, the BASL polarity pole predicts the division plane to be placed distally (Figure 2) [4], yet the underlying mechanism has not been elucidated. In plant cells, MAPKs were found to regulate MT dynamics and organization, for example, Arabidopsis MPK4 phosphorylates the MAP65 bundling factors to promote MT turnover of the phragmoplast during cytokinesis [52]. With a MAPK-scaffolding function, BASL might regulate PPB positioning through the established function of MAPKs on the MT regulatory proteins, such as MAP65s [53]. Alternatively, at one end of the cell, high MAPK signaling may counteract the PP2A complex-mediated de-phosphorylation events, which promote MT ordering and bundling [54-56], to prevent proximal PPB formation.

Could these polarity complexes at the cell cortex impinge on the cytoplasmic MTOC-mediated spindle positioning system? At least for BASL, its polarity persists throughout the cell cycle [[45••]], allowing possible regulations on spindle positioning. Then, how could cytoplasmic MT nucleation contribute to the bipolar spindle establishment in plants? In the PPB-expressing angiosperms, ‘polar caps’ were suggested to be analogous structures of the cytoplasmic MTOCs [57], as ‘gametosomes’ in *Physcomitrella patens* [45••]. The formation of polar caps involves aggregation of MT nucleation at the nuclear periphery, and the two polar caps are segregated along an axis that is perpendicular to the PPB and decisive to the spindle orientation (Figure 2) [58]. In plant cells, microtubule nucleation is mainly mediated by an evolutionarily conserved γ-tubulin-containing ring complex (gTuRC) [59] that can be regulated by the augmin complex [60]. Recent studies showed that activated MPK6 localizes to MTs, physically binds to γ-tubulin and phosphorylates the MT plus end protein EB1c

[61]. Defective MAPK signaling and lowered expression of *EB1c* in Arabidopsis resulted in mitotic abnormalities in chromosomal segregation and spindle orientation [61]. These findings hint a potential role of asymmetric MAPK signaling (e.g., induced by the BASL/YDA polarity complex) in the regulation of cytoplasmic MT nucleation, spindle orientation, thus division plane orientation in plants.

Plant cell differentiation: a link between cell polarity and localized cell wall modifications

Cell walls not only provide strength and mechanical support, but they also play diverse functions in plant growth, development, cell–cell communication, and cell morphogenesis. Specialized cell functions can be easily revealed by cell shape and structure. One prominent example is the production of a water-impermeable Casparian strip in the root endodermis.

The Casparian strip is a central ring-like lignin structure embedded in the root radial and transverse walls to physically prevent apoplastic diffusion of nutrients from the stele. The identification of key regulators and mechanisms for constructing the Casparian strip in Arabidopsis roots suggested that the formation of this central ring structure is tightly controlled by cell polarity signaling [9,62-64,65••]. An asymmetric signal triggered by the sulfated CIF peptides is perceived by the receptor-like kinase SCHENGEN3 (SGN3) [63,64], whose expression partially overlaps with a polarly distributed receptor kinase SGN1 (Figure 3a) [9]. The CIF1/2-SGN3/SGN1 signaling module defines the Casparian strip positioning. Lignin deposition at the Casparian strip depends on the EXO70A1 exocyst-mediated targeted delivery of the PM-localized CASPARIAN STRIP MEMBRANE DOMAIN PROTEINS (CASP1–5) that scaffold the enzymes for lignin biosynthesis [62,65••]. EXO70A1 appears to specifically function in directed targeting of CASPs to the PM, but not as a general regulator for secretion [65••]. The distribution of PtdIns(4,5)P_2 coincides with the exocyst accumulation, suggesting that phospholipid signaling may be important for exocyst-based CASP localization [65••]. The studies on the Casparian strip formation disclosed an elegant integration of signaling events in cell–cell communication and spatially organized membrane trafficking that ensure precise cell wall modification during plant cell differentiation.

Stomata are highly specialized functional units for gaseous exchange between plants and the atmosphere. Stomatal guard cell walls must be both strong, to retain changes in turgor pressure, and flexible, to allow the stomatal apertures adjustable to environmental changes. The formation of a pair of kidney-shaped stomatal guard cells in dicot plants involves an one-time symmetric division of the guard mother cell (GMC), followed by stomatal pore formation and guard cell morphogenesis; a process that heavily requires asymmetric cell wall modification (Figure 3b and specified below).

In fact, prior to the symmetric cell division, the ovalshaped GMCs are already polarized, manifested by end-wall thickening at the two poles that predict the division plane orientation (Figure 3b) [66,67], but the underlying molecular mechanisms remain obscure. In ferns, after the GMC division, stomatal pore initiation coincides with the formation of the anticlinal MT bundles along the midregion of the ventral wall. This is soon followed by

mutual separation of the adjacent plasmalemmata and stomatal pore broadening [68]. In this process, callose and pectin degradation takes place at the pore initiation sites.

Callose is produced by specialized cells in specific stages in the development of plant cell walls as well as in defense of environmental attacks [69]. During the formation of a stomatal pore, for example, in ferns and mosses, callose is deposited into the newly-synthesized cell wall, and soon degrades in a precisely controlled central-to-peripheral manner (Figure 3b) [70•,71]. An optimal level of callose in the differentiating stomatal wall seems to play an essential role in creating the central pore because chemical treatments that disturb callose synthesis or degradation inhibited normal pore formation in the fern *Asplenium nidus* [70•].

Active pectin degradation was also found in the process of stomatal pore formation. The pectin-degrading enzyme PGX3 (POLYGALACTURONASE INVOLVED IN EXPANSION3) accumulates polarly at the stomatal pore initiation sites (Figure 3b) [72••]. The loss-of-function mutant *pgx3* produces shorter and smaller stomatal pores, suggesting that PGX3-mediated asymmetric pectin degradation is important for central wall separation [72••]. How callose and pectin are degraded spatiotemporally and how cell wall modifying enzymes, for example, PGX3, are precisely targeted to the pore initiation sites remain enigmatic. Future work is anticipated to reveal polarity signaling events underlying these procedures.

The maturation of guard cells is featured by striking wall thickening at two positions: (1) the inner walls at the pore site and (2) the end walls at the two poles (Figure 3b) [66]. The pore thickening was thought to promote stomatal opening and assist stomatal bending when guard cells inflate [73]. The new technical advances in atomic force microscopy (AFM) that measures mechanical properties of the cell surface showed that functional guard cells in Arabidopsis have a surprisingly high cell wall stiffening at the GC polar poles, coinciding with the polar enrichment of de-esterified (hard) pectins [74••]. Digestion of pectins with polygalacturonase led to dramatic changes in the stiffness pattern across the GCs, as well as defective stomatal function [74••]. The GC polar stiffening and polar enrichment of de-esterified pectin likely require an as yet unknown polar secretion of pectin methylesterases, for example, PME6 [75•]. Again, the final stage of guard cell differentiation requires the regulation of cell polarity, but the mechanisms for this regulation remain mysterious.

Concluding remarks

Research in recent years has greatly expanded our understanding of the molecular mechanisms underlying protein polarization, which unifies and cross-links polar cell expansion, asymmetric cell division, and cell differentiation in plants. This mechanistic understanding has been greatly aided by the analysis of *in vivo* protein dynamics using the FRAP microscopy technique. FRAP has the potential to uncover new principles of protein dynamics when applied to other polarity proteins, for example, D6PK that interacts with the PM through electrostatic interactions [76] and BIK1/MARK that localizes to the PM by interacting with the transmembrane receptor FLS2 [77].

It is clear that polarity signaling plays critical roles in the regulation of division orientation and cell differentiation in plants, and some relevant polarity signaling components and pathways have been identified in recent years. Yet many challenges remain towards elucidating the mechanisms behind protein polarization, the dynamics of polarity signaling machinery, and in understanding how polarity signaling controls cell division orientation and cell differentiation. In the regulation of cell division orientation, polarity signaling may distantly impinge on MT organization, either at the cell cortex or in the cytoplasm. Elucidating the composition and regulation of the polarity signaling complexes holds the promise to functionally connect the polarity module to the cell division machinery. In differentiating cells, how cell signaling pathways lead the way to ultimately achieve specific cell shape and specialized function remains a fascinating and fundamental question in biology. An emerging theme in this area is the importance of polar deposition, deformation and modification of cell wall components, such as lignin, suberin, pectin, and callose, and polar secretion of factors modifying them, as shown in root endodermal cells and aerial guard cells. The new advances raise exciting challenges in understanding the polarity signaling machinery that orchestrates multiple polarization processes and the mechanisms that coordinate these processes with cellulose microfibril deposition, which is spatially controlled by cortical MTs.

Acknowledgment

This work is supported by National Institute of General Medical Sciences of the National Institutes of Health under award number R01GM109080.

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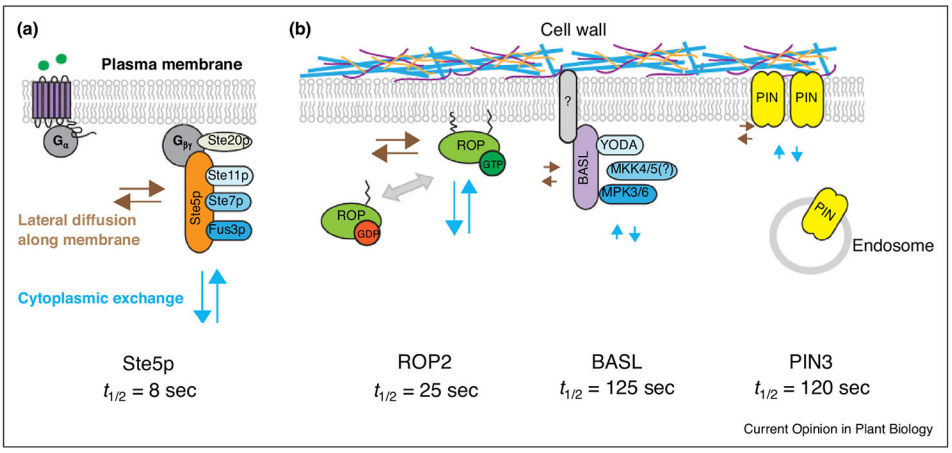

Figure 1.

Differential dynamics of polarity proteins in yeast and plants. **(a)** Ste5p scaffolds the MAPKKK Ste 11p, MAPKK Ste7p to activate the mating-specific MAPK Fus3p in yeast. Ste5p is highly mobile at the plasma membrane, where it binds to beta-gamma subunits of G-protein to create a lattice for activating the Ste5p–MAPK cascade protein complex. **(b)** Three polarized proteins in plants: ROP2, a lipid-modified protein for cell polarity; BASL, a scaffold protein for the YODA MAPK cascade; PIN3, a transmembrane auxin transporter. ROP2 rapidly switches between a GTP-bound and GDP-found forms. BASL and PIN3 show similarly slow protein mobility. A hypothetical BASL partner (grey with question mark) was predicted to be membrane-embedded or tightly associated with the plasma membrane. The PIN polarity is mediated by the membranetrafficking system, and plant cell walls exert constraints for lateral diffusion. FRAP measures the recovery rates ($t_{1/2}$) of the designated PM proteins, which are mainly determined by lateral diffusion along the PM and cytoplasmic exchange (dynamic protein–protein interaction and bulk transport). Brown arrows: long ones show fast lateral diffusion and short ones show slow lateral diffusion. Light blue arrows: long ones show fast cytoplasmic exchange and short ones show slow cytoplasmic exchange. Recovery rates $t_{1/2}$ of the respective polarity proteins (the time point when half of the final recovered intensity is reached) are specified at the bottom.

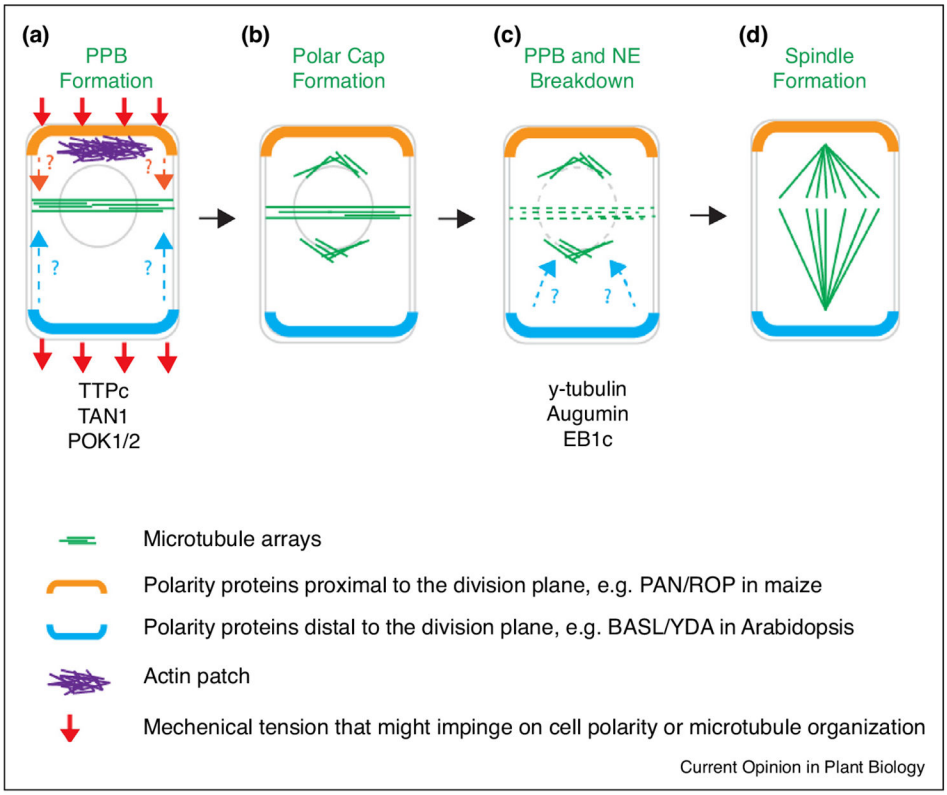

Figure 2.

Division plane orientation in plant asymmetric cell division. Diagrams show four early stages in plant cell division. In an asymmetrically dividing cell, three MT-based structures (PPB, polar cap, and spindle, green lines in **a–d**) appear successively to regulate division plane orientation. Before the PPB formation, polarized proteins (orange and blue curves) may define divisional asymmetries (a). Polarity maintenance can be regulated by extrinsic mechanical forces (red arrows). Possible functions of polarity proteins on the PPB are indicated by dashed arrows (orange and light blue). The polar caps, representing cytoplasmic microtubules nucleation centers (MTOCs), are transiently formed before the PPB and nuclear envelope (NE) breakdown to guide spindle positioning (b and c). Possible functions of the polarity proteins to the polar caps are marked by dashed arrows (c). At the metaphase, the bipolar spindle axis is perpendicular to the division plane (d).

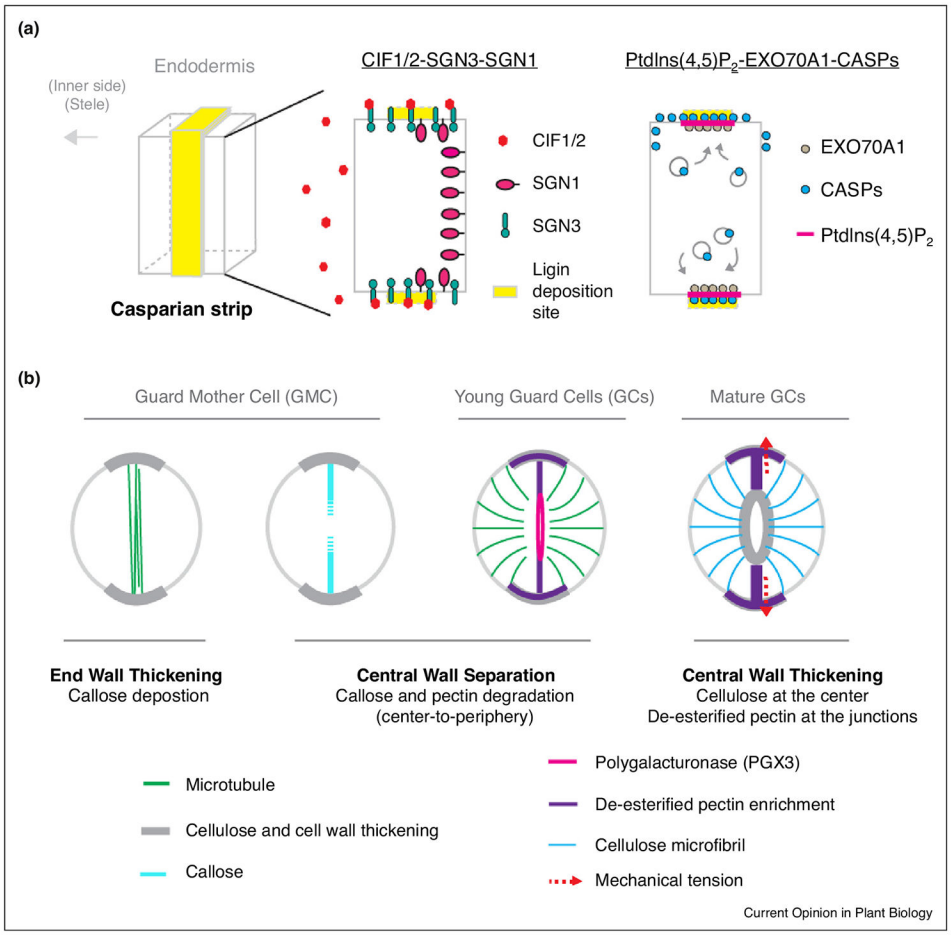

Figure 3.

Polarized cell wall specification in plant cell differentiation. **(a)** Diagrams show the formation of the Casparian strip in root endodermal development. The lignin deposition site is precisely defined by a CIF1/2-SGN3-SGN1 signaling module (left). The CASP proteins, which mark the Casparian strip site, are delivered to the PM by the exocysts containing EXO70A1 to the membrane sites coincides with the PtdIns(4,5)P_2 enrichment (right). **(b)** Diagrams show asymmetric cell wall deformation, accumulation, and modification in stomatal differentiation. The end wall thickening of GMC predicts where the PPB (green) is placed. Central wall separation involves polarized callose and pectin degradation at the pore initiation site. A functional stomatal complex requires cell wall thickening at the central pore region and modified pectins (rigid de-esterified forms) at the two poles. The pectin hardening at the polar sites imposes cell wall stiffening and mechanical force (red arrows) for functional stomatal movement. Microtubules (green lines) guide the deposition of cellulose microfibrils (blue lines) in maturing GCs.