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Protein polarization: Spatiotemporal precisions in cell division and differentiation

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Abstract

Specification of cell polarity is vital to normal cell growth, morphogenesis, and function. As other eukaryotes, plants generate cellular polarity that is coordinated with tissue polarity and organ axes. In development, new cell types are generated by stem-cell division and differentiation, a process often involving proteins that are polarized to cortical domains at the plasma membrane. In the past decade, pioneering work using the model plant *Arabidopsis* identified multiple proteins that are polarized in dividing cells to instruct divisional behaviors and/or specify cell fates. In this review, we use these polarized cell-division regulators as example to summarize key mechanisms underlying protein polarization in plant cells. Recent progress underscores that self-organizing amplification processes are commonly involved in establishing cell polarity, and cellular polarity is influenced by both tissue-level and local mechanochemical cues. In addition, protein polarization during asymmetric cell division shows a distinct feature of temporal control in the stomatal lineage. We further discuss possible coordination between protein polarization and the progression of cell cycle in this developmental context.

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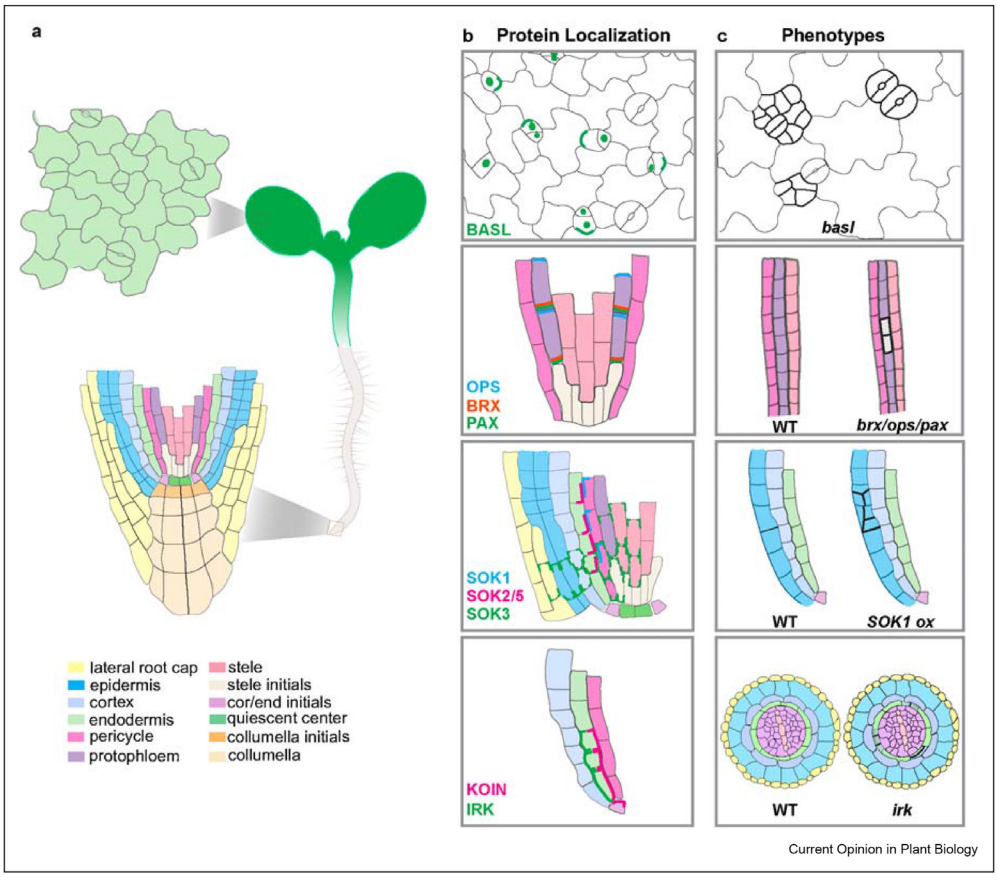
Protein polarization, Self-reinforcement, Signaling amplification, Global polarity, Temporal regulation, Stomatal development.

Cellular polarization is manifested by spatially segregated distribution of biological molecules (RNAs, proteins, and lipids), organelles, and structures. This fundamental feature of the cell contributes to cellular growth, morphogenesis, and function [1]. In asymmetrically dividing cells, cell polarity instructs division-plane orientation, influences cell-division potentials, and helps to specify distinct daughter-cell fates [2,3]. At an organizational scale, polarity of individual cells can be collectively aligned and coordinated with the tissue plane, a phenomenon known as planar cell polarity, to contribute to organ patterning and formation in development [2].

In the model plant *Arabidopsis*, well-known polarized proteins are the auxin efflux carriers, PIN-FORMED (PIN) proteins, and some of their regulatory proteins, such as the AGC kinases (see recent reviews [4,5]). Recently, several other proteins have been identified to polarize in dividing cells and their polarization associates with the divisional behaviors of the expressing cells. These polarity proteins include BASL (BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE) in the stomatal lineage cells [6], SOK (SOSEKI) proteins in the embryos and roots [7••], and oppositely polarized BRX (BREVIS RADIX) and OPS (OCTOPUS) in the protophloem [8,9]. These polarity factors are membrane-associated and attached to a subdomain of the plasma membrane through interaction with proteins and/or lipids. More recently, transmembrane cell-surface receptors IRK (INFLORESCENCE AND ROOT APICES RECEPTOR KINASE) and KOIN (KINASE ON THE INSIDE) were shown to display intracellular contrapolar localization in the root meristem [10••,11••] (Figure 1a–b). Although this is not a comprehensive list, these genes are selected in this article for further discussion because they are functionally connected with the regulation of cell division, often asymmetric, in plant development.

Here, we review recent advances on the molecular mechanisms by which protein polarization is initialized, established, and maintained in plants. We discuss new insights about how the polarity site is placed coordinately with the tissue axes. Finally, we discuss how protein polarization might be temporally controlled to coordinate with cell division and differentiation in development.

Figure 1



Polarized proteins associated with stem cells in plant development. (a) Diagrams show cell division and distinct cell types in developing leaf and root, respectively. **(b)** Diagrams depict polarization of the selected proteins in dividing cells. BASL localizes in the nucleus and accumulate to a polar crescent at the cell cortex of stomatal lineage cells. BRX and PAX co-polarize at the rootward plasma membrane in developing protophloem, while OPS is polarized to the shootward site. SOSEKI (SOK) family proteins show unique localization at polar cell edges in different root cells. Receptor kinases IRK and KOIN display opposite polarization to the outer and inner endodermal membrane domain, respectively. **(c)** Diagrams show abnormal cell division and/or differentiation when the designated polarity protein is absent or ectopically overexpressed. Abnormal cell division/differentiation are tranced with bold lines. Stomatal ACDs are disrupted in *basl* mutants, leading to abnormal division and cell-fate determination. A smaller root meristem with discontinued phloem differentiation was observed in a loss-of-function *brx* or *ops* mutant. Ectopic overexpression of *SOK1* (ox, driven by the *RPS5A* promoter in dividing cells) induces extra and misorientated cell divisions. A loss-of-function *irk* mutant produces excess periclinal cell divisions.

Protein polarization associated with cell division and differentiation

In invertebrate model systems for studying asymmetric cell division (ACD), for example, *C. elegans* zygotes and *Drosophila* neuroblasts (neural stem cells), the polarization of antagonistic anterior and posterior PAR modules is instrumental to asymmetric spindle positioning and unequal segregation of cell-fate determinants [12]. The most conceptually comparable system in plants is probably the *Arabidopsis* stomatal lineage cells that possess stem cell-like activity and divide asymmetrically to generate stomatal guard cells and pavement cells in the epidermis. In the stomatal lineage precursor, meristemoid mother cell (MMC), BASL protein is polarized to direct the asymmetric placement of cell-division plane, a process mediated by microtubule-based nuclear migration [13•,14•]. After an ACD, the polarization of BASL is only inherited by the large daughter cell, stomatal lineage ground cell (SLGC), in which polarized BASL eventually suppresses the protein abundance of bHLH transcription factor SPCH (SPEECHLESS) and stomatal differentiation [14•,15]. In the absence of *BASL*, stomatal lineage divisions become less asymmetric and lose unequal daughter-cell fates [6] (Figure 1c). Therefore, although BASL is a recently evolved, plant-specific scaffold protein [16], as its counterpart PAR proteins in animals, polarization of BASL specifies two daughter-cell fates by generating unequal distribution of cell-fate regulators or determinants.

BRX, before being established as one of the BASL physical partners in stomatal development [17•], was initially discovered for its function in root growth and localization at the rootward polarity site in developing protophloem [8,18]. Interestingly, the other membrane-associated protein OPS is polarized shootward in these cells, although its polarization was later found not absolutely required for function [19] (Figure 1b). The loss-of-function *brx* and *ops* mutants display similar phenotype of discontinued phloem differentiation (Figure 1c). Genetic analyses placed their parallel functions in phloem differentiation with BRX's contribution as a molecular rheostat in modulating auxin flux [20] and OPS's debatable contribution in activating brassinosteroid (BR) signaling [21,22]. More specifically, BRX co-polarizes with the AGC-type PROTEIN KINASE ASSOCIATED WITH BRX (PAX) but inhibits PAX's activation of PIN-mediated auxin efflux, so that auxin levels can accumulate high [20]. However, when the intracellular auxin levels are high enough, the inhibition of BRX on PAX can be released by BRX dissociation from the membrane, allowing the activation of PAX and auxin efflux [20]. The absence of PAX results in phloem phenotypes resembling those of the *brx* mutant (Figure 1c). Functions of OPS were recently connected to the CLE45-mediated peptide signaling by directly interfering with the CLE45 signaling component interactions [23].

The five SOSEKI ("cornerstone" in Japanese) proteins were identified as targets of the MP/ARF5 (MONOPTEROS/AUXIN RESPONSE FACTOR 2) transcription factor that regulates cell-division patterning in *Arabidopsis* early embryos [7••]. Each SOK protein exhibits specific localization to polar cell edges of multiple cell types in developing embryos and roots [7••]. Interestingly, the local polarization of SOK proteins was found to interpret global polarity along the body axes [24] (Figure 1b). While knocking-out the individual *SOK* genes did not result in discernable phenotypes, ectopically expressing *SOK1* in root meristem induced altered cell division orientation [7••] (Figure 1c). It is not clear how SOK proteins orient cell division, but one of the effectors might be the membrane-associated protein AN (ANGUSTIFOLIA) that interacts with SOK1 and polarizes with SOK1 to the cell edges [25••].

Two transmembrane receptor kinases, IRK and KOIN, were recently identified to polarly localize in specific cells at the root tip [10••,11••]. In the endodermal cell layer, IRK accumulates to the outer side, whereas KOIN enriches to the inner side of the plasma membrane [10••,11••]. The major defects caused by the absence of *IRK* or *KOIN* are similar, both produce excessive cell divisions in the ground tissue, leading to an enlarged root meristem [11••] (Figure 1c). The role of IRK in restricting cell division was genetically linked to CYCD6; 1 that promotes formative cell divisions in the ground tissue lineage [10••].

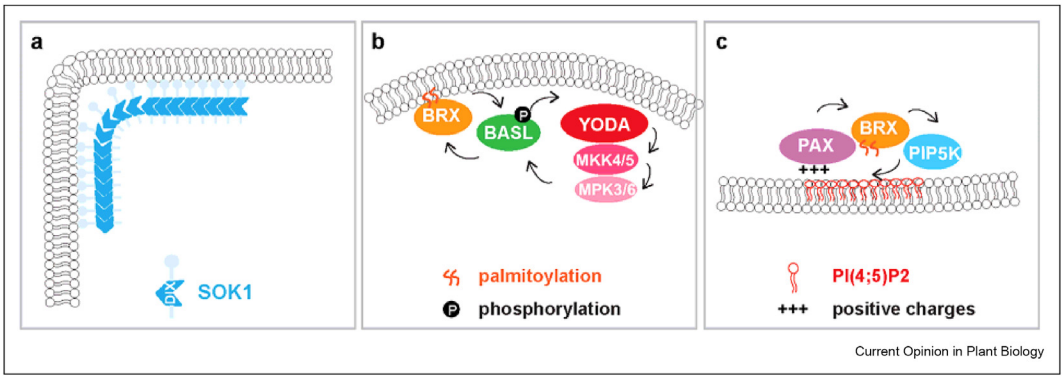
Self-reinforcing is central to protein polarization

Although almost all cell types exhibit some form of polarity (shape, structure, and function), their polarization status may not be readily revealed by size or shape, but by the expression of a molecular marker. For example, when the wall-less, spherical tobacco protoplasts were used to express exogenous proteins, *Arabidopsis* BASL appeared in a polarized manner at the cell cortex and in a randomly oriented axis [26•]. The polarization process of BASL in protoplasts is likely induced by spontaneous protein fluctuation and accumulation [26•], and facilitated by feedback regulations with the components of a conserved Mitogen-activated protein kinase (MAPK) pathway and the partner BRX proteins [17•,27]. The pursuit for the origin of cell polarization, or "symmetry breaking," has never been trivial because these events can be transient, dynamic, and often involve self-organization feedback processes [28]. Interestingly, recent studies suggested that the polarization information can simply be encoded by intrinsic properties of the protein.

The SOK proteins are membrane-associated and deeply conserved in land plants [25••]. Intriguingly, a conserved DIX domain in SOK proteins is biochemically and structurally equivalent to the DIX domain in Dishevelled, a key signaling molecule in the establishment of planar cell polarity in the *Drosophila* epithelia [29]. In a reciprocal experiment, the chimeric SOK1 protein with its DIX domain replaced with the human DVL2 DIX can polymerize and polarize to the cell edges, and function indistinguishably from the wild-type SOK1 in *Arabidopsis* [25••]. Furthermore, protein oligomerization of PAR3 was found to generate subcellular clustering and polarization in *Drosophila* epithelial cells [30,31]. Similarly, when expressed in *Arabidopsis* early embryos, SOK1 aggregated into puncta at the plasma membrane and enriched at the polar edges [25••] (Figure 2a). Thus, protein oligomerization provides a common mechanism to induce protein clustering at the peripheral membrane, contributing to cell polarization.

While self-oligomerization may provide an access to evolving simple polarity circuits, additional positive and negative feedback regulations are essential to form and stabilize protein polarization. Positive feedback loops arise from the assembly of partners to form a protein complex, interactions with regulatory proteins, and

Figure 2



Self-reinforcement is central to protein polarization in plants. (a) SOK1 polarization at the cell edges is induced by the DIX domain-mediated protein oligomerization and clustering. **(b–c)** Positive feedback loops contribute to the polarization of BASL and PAX-BRX complexes, respectively. (b), in stomatal lineage cells, BASL is phosphorylated and activated by MAPK 3 and 6 to become polarized at the cell cortex, where it enriches the MAPKKK YODA and MPK3/6 to establish a polarity complex in stomatal ACD cells. BASL also interacts with the BRX proteins and they help each other to reinforce polarization. Palmitoylation of BRX, a reversible lipid modification, is essential for the polarization. (c), in root protophloem, the BRX–PAX complex recruits the phosphatidylinositol-4-phosphate 5-kinases (PIP5Ks), which catalyze the formation of PI(4,5)P2 at the basal side of the membrane. While the membrane association of PAX depends on a polybasic motif (+++) to interact with PI(4,5)P2, the polar localization of the PAX–BRX–PIP5K module is reinforced by both protein-protein and protein-lipid interactions.

reorganization of structural components (cytoskeleton and membranes) to amplify and stabilize the polar domain. In stomatal lineage cells, polarization of BASL involves a positive feedback loop with the MAPK cascade, in which BASL nuclear export and polarization is promoted by MAPK6-mediated phosphorylation, in turn polarized BASL interacts with the MAPKK Kinase YODA to concentrate MAPK signaling that further promotes BASL polarization [27]. In addition, when BASL is expressed simultaneously with BRX in stomatal lineage cells, the nuclear pool of BASL is greatly alleviated and both proteins strongly polarize at the cell periphery, indicating an self-reinforcing system for polarization [17•] (Figure 2b).

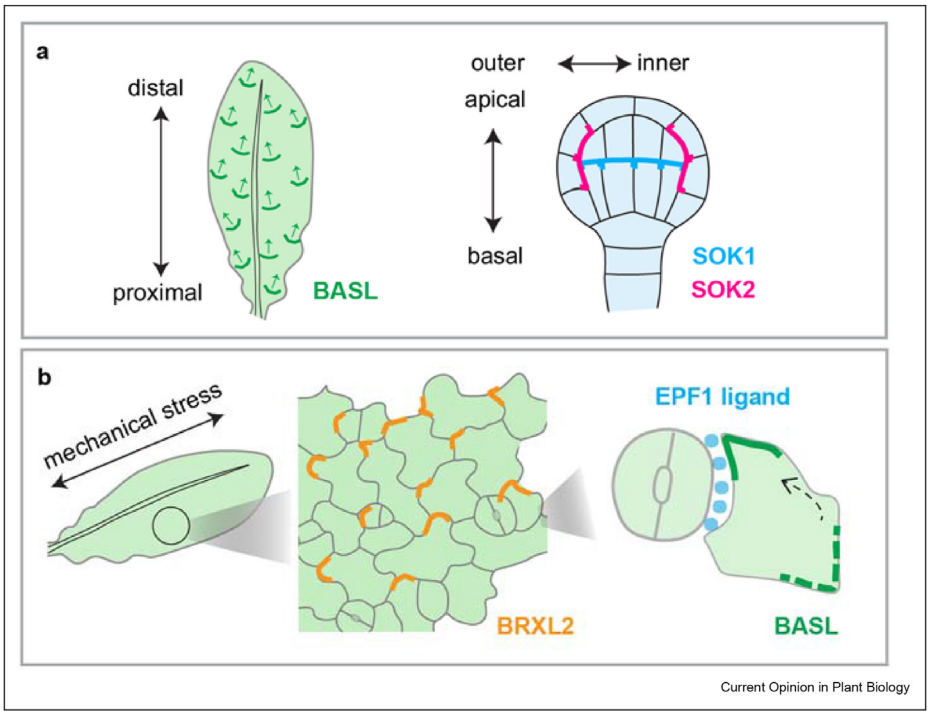
On the other hand, quantitative live-cell imaging showed that BRXL2, the representative BRX member in the stomatal lineage, becomes polarized earlier than BASL, indicating the presence of a BASL-independent pathway for BRX proteins to polarize [32]. Indeed, in root protophloem where no BASL function was established, the AGC kinase PAX interacts and polarizes rootward with BRX [20]. The BRX–PAX complex recruits the phosphatidylinositol-4-phosphate 5-kinases (PIP5Ks), which catalyze the formation of phosphatidylinositol-4,5-bisphosphate (PI(4,5)P2) [33]. Because the membrane association of PAX largely depends on its basic-hydrophobic patch to interact with PI(4,5)P2, the recruitment of PIP5Ks reinforces the polar accumulation of PAX–BRX at the plasma membrane in root protophloem [34••] (Figure 2c). Whether the BRX-PAX-PIP5K positive feedback loop exists in the stomatal lineage awaits further exploration.

Apparently, to establish stable polarization at the plasma membrane, self-organizing amplification processes require the presence of inhibitory mechanisms to ensure spatial restriction and formation of a singular pole of the polarity site [35]. Global inhibition can be achieved by dissociation of the polarity proteins from the plasma membrane. Interestingly, putative palmitoylation sites were frequently detected in the polarized proteins, such as BRX, OPS, and SOK [7••,9,17•]. Mutating the palmitoylation site is disruptive for BRX polarization [17•]. In addition, polarization of PAX is abolished when its polybasic motif is mutated or the PI(4,5)P2 abundance is reduced [34••]. Thus, lipid modification and lipid binding provide inhibitory basis for the reversibility of protein association with the plasma membrane that is required for self-organization of protein polarization.

Coordination of cell polarity globally and locally

Cells exhibiting an intrinsic orientation need to coordinate with their neighbors with respect to a tissue axis. In *Arabidopsis*, a proximodistal polarity field in leaf development is revealed by ectopic expression of BASL in the epidermal cells [36], and the apical-basal and radial organismal axes in embryos and roots are reflected by SOKs' polarization at the cell edges [7••] (Figure 3a). In the well-studied planar cell polarity systems, one major mechanism driving the alignment of cell polarity is connected to mechanical forces at the tissue scale [37]. Plant cells are encased in the cell walls that play a key role in sensing and responding to mechanical changes during cell growth. Strikingly, the plasma membrane association of SOK proteins was

Figure 3



Coordination of cellular polarity with tissue axes and local signals. (a) Ectopic expression of BASL (driven by the *35S* promoter) in pavement cells reveals a distal-proximal polarity field at the tissue level. Arrows indicate BASL polarity orientation. Polarization of SOK proteins reflects the global outer-inner and apical-basal polarity axes in developing embryos. **(b)** Tissue-wide mechanical forces influence the global pattern of BRXL2 polarization (orange crescents) in a leaf. Local chemical signaling overrides global cues to reposition the BASL polarity site. Peptide ligands, e.g. EPF1 (Epidermal Patterning Factor 1) secreted from the adjacent developing guard cells, may trigger signaling to induce the BASL polarity shift (dashed lines, disappearing BASL polarity).

found greatly disturbed when the cell walls are detached or removed [7••]. It was also shown that the cortical microtubes accurately align with the tissue mechanical patterns [38]. Knocking-out the microtubule severing protein KATANIN results in the loss-of-coordination between growth related mechanical tension and tissue-wide cellular polarity exhibited by BRXL2 in the leaf [39]. Despite the lack of mechanistic understanding of how mechanical signals instruct protein polarization, the emerging view is that mutual and dynamic interplay between mechanical and biochemical signaling across the cell wall-membrane-microtubule continuum underlies cell polarity, growth, and morphogenesis in plants (see recent reviews [39,40]).

It is also evident that short-distance chemical cues can guide protein polarization. In stomatal lineage cells, the reiterative ACD of meristemoids requires the reinitiation of the polarity site after each round of division. Positioning of the new polarity site is likely propelled away by unidentified components in the newly formed cell walls [41]. However, when SLGC undergoes another round of ACD, the post-mitotic polarity site is spatially switched to become adjacent to a developing guard mother cell, a process likely involving local peptide-receptor interactions [6,42•] (Figure 3b). The mechanisms underlying the contrasting polarization of IRK and KOIN in the root endodermis is unknown but might depend on cell identity and informed by adjacent cells, instead of the global radial axis [10••]. Domain swapping results also suggested the extracellular domain of IRK and the intracellular domain of KOIN contain information required for their polarization [11••].

Temporal control of protein polarization during cell division

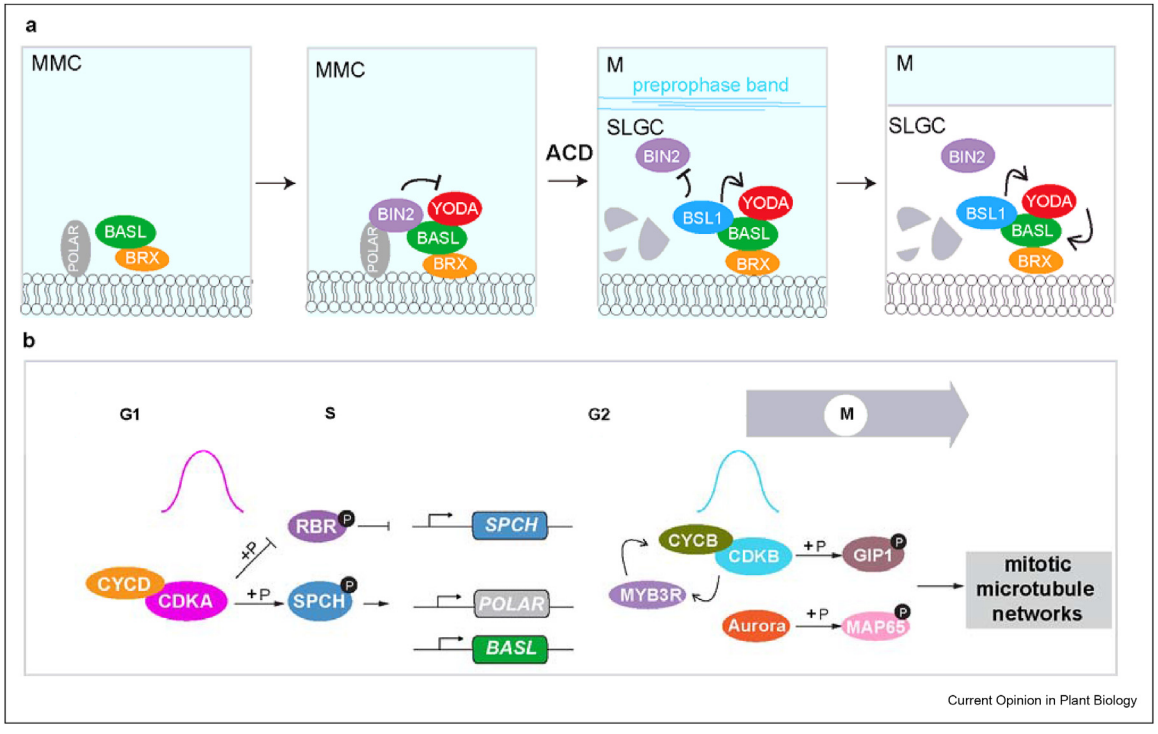
Cell polarization, as one major mechanism regulating stem-cell ACD, must be coupled to the progression of cell division and cell-fate differentiation. In recent years, exciting insights have been obtained about the dynamic assembly and function of the polarity components during stomatal ACD in *Arabidopsis* [43•,44••], mirroring those of the core polarity modules in animals [45,46]. Thus, in this section we focus discussion on stomatal lineage divisions.

Polarization of BASL was observed before, during, and after a stomatal ACD [6]. By temporally restricting

BASL's expression before or after stomatal ACD, pre-division BASL was shown to dictate directional nuclear migration and division orientation, but post-division BASL specifies cell fate [13•,14•], indicating that the seemingly same polarity site contains distinct molecular components at different stages. Indeed, a few key signaling molecules have been identified in the stomatal polarity site to control subsequent cellular events during ACD (Figure 4a). The precursor cell (MMC) is distinct from the daughter cell (SLGC) by its high cell-division potential that is connected to high protein abundance of SPCH and its partner SCRM (SCREAM)/ICE1 [27,43•,44••]. Both transcription factors are negatively regulated by MAPK-mediated phosphorylation [47,48]. It was recently determined that, in MMC, SPCH level is maintained high through the inhibition of the inhibitory MAPK signaling, which is originated from the polarized protein complex. More specifically, the MAPKK Kinase YODA that activates MAPK signaling is recruited to the polarity complex through physical interaction with BASL [27]. Nevertheless, in MMC, the kinase activity of YODA is suppressed by the presence of its inhibitor, the GSK3-like BIN2 (BRASSINOSTEROID INSENSITIVE 2) kinases that are brought in proximity by the scaffold protein POLAR [43•,49]. Therefore, SPCH is maintained high, so is the MMC division capability, by BIN2-mediated inhibition of YODA at the polarity site in MMC.

However, following the MMC division, BIN2-mediated suppression of YODA activity must be attenuated in the daughter cell SLGC to ensure lowered SPCH and lowered division potential. This transition is made possible by the synergistic activities of multiple components in the polarity complex. First, polarized BIN2 can phosphorylate POLAR for protein turnover that alleviates BIN2's association with the polarity site [43•]. Secondly, BASL recruits an inhibitor of BIN2, the BSL1

Figure 4



Temporal control of protein polarization in stomatal ACD and the cell-cycle regulators. (a) Firstly, in early MMCs, BASL, BRX, and POLAR are polarized scaffold proteins. BASL association to the plasma membrane requires the BRX proteins to be palmitoylated, whilst POLAR polarization dependents on BASL. Secondly, before stomatal ACD, the polarity complex employs POLAR to recruit the GSK3-like kinase BIN2 that suppresses YODA's activity to allow stomatal ACD. Thirdly, upon the entry into mitosis (preprophase band, blue lines), the Ser/Thr-protein phosphatase BSL1 interacts and colocalizes with BASL at the cell periphery. By joint regulation of the BIN2 GSK kinase and the YODA kinase, BSL1 functions as a spatiotemporal molecular switch to promote the transition from cell division to cell-fate differentiation. Broken piece of POLAR indicate protein degradation. Lastly, after ACD, the BASL polarity complex is only inherited by SLGC, where elevated YODA and MAPK signaling inhibits stomatal differentiation. Pink and blue shades indicate distinct daughter-cell fates. **(b)** Cell-cycle regulators implicated in stomatal ACD. During the G1/S phase, CDKA through a CDKA; 1-CYCD complex inhibits RBR1 to allow the expression of SPCH. CDKA; 1 also directly activates SPCH activity through phosphorylation. Activated SPCH induces the expression of BASL and POLAR. During the G2/M phase, microtubule networks need to be reorganized for mitosis. A positive feedback regulation between CDKB-CYCB and R1R2R3 MYB (MYB3R) transcription factors may regulate functions of the microtubule nucleation factor GIP1. In addition, the mitotic Aurora kinases directly regulate the MAP65 bundling factor to organize dynamics of microtubule networks needed for mitosis.

(BRI1 SUPPRESSOR-LIKE 1) protein phosphatase, leading to the dissociation of BIN2 from the plasma membrane [44••]. Then, the preferential nuclear localization of BIN2 results in reduced suppression on YODA at the cortex and increased suppression on SPCH in the nucleus [50]. Thirdly, BSL1 directly dephosphorylates YODA to further enhance MAPK signaling that suppresses SPCH [44••]. Thus, collectively, the SLGC is produced with low cell-division potential enabled by elevated MAPK signaling and lowered SPCH abundance.

Apparently, these successive signaling events need to be temporally controlled along the cell cycle of stomatal ACD. Live-cell imaging combined with genetic analyses suggested that in MMC, POLAR polarizes before BIN2 [43•] and BRXL2 polarizes before BASL but the exact timing for POLAR, BRXL2, and BASL in polarization has not been defined yet. In a protein co-expression experiment, BSL1 showed delayed polarization than that of BASL in MMC and strikingly, polarization of BSL1 coincides with the formation of the pre-prophase band [44••], a microtubule array appearing at the entry of mitosis. The temporal control of BSL1 polarization is critical because its activity promotes the progression of stomatal ACD by bridging up the pre-division activity of BIN2 to the post-division activity of YODA [44••].

The coordination of cell polarity and cell-cycle progression has been found crucial for ACD and metazoan development [51]. For example, the mitotic kinases Polo and Aurora A were suggested to regulate PAR proteins, cell polarity, and asymmetric division [52,53]. As in other eukaryotes, the cell-cycle progression in plants is driven by the rise and fall of cyclin-dependent kinases (CDKs) whose activity depends on the activator cyclins. Functions of the core cell-cycle components (CDKAs and CDKBs) and coordination with plant developmental have been recently reviewed [54,55]. In stomatal development, significant progress has been made towards understanding the terminal, one-time symmetric cell division (reviewed in [56]), therefore is not further discussed in this review. Here, we highlight some of the components that might regulate G1/S and G2/M phases in stomatal ACD (Figure 4b) prior to the terminal division. *Arabidopsis* CDKA; 1 is the sole ortholog of mammalian Cdk1 that controls the S-phase entry in plants, including the stomatal lineage cells. It does so through collectively positive regulation of SPCH. On one hand, CDKA; 1 phosphorylates to inhibit RBR1 (Retinoblastoma-replated protein 1) that drives the cell cycle and suppresses *SPCH* gene expression [57]. On the other hand, CDKA; 1 directly phosphorylates SPCH protein for activation [58]. Thus, SPCH is collectively promoted by CDKA; 1's activity, leading to increased expression of the polarity proteins, such as POLAR and BASL, for the execution of ACD [59]. Whether CDKA; 1 directly regulates cell polarity is unknow but its localization was detected at the microtubule arrays that must coordinate with cell polarity during mitosis [60]. Next, to enable the G2/M transition, a positive feedback loop is necessary, involving B-type CDKs and the activating MYB3R (R1R2R3-type MYB) transcription factors that induce the expression of mitotic cyclins, the activator of CDKB [55]. One of the downstream events under the CDKB2-CYCB1 complexes is to orchestrate mitotic microtubule networks through regulating GIP1 (GAMA-TUBULIN COMPLEX PROTEIN 3-INTERACTING PROTEIN 1), a component of the microtubule nucleation complex [61•]. Furthermore, the evolutionarily conserved mitotic regulator in plants, alpha Aurora kinases, has a role in specifying stem-cell asymmetric divisions, including in the stomatal lineage [62]. The Aurora kinases function in part through regulating the microtubule-bundling protein MAP65-1 [62,63]. Whether the mitotic regulators mentioned above contribute to cell polarity in ACD is an important future research direction.

Conclusion and perspectives

Recent progress in the identification of polarized proteins participating in stem-cell divisions in plants inspired an unprecedently exciting momentum to pursue the underlying molecular mechanisms for protein polarization. We have learned that positive feedback regulations involving protein oligomerization and signaling amplification play a central role in polarizing membrane-associated proteins in plants. Due to the space limit, we did not discuss membrane trafficking-based mechanisms for polarizing proteins in plants. It should be noted that polarization of BRX, PAX, IRK, and BASL is disturbed when membrane trafficking is disturbed the ARF GEF inhibitor, Brefeldin A, [11••,20,64]. Members of a protein family called PRAF/RLD (RCC1-like domain) were identified as interactors of BASL and the ARF GEF GNOM [64]. Four PRAF/RLD members are redundantly required for the establishment of BASL polarity in stomatal lineage cells. Whether and how the connected function of PRAF and GNOM may regulate the other polarity proteins should be studied in the future. Furthermore, accumulating evidence suggested that multiple polarity domains along the cell periphery can be defined by distinct proteins in one single plant cell. How multiple polarity poles are formed and coordinated intracellularly and intercellularly is a key challenging question. At the tissue scale, new regulators that set up planar or axial polarity can be identified by genome-wide genetic screens and/or protein interactome mapping strategies. Furthermore, the polarization process is complicated since one single polarity site can be dynamically occupied by distinct components at different time point, especially during cell division and differentiation. How ACD is coordinated in time with other events of the cell cycle and whether surveillance mechanisms exist to ensure an

ACD takes place only when polarity is established are all outstanding questions in the field.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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- • of special interest
- •• of outstanding interest

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The authors studied the localization and function of the IRK receptor-like kinase in *Arabidopsis* root. The loss-of-function mutant show excessive cell divisions in the ground tissue. The IRK protein was determined to polarize to the outer membrane of endodermal cells. It was suspected that IRK1 perceives a directional cue from neighboring cells and represses the activities of CYCD6; 1 in the root apical meristem.

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This study identified a new receptor-like kinase KOIN which shows contra-polarization with receptor kinase IRK in the endodermal cells. Both polarized receptor kinases are involved in restricting endodermal cell divisions but are delivered to plasma membrane by different trafficking pathways. It was also suggested that distinct domains of IRK and KOIN direct respective polarization patterns.

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