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Review article

Polarity in plant asymmetric cell division: Division orientation and cell fate differentiation

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ABSTRACT

Asymmetric cell division (ACD) is universally required for the development of multicellular organisms. Unlike animal cells, plant cells have a rigid cellulosic extracellular matrix, the cell wall, which provides physical support and forms communication routes. This fundamental difference leads to some unique mechanisms in plants for generating asymmetries during cell division. However, plants also utilize intrinsically polarized proteins to regulate asymmetric signaling and cell division, a strategy similar to the differentiation mechanism found in animals. Current progress suggests that common regulatory modes, i.e. protein spontaneous clustering and cytoskeleton reorganization, underlie protein polarization in both animal and plant cells. Despite these commonalities, it is important to note that intrinsic mechanisms in plants are heavily influenced by extrinsic cues. To control physical asymmetry in cell division, although our understanding is fragmentary thus far, plants might have evolved novel polarization strategies to orientate cell division plane. Recent studies also suggest that the phytohormone auxin, one of the most pivotal small molecules in plant development, regulates ACD in plants.

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1. Introduction

Asymmetric cell divisions (ACDs) are imperative for multicellular organisms to generate diversified cell types, while maintaining self-renewal stem cell pools (Abrash and Bergmann, 2009; Knoblich, 2008). Both intrinsic and extrinsic factors cause

Abbreviations: ACD, asymmetric cell division; PM, plasma membrane; MT, microtubule; F-actin, filamentous actin; M, meristemoid; SLGC, stomatal lineage ground cell; GC, guard cell; SMC, subsidiary mother cell

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asymmetries during cell division. Intrinsic mechanisms refer to those of which cell fate differentiation occurs prior to cytokinesis of the parental cell (Goldstein and Macara, 2007; Knoblich, 2008). Extrinsic factors drive differentiation outcome by asymmetric placement of the daughter cells into two distinct microenvironments (Fuller and Spradling, 2007). While both processes are important in complex organisms, intrinsic or extrinsic may have more weight over the other under certain conditions.

“Cell polarization”, or “symmetry breaking”, describes spontaneous assembly of cell cortical membrane domains. During this process proteins, mRNAs, organelles, cytoskeletal components among other molecules become distributed unevenly. Polarization occurs in essentially all cellular organisms and is required for fundamental processes in morphogenesis, cell division as well as cell differentiation (Freisinger et al., 2013; Yang and Lavagi, 2012). Cell polarization is a typical intrinsic regulatory measure that is required early in an ACD and is significant for subsequent checkpoints, e.g. mitotic spindle alignment, division plane positioning and daughter cell fate specification. The molecular mechanisms underpinning stem cell ACD have been described in model systems, such as the worm *C. elegans* embryos, the fruit fly *Drosophila melanogaster* nervous systems, and the budding yeast *Saccharomyces cerevisiae* (Inaba and Yamashita, 2012). In higher plants, the key roles of cell polarization in stem cell ACD are manifested by asymmetrically distributed proteins and signaling pathways. Contrary to the dominant roles of animal polarity proteins being almost entirely intrinsic cues, plant polarity proteins seem to participate in both intrinsic and extrinsic pathways to regulate divisional asymmetries in development. In addition, the phytohormone auxin is recognized as an important regulator of ACD in multiple developmental contexts (Balcerowicz et al., 2014; Le et al., 2014; Zhang et al., 2014). This review mainly focuses on the current understanding of how ACD participating proteins and related signaling pathways are asymmetrically distributed in plant cells and how auxin activity in addition to these pathways may regulate physical asymmetry and differential identity in cell division. Other mechanisms underlying cell fate asymmetry in plant ACD, but not necessarily associated with cell physical asymmetry, have been discussed previously (Abrash and Bergmann, 2009; Fisher and Sozzani, 2016; Petricka et al., 2009; Pillitteri et al., 2016; Ten Hove and Heidstra, 2008; Wu and Gallagher, 2011).

2. Symmetry breaking at the cortical membrane

Hallmark polarity proteins in animals, e.g. the Cdc42 small GTPase (Chant, 1999; Slaughter et al., 2009) and PAR proteins (Goldstein and Macara, 2007; Nance and Zallen, 2011), have been investigated intensively for the past decades. However, many of these highly conserved proteins found in animals are missing from the plant genome. The discoveries of polarized proteins in higher plants, e.g. the PANGLOSS (PAN) receptor-like proteins and the plant-specific, novel protein BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE (BASL) that participate in the regulation of plant ACD, have not been made until a few years ago (Cartwright et al., 2009; Dong et al., 2009). But excitingly, recent progress disclosed new genetic and physical partners in the PAN-mediated pathway (Humphries et al., 2011; Zhang et al., 2012; Facette et al., 2015) and BASL-centered polarity system (Pillitteri et al., 2011; Zhang et al., 2015). Current data suggest that, despite these polarity proteins being plant-specific, common regulatory themes, such as positive feedback loops, cytoskeletal reorganization and spatially organized cell signaling, underpin the cell polarity-driven ACD in both animals and plants.

2.1. Protein polarization: positive feedback loops

The yeast *S. cerevisiae* is a single-celled organism that continuously produces small daughter cells via polarizing the mother cell to form a bud, which expands and detaches from the mother. Polarization of the mother cells can be easily recognized as a patch of enriched cytoskeleton and membrane trafficking components at the polarity site, which promote the growth of the daughter cell. The polarity regulator, small Rho GTPase Cdc42, was first identified by Adams and others (Adams et al., 1990) and later established as “the center of cell polarization” ubiquitously from yeast to humans (Etienne-Manneville, 2004; Park and Bi, 2007). Loss of Cdc42 function leads to polarization failure of the mother cell and causes division problems (Adams et al., 1990). The cycling of Cdc42 between active guanosine triphosphate (GTP)-bound and inactive guanosine diphosphate (GDP)-bound forms is controlled by orchestrated activity of activators (guanine nucleotide exchange factors, GEFs), inhibitors (ATPase-activating proteins, GAPs) and other modulators (Rho GTPase-dissociation inhibitors, GDIs) (Vetter and Wittinghofer, 2001). One of the two major pathways that distribute Cdc42 to a highly polarized fashion at the cell cortex is actin-independent and requires a Cdc42-Bem1-Cdc24 centered positive feedback loop (Fig. 1). Bem1 is a scaffold protein and Cdc24 is a GEF activator of Cdc42. In the absence of any spatial cues, stochastically activated Cdc42 molecules may spontaneously cluster to initiate a cortical site where Bem1 is recruited, which locally accumulates Cdc24 that further activates Cdc42 to expand the polarity domain (Butty et al., 2002; Smith et al., 2013). More recently, a Cdc42 effector p21-activated kinase PAK was also found as a part of the complex that binds to Cdc24 and contributes to spontaneous polarization of yeast cells (Kozubowski et al., 2008; Woods et al., 2015). Thus, positive feedback loops provide a base for spontaneous initiation of Cdc42 polarization.

In *Arabidopsis*, the stomatal lineage cells divide asymmetrically to produce highly specialized guard cells that conduct gas exchange between the plant and the atmosphere (Bergmann and Sack, 2007). The initiation of stomatal precursor cells occurs in young developing leaves and their division orientation appears random relative to the leaf axis (Lau and Bergmann, 2012), suggesting an intrinsic polarization property of the cells. This is strongly supported by the discovery of the plant-specific BASL gene (Dong et al., 2009). In the absence of BASL, the stomatal lineage cells lost their full capacity to divide asymmetrically, thus producing an enlarged proliferating population with equal division pattern and disturbed daughter cell fate segregation (Dong et al., 2009). The localization of BASL protein is mainly featured by its strong polar accumulation at the cell cortex that is indispensable for its function (Dong et al., 2009). In plant cells, the molecular mechanisms for protein polarization are better understood for PIN-FORMED (PIN) auxin transporters. PINs are membrane integral proteins and often occupy distinct plasma membrane domains in *Arabidopsis* plants (Friml, 2003). PIN polar targeting and maintenance at the plasma membrane involve rapid and constitutive vesicular recycling between the PM and the endosomes (Feraru and Friml, 2008; Grunewald and Friml, 2010).

How the non-membrane protein BASL polarizes and associates to the PM remains unknown, but interestingly, both positive feedback loop and protein spontaneous clustering appear to be part of the process (Fig. 1). The positive feedback loop involves BASL, the Mitogen Activated Protein Kinase (MAPK) Kinase Kinase YODA (YDA) and MAPK 3 and 6 (MPK3/6), both of which are pivotal signaling molecules in stomatal development (Bergmann et al., 2004; Wang et al., 2007). Activation of BASL polarization can be mediated by MPK3/6 phosphorylation, which promotes nuclear export and cortical polar accumulation (Zhang et al., 2015). At the cell cortex, phosphorylated BASL functions as a scaffold protein

and binds to both YDA and MPK3/6, likely promoting signaling specificity and/or efficiency of the YDA-MPK3/6 pathway (Zhang et al., 2015). With the interactions established, activating more MPK3/6 can induce further activation of BASL to amplify the polarization effect. In mature tobacco pavement cells, overexpressing BASL protein showed even distribution along the PM. However, when YDA and MPK6 were co-expressed, the three proteins clustered together and accumulated into patches at the cell cortex (Zhang et al., 2015). This suggests a spontaneous self-catalyzing property of the BASL-YDA-MPK3/6 module, which to some extent resembles self-organizing polarization of Cdc42-Bem1-Cdc24 in yeast (Fig. 1).

POLAR is another polarized protein of unknown function in the *Arabidopsis* stomatal lineage. The cortical polarization pattern of POLAR mirrors that of BASL and is dependent on the presence of BASL (Pillitteri et al., 2011). But how POLAR contributes to the polarity pathway to regulate stomatal ACD remains unknown because mutations in POLAR did not yield discernable defects.

2.2. Cell polarization: cytoskeletal reorganization

In yeast, the second positive feedback loop in Cdc42 polarization involves active Cdc42-guided, actin-dependent exocytosis (Pruyne et al., 2004; Rohatgi et al., 1999). Two forms of actin structure (patches and cables) are positively regulated by Cdc42. Cdc42-GTP promotes the formation of actin cables, which are nucleated by formin-family proteins (Pruyne et al., 2004) and provide tracks for the delivery of exocytotic vesicles carrying Cdc42-GTP (Evangelista et al., 2002; Pruyne et al., 2004). This in turn enhances Cdc42 enrichment at the polarization site (Marco et al., 2007; Wedlich-Soldner et al., 2003; Wedlich-Soldner et al., 2004) (Fig. 2). Actin patches, a Cdc42-dependent platform for endocytosis and cell wall remodeling, contain a dynamic network of branched actin filaments that are polymerized by the Arp2/3 complex (Moseley and Goode, 2006).

In plants, the Rho family is diverged into the Rho-like GTPase from Plants (ROP) family that shares the common ancestor of Cdc42 (Gu et al., 2004). Similar to their counterparts in yeast and animals, ROP polarization also involves feedback loops (Hazak et al., 2010; Zhang and McCormick, 2007) that coordinate cytoskeleton organization and vesicular trafficking to establish cell polarity as well as to promote polar growth in plants (Fu et al., 2005; Gu et al., 2005). The roles of ROPs in plant ACD were demonstrated by their functional connection with the PAN leucine-rich repeat receptor-like kinases in maize stomatal development. The polarization of PANs represents an extrinsic-cue induced process.

In maize (*Zea mays*), each stomatal complex is composed of two guard cells flanked by two subsidiary cells (Fig. 2). The production of subsidiary cells requires an ACD of the precursor subsidiary mother cells (SMC). Prior to an ACD, the SMC polarization is demonstrated by asymmetric accumulation PAN proteins at the plasma membrane abutting the neighboring guard cells, followed by the formation of actin patch at the polarity site (Cartwright et al., 2009). Loss of *PAN1* or *PAN2* led to failures of SMC premitotic polarization and abnormal SMC division (Cartwright et al., 2009; Zhang et al., 2012). Recent work disclosed that prior to PAN polarization, the SCAR/WAVE complex (activator of the actin nucleating Arp2/3 complex) polarizes in SMCs independent of PANs and is required for PAN polarization (Facette et al., 2015). The early polarization of SCAR/WAVE was unexpected because PAN proteins physically interact and polarize ROP proteins (Humphries et al., 2011), which activate the downstream SCAR/WAVE complex and thus the Arp2/3 complex for promoting formation of actin patches in SMCs (Facette et al., 2015). It remains obscure how the SCAR/WAVE complex becomes polarized and how it functions upstream

to determine PAN polarization. As membrane embedded receptors, PANs may require endosomal recycling, during which actin plays important function (Kaksonen et al., 2006; Samaj et al., 2004). It is tantalizing to suspect that the actin network is inconspicuously reorganized by locally enriched SCAR/WAVE, which then influences membrane trafficking and polarization of PAN proteins in SMCs. What initial cue derived from GCs to induce polarization of SCAR/WAVE and SMCs remains a fascinating question in the field.

3. Divisionally physical asymmetry

Because plant cells are restricted by rigid cell walls, their coordinated division orientation and cell expansion are crucially important for tissue formation and organ patterning in plant development. Division placement has been noted long ago that it is closely related to cell shape in symmetrically dividing cells. In the late 1800's, Errera hypothesized that the new cell wall takes the shortest path that divides the mother into halves (Smith, 2001). This classic "Errera's rule" has been re-evaluated by several studies in recent years that integrated microscopic examination and computational simulation (Besson and Dumais, 2011; Sahlin and Jonsson, 2010). The current understanding is that the majority of symmetric cell divisions comply the shortest distance rule, in particular in cells with pronounced length differences in axis. On the other hand, in polygonal cells, the orientation of cell division is stochastic and random, and can be guided by surrounding mechanical forces (Besson and Dumais, 2011; Prusinkiewicz, 2011). However, ACDs that are coordinated with polarity of the mother cells override the influence of cell shape on selection of the division plane and cortical polarity proteins contribute heavily to defining PM domains and thus division plane establishment.

In animals, the cleavage plane that separates two daughter cells is defined by the contractile actomyosin ring, which uses the mitotic spindle as a positional cue (von Dassow, 2009). Correct spindle positioning is the linchpin to deciding placement of the cytokinesis furrow and ensuring cell fate determinants appropriately segregated (Williams and Fuchs, 2013). Conserved Partitioning defective (PAR) polarity proteins play fundamental roles in the regulation of ACD orientation in *C. elegans*, *Drosophila*, and vertebrates (Goldstein and Macara, 2007). In *Drosophila* neuroblast ACD, the PAR complex (PAR3 and PAR6 scaffold proteins and aPKC kinase) is polarized at the apical cortex and accumulates another apical complex (Pins, Gai, Mud/NuMA) that directs and establishes spindle orientation. Mud/NuMA is a MT-binding protein that localizes to spindle poles and interacts with the dynein motor complex (Siller et al., 2006). The cortical-attached, MT minus-end directed motility of dynein exerts a pulling force to help navigate the mitotic spindle (Kiyomitsu and Cheeseman, 2012). Alternatively, dynein may serve as a cortical anchor of the plus-end depolymerizing microtubules that indirectly allow spindle positioning (Grill et al., 2003; Kozlowski et al., 2007). A number of MT plus-end binding proteins, e.g. CLASP, EB1 and the MCAK MT depolymerase have also been identified to contribute to MT dynamics and spindle orientation in animals (reviewed in Lu and Johnston, 2013).

In plant cells, by contrast, the selection of cell division plane in plants is determined prior to the formation of mitotic spindle and relies on a specialized MT apparatus, the preprophase band (PPB), to organize cell division plane. The PPB is a cortical array of cytoskeletal filaments that are composed of microtubules (MTs) and filamentous actins (F-actins) and transiently assembled at G2 phase and disassembled at mitosis (Mineyuki, 1999). The cortical demarcation of the PPB loyally predicts where the division plane is placed, though the formation of PPB is not absolutely required

across plant species. But in plant cells where PPBs are normally formed, i.e. in *Arabidopsis*, the lack of PPB results in severely misorientated cell division planes (Azimzadeh et al., 2008; Camilleri et al., 2002; Kawamura et al., 2006).

3.1. Formation of the PPB

The MT network undergoes tremendous reorganization during plant cell division (Rasmussen et al., 2013). The PPB formation requires disassembly of the interphase cortical MT network, particularly outside of the PPB zone, and concomitant organization of a belt-like array at G2 phase (Rasmussen et al., 2011). In consonance with that, a number of microtubule-associated proteins (MAPs) co-localize with the PPB to initiate and maintain its organization (Rasmussen et al., 2013). Recent research have shed light upon a number of advances in understanding the PPB disassembly, the cortical division zone (CDZ) establishment, phragmoplast guidance and expansion, as well as cell plate synthesis (see recent reviews (Lipka et al., 2015; Muller and Jurgens, 2015; Rasmussen et al., 2011; Rasmussen et al., 2013)). Here we discuss the very early events in PPB formation and how cell polarization might play a role in orientating the PPB.

The initiation of PPB formation is not fully understood yet, but drug treatment and genetic analysis placed protein phosphorylation/dephosphorylation events to a critical position in this process. Application of kinase and phosphatase inhibitors interferes with PPB formation in plants (Ayaydin et al., 2000; Katsuta and Shibaoka, 1992). Two *Arabidopsis* mutants *tonneau1* (*ton1*) and *fass/ton2* do not produce PPBs and cell division planes are randomly placed (Azimzadeh et al., 2008; Kirik et al., 2012; Torres-Ruiz and Jurgens, 1994). TON2/FASS belongs to a heterotrimeric protein phosphatase 2A (PP2A) complex, functioning as a regulatory B subunit (Camilleri et al., 2002). Later, other subunits of the PP2A complex, including the scaffold A subunits and the catalytic C subunits, were co-purified with TON2/FASS from *Arabidopsis* plants and mutations in these genes recapitulated the cell division defects in *ton2* (Spinner et al., 2013). The PPB association of the PP2A holoenzyme is achieved by forming a so called TTP complex (TON1/TRM/PP2A) with two other MT-binding proteins, TON1 and TON1 RECRUITING MOTIF (TRM), both of which share sequence similarity with animal centrosomal proteins (Drevensek et al., 2012). Loss of TTP components results in the absence of PPB and thereby abnormal division plane in *Arabidopsis*, but spindles and phragmoplasts are not discernably affected (Spinner et al., 2013).

It remains a challenge to identify downstream substrates of the PP2A complex for PPB formation and maintenance. TON1 itself was recognized as a phosphoprotein (Benschop et al., 2007) and can be a direct target of PP2A. Putative candidates also include other PPB-associated MT regulators, e.g. MOR1, SABRE and CLASP, mutations in all of which gave rise to defects in PPB formation and positioning (Ambrose et al., 2011; Kawamura et al., 2006; Pietra et al., 2013; Twell et al., 2002). A conserved eukaryotic protein of unknown function in *Arabidopsis*, SABRE, weakly associates with the PPB MTs and functions downstream of CLASP (Pietra et al., 2013). The genetic interaction and functional connection of these PPB binding proteins with the TTP family and phosphorylation activity require further in-depth investigation.

The initial position of PPB formation at the cell cortex seems to be uneven and might relate to where the nucleus is located. Three-dimensional analysis of MT array in *Arabidopsis* leaf epidermal cell divisions disclosed that PPB development initiates from the inner cell cortex (the mesophyll side), but not from the outer side (near the atmosphere). This polarized developmental manner is closely related to the preprophase nuclei that are mainly positioned near the inner cell wall (Lucas and Sack, 2012). Indeed, spatial co-alignment of the nucleus and the PPB in early prophase cells has

been noticed for a long time. Early centrifugation experiments using the fern *Adiantum protonemata* showed that a second PPB is induced around the displaced nucleus (Murata and Wada, 1991). However, the position of the nucleus alone can not determine PPB positioning because when more than one PPB was induced in synchronization of tobacco BY-2 cells, some PPBs do not encircle the nucleus (Granger and Cyr, 2001; Yoneda et al., 2005). In addition, in asymmetrically dividing *Arabidopsis* stomatal cells, PPB formation precedes nuclear migration and is independent of nucleus position (Geisler et al., 2003). Thus, whether the nucleus leads the PPB or *vice versa* may depend on cell type and biological context. Particularly in asymmetric divisions, cell polarity is an important factor that influences division plane orientation.

3.2. Orientating the PPB – polarity protein complexes

In *Arabidopsis* stomatal ACD precursor cells, polarization of BASL seems to propel the division plane to form distally (Fig. 3). How BASL functions to orientate the division plane remains a major mystery. However, as the YDA MPK3/6 cascade is tightly associated with polarized BASL (Zhang et al., 2015), it is worth speculating that a MAPK signaling gradient has a potential function in this process (Fig. 3). Besides the BASL positive feedback loop mentioned earlier, a YDA MPK3/6 gradient can also be set up by extrinsic signals triggered by ligand-receptor interaction (Torii, 2012). The extracellular peptide ligands, Epidermal Patterning Factors (EPFs), act upstream of the MAPK cascade in stomatal development (Abrash and Bergmann, 2010; Hara et al., 2007; Hara et al., 2009; Hunt and Gray, 2009; Kondo et al., 2010; Sugano et al., 2010). EPF1 and EPF2 interact with the leucine-rich repeat receptors, Too Many Mouth (TMM) and ERECTA receptor-like kinases (ER, ER-like 1 and ER-like 2), to activate the YDA MPK3/6 pathway (Jewaria et al., 2013; Lee et al., 2015; Nadeau and Sack, 2002; Shpak et al., 2005). In particular, in the loss-of-function mutants *epf1* and *tmm*, the orientated ACD abutting a differentiating stomatal cell cannot fully navigate its polarity and fails to divide properly (Hara et al., 2009; Nadeau and Sack, 2002).

More evidence supported that the levels of YDA MPK3/6 activity need to be tightly controlled in plant development, especially in cell division orientation. Both loss-of-function *yda* and gain-of-function $\Delta Nyda$ (constitutively active YDA, the N-terminal inhibitory domain deleted) showed similarly dis-orientated cell division profile in *Arabidopsis* roots (Smekalova et al., 2014). Consistently, mutations in *MPK6* recapitulated this phenotype (Muller et al., 2010). Despite genetic data placing YDA as an upstream activator of MPK3/6 in stomatal development and embryogenesis (Bergmann et al., 2004; Lukowitz et al., 2004; Wang et al., 2007), surprisingly, *MPK6* protein level was found increased in *yda* null mutants and that elevated *MPK6* may contribute to the phenotype of oblique cell walls in roots. Careful examination of *MPK6* localization in *yda* and $\Delta Nyda$ showed that YDA activation promotes more physical association of *MPK6* with the mitotic MT arrays (Smekalova et al., 2014). It was hypothesized that microtubule-associated protein (MAP) regulators, e.g. MAP65 and MOR1, might be subject to *MPK6* phosphorylation and regulation (Muller et al., 2010). The identification of new *MPK6* substrates is anticipated to improve our understanding of how YDA MPK3/6 signaling may regulate divisional plane orientation.

An ungrouped plant-specific Kinesin motor-protein, *ARK3/AtKINUa*, is enriched at the PPB prior to the nuclear envelope breakdown (Malcos and Cyr, 2011; Sakai et al., 2008). Cell type-specific RNAi knocking down the expression of *ARK3* in *Arabidopsis* generated mildly clustered stomatal lineage divisions and paired guard cells, phenocopying that of *basl* mutants (Lau et al., 2014). The major defects of *ark3* mutants seem to center on the orientation of the PPB, but not the formation of the PPB and cell

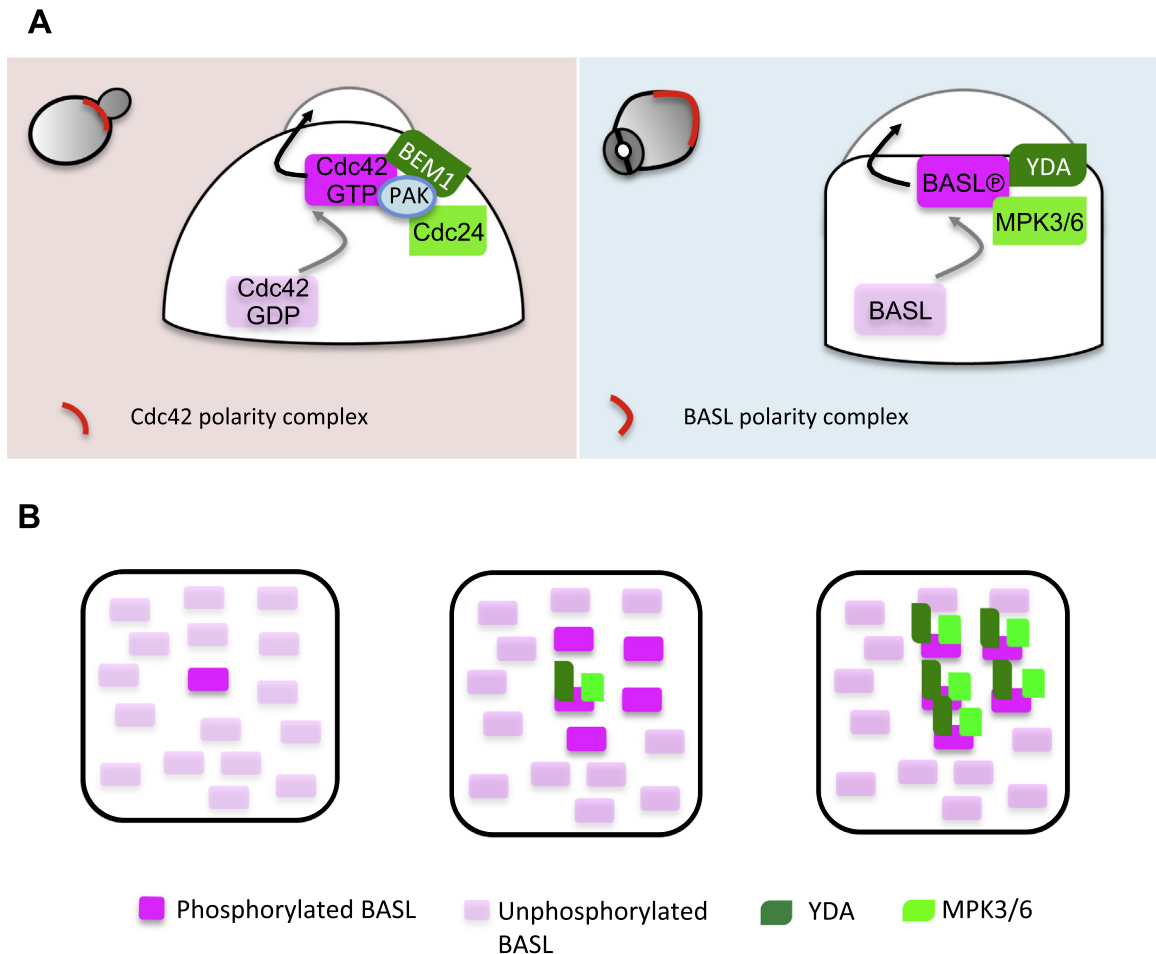


Fig. 1. Symmetry-breaking polarization of Cdc42 and BASL in yeast and *Arabidopsis* stomatal lineage cells. (A) *Left panel:* Spontaneous polarization of the Cdc42-Bem1-Cdc24/PAK feedforward loop. In budding yeast, stochastically activated Cdc42-GTP (gray arrow) recruits the scaffold protein Bem1, which locally enriches Cdc24, the Cdc42 GEF activator, to amplify activated Cdc42-mediated protein clustering. This intrinsic positive feedback loop polarizes Cdc42 to the cortical site and promotes daughter cell expansion (black arrow). *Right panel:* In *Arabidopsis* stomatal ACD precursor cells, a positive feedback loop of BASL-YDA-MPK3/6 promotes cell polarization. MPK3/6-mediated phosphorylation triggers BASL cortical polarization (gray arrow). At the cell cortex, phosphorylated BASL functions as a scaffold to recruit both YDA and MPK3/6, which further produces more activated MPK3/6, and thus more activated BASL to polarize. Polarization of the BASL complex was hypothesized to promote local cell expansion (black arrow). (B) Working model for spontaneous clustering of BASL-YDA-MPK3/6 in plant cells. We hypothesize that, similar to Cdc42 polarization, initially activated BASL molecules organize a positive feedback loop by recruiting its activators YDA-MPK3/6, which allows the conversion of neighboring molecules to become active, eventually leading to the formation of a polarized cluster.

plate per se (Lau et al., 2014). Future genetic analyses between the BASL-MAPK polarity pathway and ARK3 should delineate whether and how they function together to orientate stomatal ACD.

The position of PAN-ROP polarity relative to the cell division plane in maize SMCs opposes how BASL is positioned in the *Arabidopsis* stomatal lineage cells (Fig. 2B). New cell walls are placed proximately to where PAN is polarized (Cartwright et al., 2009; Zhang et al., 2012). Following PAN polarization and actin patch formation, the nucleus migrates to the division side. Based on inhibitor tests, the directional movement of the nucleus is actin-dependent (Panteris et al., 2006). The microtubule arrays are organized into a specialized prophase “half-spindle” that is connected with the PPB. This perinuclear MT complex may spatially confine the nucleus adjacent to the SMC (Panteris et al., 2006) (Fig. 2B). Thus far, it is not known whether the PAN-ROP pathway contributes to the formation of “half-spindle”-PPB complex, though this complex seems to be stabilized by actin patches (Panteris et al., 2006).

3.3. Orientating the PPB – auxin pathways

Auxin has profound influences on plant embryogenesis and post-embryonic development (Dharmasiri et al., 2005; Friml et al.,

2003; Jürgens et al., 1991; Reinhardt et al., 2000). Recent progress in *Arabidopsis* stomatal development and early embryo patterning disclosed important roles of auxin signaling in establishing asymmetric division plane placement. By 3-D high-resolution confocal imaging and computational analysis, a complete map of *Arabidopsis* early embryogenesis (from the zygote to the late heart stage) was constructed (Yoshida et al., 2014). This method greatly enhanced our ability to evaluate cell volume and cell wall surface asymmetries during cell divisions. Interestingly, new ACD patterns in embryos of eight cells or larger were recognized because in the lower tier, the volume of the outer cell (protoderm) is two times larger than that of the inner cell (precursor of vasculature and ground tissues) (Fig. 4). Such a volume difference cannot be revealed by conventional 2-D cross-sections. Furthermore, mutations in *BODENLOS (BDL)/IAA12*, an inhibitor of auxin response, reverted these divisions to be symmetric, suggesting that asymmetric placement of the division plane is linked to auxin-related pathways (Yoshida et al., 2014). It remains a mystery how auxin response may orientate cell division in embryos, but a concentration gradient may play a role. Indeed, the newly developed auxin markers DR5v2 and R2D2 disclosed a slightly elevated auxin signaling in the inner cells of the early globular embryo (Liao et al., 2015). In addition, both local auxin production and PIN

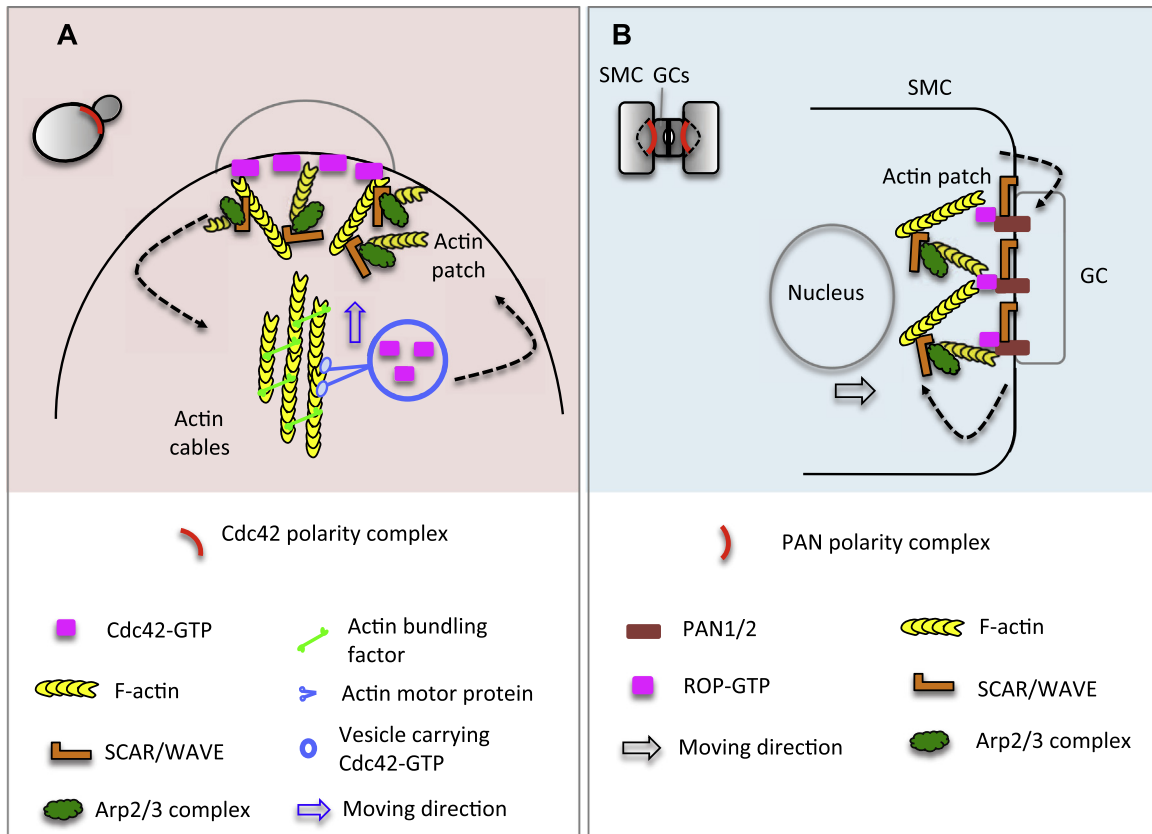


Fig. 2. Actin-dependent positive feedback loops for symmetry-breaking polarization in yeast and maize stomatal cells. (A) Cytoskeletal-dependent polarization of Cdc42 in yeast. Cortically localized active Cdc42-GTP triggers Formin activity (bundling factor) to form actin cables. Actin cables provide tracks for the delivery of exocytotic vesicles carrying Cdc42-GTP to the polarizing site, resulting in further nucleation of actin cables, which in turn enhances the Cdc42 polar enrichment (dashed arrows). Blue arrow shows the moving direction of Cdc42-GTP vesicles driven by actin motor proteins. The formation of actin patches is stimulated by Cdc42-GTP activated SCARE/WAVE and Arp2/3, which promote actin nucleation and branching. Actin patches are necessary for dynamic delivery of PM and cell wall materials. (B) PAN1/2 polarization in maize Subsidiary Mother Cells (SMCs). In SMC, polarized PAN1/2 physically bind to ROP and lead to ROP activation. Polarized ROP activates the SCAR/WAVE and Arp2/3 activity and thus nucleation of actin to form a dense actin patch at the polarity site. Actin patches may help directional nuclear migration (black arrow) during SMC ACD. Interestingly, polarization of SCAR/WAVE is also required for PAN1/2 polarization, suggesting an interdependent activation (dashed arrows).

polarization are required for the cell polarization and subsequent apical-basal axis establishment in early embryos (Robert et al., 2013). Alternatively, MP/ARF5 may act through target genes to promote other hormonal pathways, i.e. auxin-elevated cytokinin biosynthesis triggers division plane shift in vasculature tissue (De Rybel et al., 2014; Ohashi-Ito et al., 2014). The major challenge in the field is to provide a mechanistic understanding of how auxin gradient, most likely very small within a cell or between neighboring cells, may alter cell polarity and the PPB positioning.

Additionally, recent discoveries of the interconnection between mechanical stress and auxin signaling shed new lights into the intricate crosstalk between cell growth/expansion and auxin function. In both *Arabidopsis* and tomato shoot apex, changes in mechanical tension were found to influence auxin transport and accumulation. In addition, the amount and intracellular localization of PIN1 and the cortical MT orientation are also modulated (Heisler et al., 2010; Nakayama et al., 2012), indicating that cell growth strain feeds back to auxin signaling. Apparently, in our future studies, consideration of auxin signaling should be coupled with growth and division parameters of the cells to interpret the mechanisms of plant ACD.

4. Cell fate asymmetry

Cell-to-cell communication in plants can be achieved through ligand-receptor interactions, intercellular trafficking of hormones,

RNAs and proteins that include transcription factors (Wu and Gallagher, 2011). Mobile transcription factors play pivotal roles in plant development (Han et al., 2014) and ACD-involved tissue patterning (Petricka et al., 2009; Ten Hove and Heidstra, 2008). A well-studied case showed that directional movement of the GRAS transcription factor SHORT ROOT (SHR), from the stele into the endodermal cells, helps to establish asymmetric identity of the cortex and endodermis in *Arabidopsis* roots (Helariutta et al., 2000; Nakajima et al., 2001). Another example showed that the bHLH transcription factor TARGET OF MONOPTEROS 7 (TMO7) travels from the provasculature of the early embryo into the hypophysis to direct asymmetric division (Schlereth et al., 2010). Recently emerging evidence showed that polarizing signals, e.g. proteins and auxin, play important roles in differentiating daughter cells. Here we discuss how polarized cues and auxin signaling may contribute to cell fate specification in plant ACDs.

4.1. Polarized proteins

In animals, the PAR proteins can asymmetrically segregate cell fate-determining factors. In *Drosophila* neuroblast ACD, the apically polarized PAR complex directs the fate-determining complex, including Numb (an endocytic protein), Brain tumor (BRAT, a translation inhibitor) and Prospero (a transcription factor) to partition into the basal cell (Knoblich, 2008). After mitosis, the cell fate determinants act together to prevent stem cell self-renewal while promoting differentiation (Berdnik et al., 2002; Knoblich

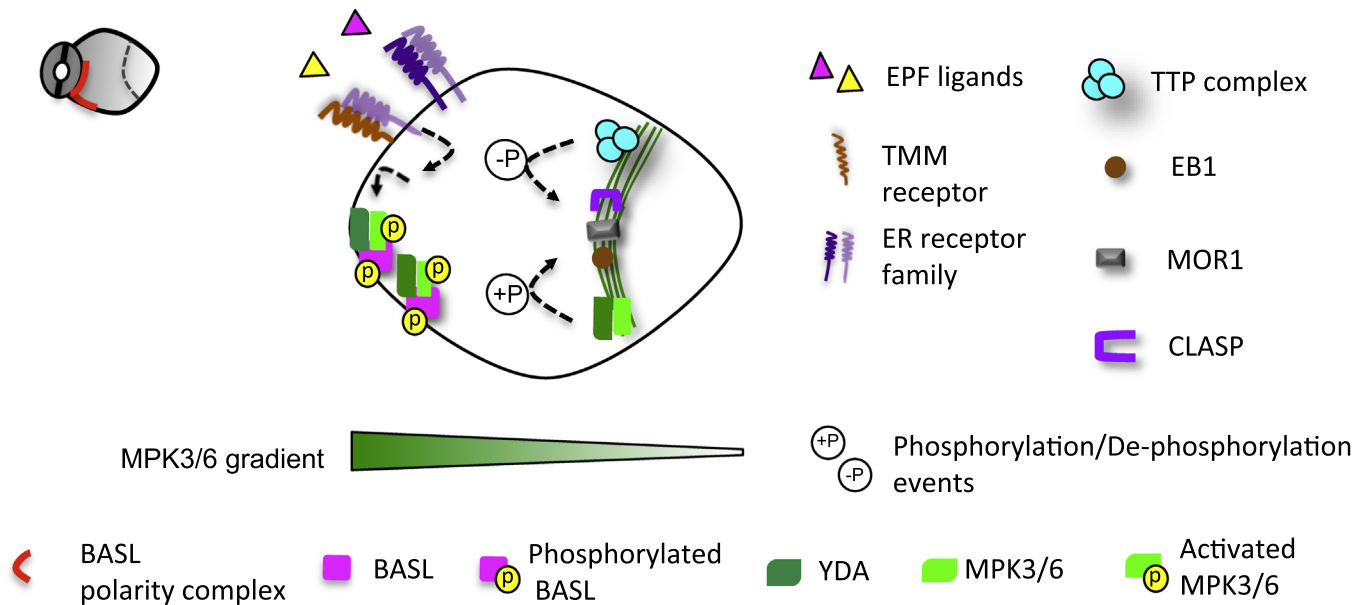


Fig. 3. A hypothetical MAPK gradient and asymmetric placement of cell division plane in the *Arabidopsis* stomatal lineage. In *Arabidopsis* stomatal ACD, we hypothesize that the YDA MPK3/6 signaling forms a gradient. Two possible mechanisms may contribute to this gradient. The positive feedback loop with BASL maintains a high concentration of MPK3/6 at the crescent. Extrinsic peptide ligands EPF1/2, derived from the neighboring stomatal cells, are perceived by the membrane receptors TMM and ERECTA family, which may locally activate the downstream YDA-MPK3/6 pathway. The formation of the PPB requires a core TTP complex containing protein phosphatase PP2A enzymes with two MT-binding proteins, TON1 and TRM. The PPB-associated MT regulators, e.g. MOR1, CLASP1 and EB1, might be subject to MAPK- and PP2A-mediate phosphorylation and dephosphorylation, respectively, to initiate and orientate the PPB formation.

et al., 1995; Sonoda and Wharton, 2001; Spana and Doe, 1995).

In *Arabidopsis*, a stomatal lineage precursor cell, Meristemoid Mother Cell (MMC), divides asymmetrically to produce a Meristemoid (M) and a Stomatal Lineage Ground Cell (SLGC). The two daughter cells differ in their stem cell division potential and terminal cell fate. An M divides 3–4 times before terminating into a pair of guard cells and a SLGC may divide one more time before it expands to become a pavement cell. The BASL-YDA-MPK3/6 is polarized premitotically and inherited to the SLGC only. The two daughter cells differ in BASL polarization and the associated MAPK cascade (Zhang et al., 2015). Downstream of MPK3/6, the bHLH transcription factor SPEECHLESS (SPCH) is a direct substrate being phosphorylated for degradation (Lampard et al., 2008). Expression of SPCH initiates stomatal ACD and promotes fate differentiation (Lau et al., 2014; MacAlister et al., 2007). Therefore, the BASL-YDA-MPK3/6 polarity module in the SLGC was hypothesized to exert stronger negative regulation on SPCH expression, thus inducing lowered division potential and cell fate departed from stomatal terminal differentiation (Fig. 5).

Spatially organized MAPK activity by polarity proteins is not entirely novel in animal ACD systems (Fig. 5). Mouse muscle satellite cells (quiescent stem cells) are activated by injury and divide asymmetrically to replenish damaged cells (Jones et al., 2005). Polarized PAR proteins were found to concentrate or activate p38 α / β MAPK activity in only one daughter cell, which turns on the expression of bHLH transcription factor MyoD to promote amplifying divisions and terminal fate differentiation (Troy et al., 2012). The other daughter cell retreats back and maintains the quiescent status. In plants, MAPK-mediated suppression of SPCH seems to provide more flexibility in stomatal development. When MAPK activities are modulated by environmental changes, stomatal lineage cells are capable of adjusting SPCH level and adapting their division potential to optimize stomatal density and improve growth.

4.2. Asymmetric auxin signaling

Auxin levels and responses fluctuate in *Arabidopsis* stomatal

development. Auxin signaling peaks in the Ms that undergo active stomatal ACD, and drops when an M differentiates into a guard mother cells (GMCs) (Le et al., 2014). Lowered auxin level in the Ms might be mediated by elevated expression of the auxin effluxer PIN3 and was hypothesized to induce stomatal terminal differentiation (Fig. 4). The loss-of-function auxin transporter mutants *pin2 3 4 7* and *pin1 3 4 7*, similar to the receptor mutants *tir1 afb1 2 3*, showed elevated stomatal index and clustered patterning, suggesting that auxin negatively regulates stomatal production (Le et al., 2014). This connection was further corroborated by Zhang et al. (2014) and Balcerowicz et al. (2014). The crosstalk between auxin signaling and the core stomatal development pathway is partially achieved by the activity of Auxin Response Factor (ARF) 5/MONOPTEROS (MP) that directly binds to the promoter of *STOMAGEN* and suppresses its expression (Zhang et al., 2014). *STOMAGEN* is a positive regulator of stomatal development (Kondo et al., 2010; Sugano et al., 2010). As an EPF ligand, *STOMAGEN* competes with EPF1 and EPF2 to inhibit the TMM/ER receptor-mediated signal transduction (Jewaria et al., 2013; Lee et al., 2015). However, this functional connection cannot well address how auxin depletion from the Ms is related to stomatal differentiation because *STOMAGEN* is not expressed in the epidermis, but rather functions non-cell-autonomously from the inner mesophyll cells. Future work is anticipated to resolve what determines PIN asymmetric distribution in stomatal ACD pairs and how ARFs crosstalk with the core stomatal fate factors to control cell fate asymmetry.

5. Concluding remarks and perspectives

Orientated cell division and diversified cell fate are key to plant growth and developmental patterning. The discovery of polarized proteins in plant ACD provided a unique opportunity to study the molecular mechanisms underpinning protein polarization at the membrane and how they organize cellular contents to orientate cell division plane and determine differential identity of daughter cells. Although both plants and animals utilize common regulatory

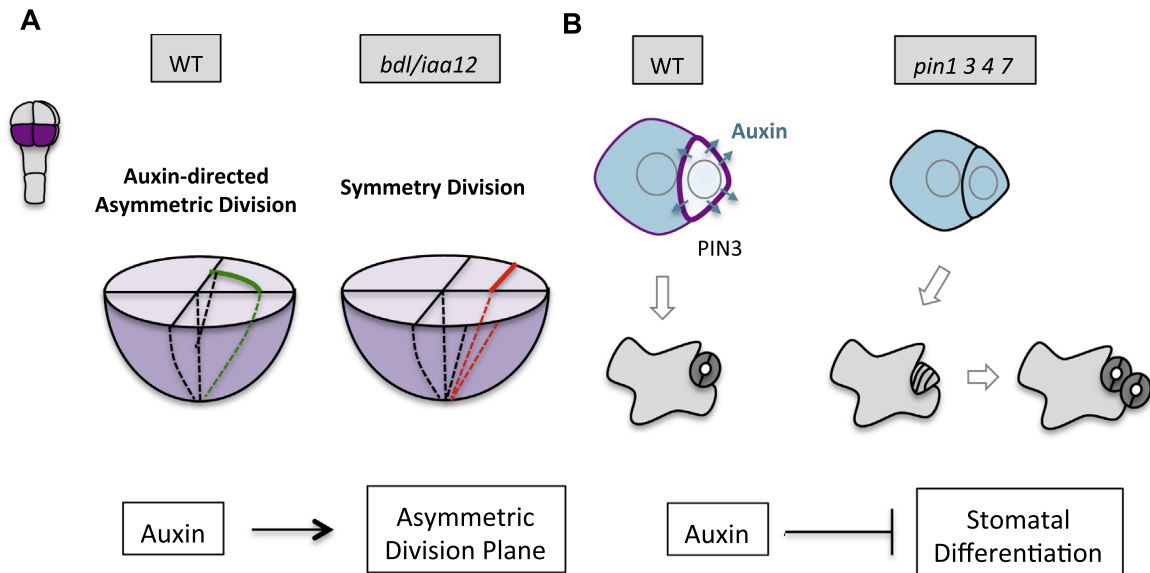


Fig. 4. Auxin signaling participates in plant ACD. (A) Auxin pathway promotes ACD in early embryos. In 8-cell embryos, ACDs were detected in the lower tier (purple) by 3D high-resolution confocal imaging and computational analyses. The volume of outer cells (protoderm) is about two times that of the inner cells (precursor of vasculature and ground tissues). However, in the auxin inhibitor mutant *bdl/iaa12*, ACDs were reverted to symmetric. How auxin signaling promotes asymmetric placement of the division plane is unknown. (B) Auxin depletion from the M is linked to stomatal differentiation after an ACD. In the wild-type (WT), auxin level drops when the M differentiates into stomatal guard cells. Auxin depletion is likely mediated by elevated expression level of the PIN3 auxin effluxer (purple). In the loss-of-function mutant *pin1 3 4 7*, the M maintains high level of auxin and does not differentiate, but divide more times before terminal fate adoption.

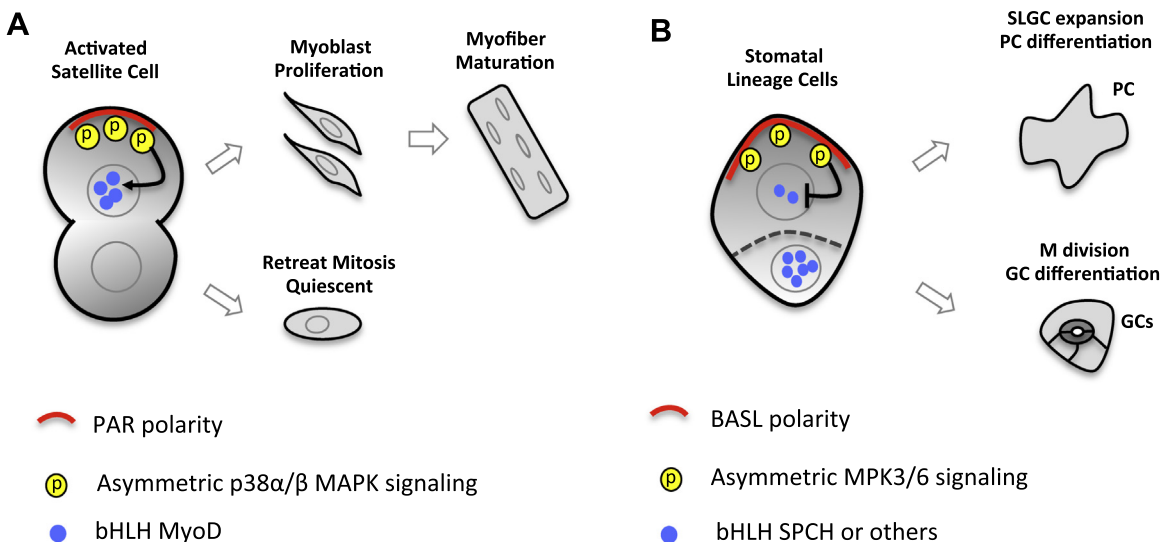


Fig. 5. Asymmetrically activated MAPK signaling in muscle satellite cell ACD and Arabidopsis stomatal ACD. (A) Asymmetric p38α/β MAPK activity in mouse muscle satellite cell ACD. Polarized PAR protein complex activates p38α/β MAPK activity in only one daughter cell, which turns on the expression of bHLH transcription factor MyoD to promote amplifying divisions followed by terminal fate differentiation. The other daughter cell exits cell division and returns to the quiescent state. (B) Asymmetric distribution of the YDA-MPK3/6 cascade in *Arabidopsis* stomatal ACD. Premitotically polarized BASL-YDA-MPK3/6 complex is only inherited to the large daughter cell (SLGC, stomatal lineage ground cell), which results in phosphorylation of the nuclear bHLH SPCH for degradation. Differential SPCH expression levels in two daughters direct their distinct developmental path. The small daughter (M, Meristemoid) undergoes a few divisions and terminates into stomatal guard cells and the large SLGC expands to become a pavement cell (PC).

modes, e.g. self-organizing molecular amplification and cytoskeleton-dependent forward feedback loops, cell polarization in plants is featured with striking responsiveness to external cues. Finding the GC-derived cues in maize stomatal system will further emphasize position-dependent mechanisms in plant development. In PAR systems, the separation of cortical domains requires phosphorylation-mediated mutual exclusion. Whether polarized BASL and YDA MPK3/6 phosphorylation may propel another set of proteins into the other end is a fascinating question.

Genetic and computational analyses provided in-depth insights into the regulators and general rules in plant symmetric cell

divisions. The finding of asymmetrically activated MAPK cascade in stomatal ACD further highlighted regulatory similarities between stomatal lineage cells and muscle satellite cells, both of which are adult stem cell pools. Not only are cell fate transitions orchestrated by bHLH transcription factors in both systems (Matos and Bergmann, 2014), daughter cell differentiation is also achieved by spatially controlled MAPK signaling and differential regulation of bHLH factors. In addition, an exciting wave of research is anticipated to integrate polarized proteins and auxin signaling to the selection of cell division plane in plant ACD. Finding more MAPK substrates and downstream effectors of auxin responses that

appear prior to the PPB formation may have the potential to translate cell polarity to division orientation.

Long-term high-resolution 4-dimensional imaging combined with computational quantification has the promise to reveal more plant ACDs that are unrecognized thus far. It remains a challenge to provide a unified view on how auxin regulates plant ACD. Aided by the improved auxin sensors, DR5v2 and R2D2, many plant ACD systems should be revisited so that potential functions of auxin signaling as a general regulator can be better evaluated.

Conflict of interest disclosure

The authors declare that there are no conflicts of interest.

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