REVIEW ARTICLE

Division site determination during asymmetric cell division in plants

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Short title: Asymmetric division site determination

One-sentence summary: Plants use common strategies to establish asymmetric cell division involving processes such as morphological changes in cells, cytoskeletal dynamics, and cytoskeleton-dependent nuclear positioning.

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ABSTRACT

During development, both animals and plants exploit asymmetric cell division to increase tissue complexity, a process that usually generates cells dissimilar in size, morphology, and fate. Plants lack the key regulators that control asymmetric cell division in animals. Instead, plants have evolved two unique cytoskeletal structures to tackle this problem: the preprophase band and phragmoplast. The assembly of the preprophase band and phragmoplast and their contributions to division plane orientation have been extensively studied. However, how the division plane is positioned off the cell center during asymmetric division is poorly understood. Over the past 20 years, emerging evidence points to a critical role for polarly localized membrane proteins in this process. Although many of these proteins are species- or cell type-specific, and the molecular mechanism underlying division asymmetry is not fully understood, common features such as morphological changes in cells, cytoskeletal dynamics, and nuclear positioning have been observed. In this review, we provide updates on polarity establishment and nuclear positioning during asymmetric cell division in plants. Together with previous findings about symmetrically dividing cells and the emerging roles of developmental cues, we aim to offer evolutionary insight into a common framework for asymmetric division-site determination and highlight directions for future work.

INTRODUCTION

Asymmetric cell division (ACD) is a universal mechanism that creates cell diversity in both animals and plants. While animals employ a conserved molecular repertoire to execute ACD (Knoblich, 2010; Sunchu and Cabernard, 2020), how plants have evolved to accomplish this task at the molecular and cellular levels remains enigmatic. To date, only a few plant model systems have been thoroughly investigated. Interestingly, plant-specific polarity proteins including BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE (BASL) (Dong et al., 2009), BREVIS RADIX family (BRXf) proteins (Rowe et al., 2019), POLAR LOCALIZATION DURING ASYMMETRIC DIVISION AND REDISTRIBUTION (POLAR) (Pillitteri et al., 2011), the leucine-rich repeat-receptor-like kinases (LRR-RLKs) PANGLOSS 1 (PAN1) and PANGLOSS 2 (PAN2) (Cartwright et al., 2009; Humphries et al., 2011; Zhang et al., 2012), and RHO OF PLANTS (ROP) (Humphries et al., 2011; Yi and Goshima, 2020) have been identified as master regulators of ACD, which resemble the PARTITIONING DEFECTIVE (Par) proteins in animals. Among these, PAN1 and PAN2 are grass-specific (Chen et al., 2017). BRXf, POLAR, and BASL are present in a complex (Houbaert et al., 2018; Rowe et al., 2019; Guo et al., 2021a); however, phylogenetic analyses indicated that they have evolved separately with the emergence of BASL in eudicot species (Nir et al., 2022; Ramalho et al., 2022). ROPs are plant-specific small GTPases homologous to the Cdc42/Rho/Rac superfamily in yeasts and animals. These highly conserved proteins play fundamental roles in cell polarity and ACD in various species ranging from bryophytes to flowering plants (Humphries et al., 2011; Feiguelman et al., 2018; Yi and Goshima, 2020).

It appears that plants have evolved different molecular pathways to initiate ACD. However, an increasing number of studies have revealed some common features. For instance, in well-studied plant systems such as stomatal lineage cells (Dong et al., 2009; Muroyama et al., 2020), zygotes

(Kimata et al., 2016), and lateral root founder cells (De Rybel et al., 2010; Vilches Barro et al., 2019) in *Arabidopsis thaliana* and protonema cells and gametophore initials in mosses (Kosetsu et al., 2017; Yi and Goshima, 2020), all asymmetrically dividing cells undergo a series of subcellular events, including cell polarization, membrane expansion, nuclear migration or positioning, and asymmetric division. Functionally analogous molecules such as polarity proteins and cytoskeletal elements are also involved in ACD. It is tempting to speculate that plants have taken similar strategies to fulfill the need for ACD and development during evolution. In this review, we discuss how a division site is determined during ACD in plants with a focus on cell polarity and nuclear positioning. By providing recent updates in model plant cells and combining findings about symmetric divisions, we hope to offer evolutionary insight into the cellular mechanisms of ACD and to highlight questions that require further investigation toward obtaining a full picture of a conserved theme for the establishment of ACD in plants.

THE PREPROPHASE BAND AND CORTICAL DIVISION ZONE

Unlike animals, plants use two unique cytoskeletal structures, the preprophase band (PPB) and phragmoplast, to carry out cell division (Smertenko et al., 2017). As the PPB forms underneath the plasma membrane before nuclear envelope breakdown and marks the future division site, it has been suggested that the PPB is the key determinant of division site selection (Traas et al., 1995; Camilleri et al., 2002; Azimzadeh et al., 2008; Spinner et al., 2010; De Smet and Beeckman, 2011; Rasmussen et al., 2011). The formation and function of the PPB have been extensively studied in symmetrically dividing cells. However, few studies have focused on the relevant mechanisms during asymmetric division. In this section, we briefly discuss factors that govern division plane orientation based on the knowledge mostly from symmetric divisions and highlight evolutionarily conserved components that may also contribute to asymmetric division in a broader context. For more mechanistic details on division plane selection, the readers are referred to the following excellent reviews (Lipka et al., 2015; Rasmussen and Bellinger, 2018; Livanos and Muller, 2019).

As the cell cycle proceeds, the PPB forms at preprophase and disappears at prometaphase. The positional information of the PPB is transmitted to subsequently recruited molecules including PHRAGMOPLAST ORIENTING KINESINs (POKs) (Lipka et al., 2014), RAN GTPASE ACTIVATING PROTEIN 1 (RanGAP1) (Xu et al., 2008), PLECKSTRIN HOMOLOGY GTPASE ACTIVATING PROTEINs (PHGAPs) (Stockle et al., 2016), and TANGLED (TAN) (Walker et al., 2007) (Figure 1). These proteins define/specify a new domain termed the cortical division zone (CDZ). During cytokinesis, the CDZ guides phragmoplast expansion and eventually fuses with the expanding cell plate (Smertenko et al., 2017; Livanos and Muller, 2019). This mechanism is known as phragmoplast guidance.

A few observations suggest that the PPB is not essential for division site determination. First, many types of plant cells divide without forming a PPB, such as starchy endosperm cells (Brown et al., 1994; Olsen, 2001), meiocytes (Brown and Lemmon, 1991), and microspores (Heslop-Harrison, 1968; Otegui and Staehelin, 2000) in flowering plants, and protonema cells (Schmiedel and Schnepf, 1979; Schmiedel et al., 1981) and gametophore initials in mosses (Kosetsu et al., 2017). Second, in PPB-depleted mutants, cell division exhibits only mild detects in orientation (Schaefer et al., 2017). Consequently, plant development is largely normal in these mutants (Traas et al., 1995; Schaefer et al., 2017).

By contrast, the CDZ markers do appear in naturally non-PPB-forming cells and PPB-depleted mutant cells (Miki et al., 2014; Schaefer et al., 2017; Yi and Goshima, 2020) (Figure 2). In addition, the loss of key components required for CDZ formation, such as the kinesins POKs, causes drastic defects in development and division orientation (Muller et al., 2006). These findings suggest that phragmoplast guidance is a widely conserved mechanism in land plants and that the PPB is a promoting factor. In agreement with this notion, the phragmoplast emerged earlier than the PPB

during evolution (Buschmann and Zachgo, 2016). Moreover, all key components of the CDZ (POKs, RanGAP1, PHGAPs, and TAN) are present in basal land plants (confirmed by BLAST analysis of Arabidopsis gene sequences against the moss *Physcomitrium patens* [*P. patens*] and liverwort *Marchantia polymorpha* genomes), whereas some PPB components, such as TON1 RECRUITING MOTIF (TRM) proteins, are specific to seed plants (based on phylogenetic analyses from the TAIR database www.arabidopsis.org and the Ensembl Plants database www.plants.ensembl.org).

As the maintenance of TAN, RanGAP1, and PHGAP1/2 at the CDZ depends on POKs, these kinesins appear to be central for division plane orientation (Walker et al., 2007; Xu et al., 2008; Stockle et al., 2016). How CDZ components are recruited to the cell cortex in the absence of a PPB remains unclear. Presumably, cell polarity and nuclear positioning may directly or indirectly influence the localization of CDZ components and/or act cooperatively with them to specify the division plane during both symmetric and asymmetric divisions (see below). Connections between early regulators of ACD and CDZ components should exist. Identifying such connections would be of great interest for understanding division site determination during ACD in plants.

NUCLEAR POSITIONING AND ITS RELATIONSHIP WITH THE PPB AND CDZ

The determination of a division site in animals is largely controlled by spindle positioning (D'Avino et al., 2015; Kiyomitsu, 2019). To date, no compelling evidence suggests the existence of a similar mechanism in plants (Abrash and Bergmann, 2009; Yamada and Goshima, 2017), although the mitotic spindle has to be properly anchored and oriented (Ambrose and Cyr, 2008; Kosetsu et al., 2017; Leong et al., 2020; Kozgunova et al., 2021). Mitotic spindles in plants usually assemble at a place where the nucleus is located. Therefore, the approximate site of the division plane is determined by nuclear position. This is more obvious in asymmetrically dividing cells, whose division is preceded by directed nuclear migration or positioning (Kimata et al., 2016;

Vilches Barro et al., 2019; Yi and Goshima, 2020). Nuclear positioning may affect division site determination during both symmetric and asymmetric divisions. However, the contribution of nuclear positioning has been only studied in a limited number of cell types due to the lack of a conspicuous repositioning phase during most symmetric divisions.

Early studies in symmetrically dividing cells point to coordination between nuclear positioning and PPB formation. A number of findings suggest that nuclear positioning is instrumental for the assembly of the PPB. First, the nucleus itself is an MT-organizing center (MTOC), and nucleus-associated MTs are reoriented transversely in elongated cells to participate in PPB formation (Wick and Duniec, 1983; Flanders et al., 1990; Stoppin et al., 1994). Second, the displacement of the nucleus by centrifugation is sufficient to induce PPB formation (Murata and Wada, 1991). Third, the absence of a PPB induced by drug treatment does not affect nuclear migration (Katsuta et al., 1990). Fourth, in asymmetrically dividing cells, PPB formation is preceded by nuclear migration and occurs at a place around the nucleus (Kimata et al., 2016). However, contradictory results also indicate that the PPB could influence nuclear position. For example, when the PPB and nucleus are slightly separate from each other, the nucleus could change its morphology and move to the central region encircled by the PPB (Granger and Cyr, 2001). Interestingly, if the nucleus is distant from the PPB, it could not be aligned to the plane of the PPB (Granger and Cyr, 2001). In the stomatal lineage of cereals, under experimental conditions, the PPB could be assembled independently of nuclear position (Galatis et al., 1983; Galatis et al., 1984). Therefore, nuclear positioning and PPB formation are independent to some extent. Nevertheless, the nucleus could interact with the PPB to fine-tune its position when these structures are close to each other. Indeed, MTs connecting the nucleus and the PPB are commonly observed and play important roles in nuclear positioning (Sinnott and Bloch, 1940; Wick and Duniec, 1983; Bakhuizen et al., 1985; Venverloo and Libbenga, 1987; Flanders et al., 1990; Granger and Cyr, 2001; Ambrose and Cyr, 2008)

As mentioned above, the CDZ but not the PPB is a universal feature of land plants. The combinatory action of nuclear positioning and CDZ-dependent phragmoplast guidance would represent a conserved scheme to determine a division site in plants. First, the nucleus migrates to a place where the future division occurs. This process selects the approximate site of division as a mitotic spindle is locally assembled. Notably, the nuclear position does not precisely define where the expanding cell plate will attach to. Second, the CDZ is established following nuclear positioning. This structure acts as a guidance cue to lead phragmoplast expansion and cell plate assembly, thus determining the orientation of the division plane (Figure 2A). A mechanism must exist to coordinate nuclear positioning and CDZ establishment during both symmetric and asymmetric divisions. In bryophytes, CDZ assembly likely functions downstream of nuclear positioning because POKs (e.g., kinesin-12Ie) and PpREN (the moss homolog of PHGAP1/2 in Arabidopsis) localize to the CDZ after nuclear envelope breakdown (Miki et al., 2014; Yi and Goshima, 2020) (Figure 2B). However, all key components required for CDZ assembly in Arabidopsis, except PHGAP1/2, are recruited to the CDZ at prophase, indicating that the CDZ functions earlier in division site selection (Walker et al., 2007; Xu et al., 2008; Lipka et al., 2014; Stockle et al., 2016). Whether this difference has coevolved with the emergence of the PPB remains an open question. Nevertheless, the relationship between nuclear positioning and CDZ assembly in different plant lineages still requires functional investigation.

It is noteworthy that the formation of the CDZ can be a dynamic process. In moss caulonema filament cells, the position of PpKin12- and PpREN-labeled CDZ is slightly shifted with the progression from metaphase to telophase, leading to an oblique division plane (Miki et al., 2014; Yi and Goshima, 2020) (Figure 2B). Similar to the PPB, the CDZ may possess the ability to self-organize to some extent and is not precisely specified by signals from the nucleus. Moreover, CDZ-mediated phragmoplast guidance can be functional when the nucleus or mitotic spindle is slightly

displaced (Venverloo and Libbenga, 1987). The IQ67 DOMAIN proteins IQD6/7/8 were recently found to control division plane orientation in Arabidopsis (Kumari et al., 2021). IQD8 and related proteins promote the recruitment of POKs and PHGAPs to the CDZ via direct binding, whereas the localization of IQD8 does not require POKs or PHGAPs. In addition, in contrast to POKs, IQDs are required for PPB formation, although the underlying mechanism is not yet fully understood. IQDs are thought to function as a scaffold to coordinate PPB assembly and CDZ establishment during symmetric division. IQDs are evolutionarily conserved, and many of them are associated with MTs (Abel et al., 2005; Burstenbinder et al., 2017). Presumably, IQDs could play important roles in regulating division orientation in both PPB-forming and PPB-free cells. For example, the positional information from the nucleus may be transmitted to the cell cortex via interactions between IQDs and MTs nucleated around the nucleus. Indeed, IQD8, the major protein for division plane regulation, localized to both the nuclear envelope and MTs when transiently expressed in *Nicotiana benthamiana* (Burstenbinder et al., 2017; Kumari et al., 2021); some members of the IQD family can directly bind MTs (Wendrich et al., 2018) and MT-associated proteins (Burstenbinder et al., 2013; Wendrich et al., 2018).

In addition to POKs and IQDs, other cortex-residing molecules such as TAN (Smith et al., 2001; Martinez et al., 2020), KINESIN-LIKE CALMODULIN-BINDING PROTEIN (KCBP) (Buschmann et al., 2015), AUXIN-INDUCED IN ROOT CULTURES 9 (AIR9) (Buschmann et al., 2006; Buschmann et al., 2015), the 65-KD MICROTUBULE-ASSOCIATED PROTEIN 4 (MAP65-4) (Li et al., 2017), and Myosin VIII (Wu and Bezanilla, 2014) may also interact with the nucleus via their MT-binding ability. Although novel components of the CDZ are likely to emerge, and the interactions between the nucleus and the CDZ remain to be characterized, cytoskeletal dynamics, especially those of MTs, tend to play a crucial role in bridging these two structures. Further studies are required to understand how nuclear positioning and CDZ assembly coordinate to control division site selection. First, it is necessary to identify components of the CDZ that are

functionally conserved in the absence of a PPB and are recruited to the cortex during early stages of mitosis. Second, how these proteins interact with the nucleus and self-organize into a polar domain at the cortex remains to be determined.

NUCLEAR POSITIONING MECHANISMS

Nuclear positioning is not necessary for most symmetric divisions. However, it must be precisely controlled in cells undergoing physically asymmetric division as well as in some types of symmetrically dividing cells. Undoubtedly, cytoskeletal elements play a fundamental role in this process. For instance, in highly vacuolated cells, the pre-mitotic nucleus moves to the cell center with the aid of nucleus-associated MTs (Venverloo and Libbenga, 1987; Flanders et al., 1990; Goodbody et al., 1991) (Figure 1). As cortical MTs can sense cell geometry and tensile stress to align the division plane (Besson and Dumais, 2011; Louveaux et al., 2016), it is plausible that nuclear positioning is mediated by forces resulting from dynamic MT polymerization and reorganization. This model has not been widely tested, but it may not apply to cells that do not contain cortical MTs.

Alternatively, the active transport of pre-mitotic nuclei might involve cytoskeletal motors. Nuclear movement and positioning have been observed in various interphase cells in response to cell growth and environmental stimuli (Griffis et al., 2014; Groves et al., 2018; Wada, 2018; Fatema et al., 2019). In flowering plants, actin and myosin but not MTs appear to be the predominant regulators of nuclear movement (Ketelaar et al., 2002; Iwabuchi et al., 2010; Tamura et al., 2013; Higa et al., 2014; Kawashima et al., 2014; Nakamura et al., 2018). A similar transport mechanism could be employed for pre-mitotic nuclear positioning. Interestingly, MT-dependent transport was shown to control pre-mitotic nuclear migration in rice (*Oryza sativa*) and tobacco (*Nicotiana tabacum*) BY-2 cells, suggesting that nuclear positioning may require cell type- or organism-specific mechanisms (Frey et al., 2010). Indeed, during formative division or asymmetric division,

nuclear migration is governed by the differential action of actin filaments (F-actins) and MTs, depending on the cell type and organism (see below). Intriguingly, despite their differences, studies of various plant cells have now identified polarity proteins as upstream regulators of cytoskeleton-dependent nuclear migration (Figure 2A). The polarity-cytoskeleton-nuclear positioning pathway appears to be functionally conserved during plant ACD. Our knowledge of this model is at its early stages. In the following section, we discuss studies in model systems that may support the existence of this pathway (Table 1).

ZYGOTIC AND EMBRYONIC DIVISIONS IN ARABIDOPSIS

Divisions at the early stage of embryonic development in Arabidopsis are mostly asymmetric and invariant (Armenta-Medina and Gillmor, 2019). After fertilization, the zygote undergoes rapid polar growth and a typical asymmetric division to generate a small apical cell and a large basal cell, which will develop into the proembryo and suspensor, respectively (Mansfield and Briarty, 1991) (Figure 3A). Various studies have identified regulatory factors controlling polarity establishment and maintenance, division asymmetry, and cell fate determination in the zygote, such as the WRKY DNA-BINDING PROTEIN 2 (WRKY2) transcription factor (Ueda et al., 2011; Ueda et al., 2017), the membrane receptors SHORT SUSPENSOR (SSP) and ZYGOTIC ARREST 1 (ZAR1) (Bayer et al., 2009; Yu et al., 2016), the YODA (YDA)/MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) kinase cascade (Lukowitz et al., 2004; Zhang et al., 2017), and auxin signaling (Friml et al., 2003). However, the underlying cellular mechanism for the selection of an asymmetric division site has only recently been discovered.

Several years ago, using *in vitro*-cultured embryos and time-lapse imaging, Kimata et al. revealed the differential roles of cytoskeletal elements in coordinating cell elongation and asymmetric division (Gooh et al., 2015; Kimata et al., 2016). The disruption of MT polymerization impedes polar cell growth but not division asymmetry. By contrast, the perturbation of F-actins markedly

inhibits nuclear migration and has relatively mild effects on cell elongation. Accordingly, MTs reorganize into a transverse subapical ring during the elongation phase; F-actins form an apical cap and appear as longitudinal arrays in the cytoplasm, potentially favoring actin-dependent transport of the nucleus. The formation of the PPB is preceded by nuclear migration, indicating that nuclear positioning plays a pivotal role in determining division asymmetry. How nuclear migration is controlled mechanistically and how it is linked to the developmental context are not clear yet. Conceivably, cell polarity may affect nuclear positioning by regulating actin reorganization and/or actin-dependent transport. In support of this notion, ROP3 was shown to control zygotic asymmetric division (Huang et al., 2014). ROPs are versatile regulators involved in actin polymerization, MT dynamics, cell morphogenesis, and polar cell growth (Feiguelman et al., 2018). Therefore, ROPs may target the actin network to regulate nuclear positioning and zygotic division in response to developmental cues. Moreover, polar growth and nuclear migration are intimately coupled. Both processes could be subject to ROP regulation. Another promising candidate for the control of nuclear transport is myosin. Myosins are actin-associated motors (Nebenfuhr and Dixit, 2018). Among the 17 members in Arabidopsis, MyoXI-i is critical for interphase nuclear positioning (Avisar et al., 2009; Tamura et al., 2013); nuclear migration in sperm before fertilization also requires uncharacterized myosin members (Kawashima et al., 2014). Studying the function of myosin during zygotic division will add another dimension to our understanding of the mechanisms of pre-mitotic nuclear positioning.

MTs and actin are also required for asymmetric divisions at later stages of embryo development, although a nuclear positioning phase is not obvious (Vaddepalli et al., 2021). In a recent study, IQD6 and its close homologs IQD7 and IQD8 were shown to regulate division orientation, as is also observed during symmetric division (Kumari et al., 2021; Vaddepalli et al., 2021). Intriguingly, the determination of division plane orientation is attributable to MT- and actin-dependent cell shape but not to nuclear positioning or the polarity axis (Vaddepalli et al., 2021). This phenomenon

is thought to be due to the difference in cell morphology between the zygote and embryo (large and elongated versus small and polyhedral). However, using three-dimensional computational modeling, Moukhtar et al. found that the nucleus could constrain surface minimization to modulate the default geometric rules for division plane selection, pointing to a critical role for nuclear positioning (Moukhtar et al., 2019). Regardless of this controversy, auxin signaling components such as the auxin receptor TRANSPORT INHIBITOR RESPONSE1 (TIR1)/AUXIN-SIGNALING F-BOX (AFB) (Prigge et al., 2020), Aux/IAA protein IAA12/BODENLOS (BDL) (Yoshida et al., 2014; Vaddepalli et al., 2021), AUXIN RESPONSE FACTOR ARF5/MONOPTEROS (MP) (Schlereth et al., 2010; Moller et al., 2017), and the auxin efflux carrier PIN-FORMED (PIN) (Friml et al., 2003; Blilou et al., 2005) play profound roles in division orientation and embryonic patterning. The expression of polarity proteins (e.g., ROP3, ROP9, and RopGEF5) and cytoskeleton-associated molecules (e.g., IQD6) is positively regulated by auxin signaling (Huang et al., 2014; Vaddepalli et al., 2021). Therefore, auxin is linked to division plane selection via its transcriptional regulatory activity.

In addition to the cytoskeleton, the asymmetric distribution of organelles could affect nuclear positioning and division asymmetry. The polarization of the zygote is accompanied by the expansion of a large vacuole at the basal cytoplasmic region (Mansfield and Briarty, 1991). Pharmacological and genetic disruption of vacuole morphology and distribution blocks nuclear positioning and inhibits division asymmetry (Kimata et al., 2019; Matsumoto et al., 2021). Similar to nuclear migration, the formation of a tubular vacuole around the nucleus and its asymmetric distribution are actin-dependent (Kimata et al., 2019). Hence, zygotic nuclear positioning requires pathways involving signaling cues, cell polarity, cell morphology, the cytoskeleton, and intracellular organelle interactions.

FOUNDER CELL DIVISION DURING LATERAL ROOT INITIATION

The development of lateral roots in Arabidopsis begins with the specification of pairs of founder cells derived from xylem pole pericycle cells (Cavallari et al., 2021). The two abutting elongated founder cells undergo physically asymmetric divisions, producing two adjacent small central cells and two large flanking cells (De Rybel et al., 2010) (Figure 3B). Before division, the central domain of each founder cell expands radially to a greater extent than the peripheral domain (Vilches Barro et al., 2019). Meanwhile, the nuclei migrate toward the common cell wall and undergo an asymmetric division. Cell-autonomous and non-autonomous auxin modules are critical for founder cell specification and asymmetric division as well as lateral root initiation at later stages of root development (Dubrovsky et al., 2008; De Rybel et al., 2010; Goh et al., 2012; Marhavy et al., 2013). GOLVEN (GLV) peptide signaling also plays an important role in founder cell specification and asymmetric division (Fernandez et al., 2015; Fernandez et al., 2020; Cavallari et al., 2021). However, how division asymmetry is achieved at the mechanistic level is not well understood.

Asymmetric cell expansion is a prominent cellular event that occurs concurrently with nuclear migration. Using time-lapse imaging, Vilches Barro et al. showed that MTs are arranged along the long axis before the first asymmetric division of pericycle founder cells and become isotropic in the central domain and anisotropic in the peripheral domain after division, indicating that MTs are responsible for asymmetric expansion (Vilches Barro et al., 2019). Accordingly, the depolymerization and stabilization of MTs lead to globally enhanced and decreased cell expansion, respectively. MT organization is defective when auxin signaling is perturbed, suggesting that the auxin pathway targets MTs to induce polarity establishment. Similarly, the disruption of F-actin stability increases the level of founder cell expansion and induces defects in polar nuclear migration and division asymmetry. It appears that MTs and actin are differentially involved in founder cell morphology and nuclear dynamics, which is similar to that during zygotic division

(Kimata et al., 2016). However, the exact relationship between cell growth and nuclear positioning has not been fully clarified.

Founder cell expansion and polarization are strongly influenced by cell wall properties. For example, inhibiting cellulose synthesis increases cell expansion and MT isotropy (Vilches Barro et al., 2019). The division capacity of founder cells is restrained by the overlying endodermis (Vermeer et al., 2014; Marhavy et al., 2016). A recent study showed that the cell wall remodeling enzyme EXPANSIN A1, an auxin-responsive factor, is responsible for founder cell expansion and asymmetric division (Ramakrishna et al., 2019). Additionally, vesicle trafficking-mediated cell wall remodeling is closely associated with lateral root emergence and patterning, although how it influences cell expansion and asymmetric division remains elusive (Wachsman et al., 2020). At stage II of lateral root development, lateral root initial cells undergo periclinal divisions, a phenomenon that is strongly against the default geometric rules for division plane orientation. Such divisions are also preceded by significant lateral growth (Schutz et al., 2021). These findings together suggest that signaling cues must play a role in cell shape regulation and division plane selection. Although the direct effectors remain to be characterized, polarity proteins might be involved in these processes. For example, ROP6 displays polar membrane localization in the lateral root founder cells and initials (Poraty-Gavra et al., 2013). Dominant-negative and constitutively active forms of ROP2 could inhibit and promote lateral root initiation, respectively (Li et al., 2001). ROPs may modulate cytoskeletal dynamics as well as cell wall remodeling (as revealed in other developmental processes) to initiate polarization and/or to regulate nuclear positioning (Feiguelman et al., 2018). Moreover, the expression of *ROP6* is controlled by auxin (Poraty-Gavra et al., 2013). Therefore, lateral root founder cell division employs hormone signaling, polarity regulators, cytoskeletons, and nuclear migration, similar to cell division in the zygote; however, tissue-specific mechanisms, such as cell wall remodeling, also play fundamental roles in this process.

THE DIVISION OF STOMATAL LINEAGE CELLS

In Arabidopsis, meristemoid mother cells (MMCs) divide asymmetrically to produce a larger stomatal lineage ground cell (SLGC) and a smaller meristemoid (Guo et al., 2021a) (Figure 3C). Meristemoids can either maintain an MMC fate or directly differentiate into guard mother cells (GMCs). Similarly, the SLGCs can differentiate into pavement cells or undergo asymmetric divisions to generate a smaller meristemoid distant from the previous one, thus enabling the proper spacing of stomata. The division site of MMCs and SLGCs is predictable and is marked by the position of the PPB and nucleus (Zhao and Sack, 1999; Lucas et al., 2006).

The initiation of asymmetric division of stomatal lineage cells depends on polarly localized proteins on the plasma membrane. An increasing number of components of the polarity module have been identified, including BASL, POLAR, BRXf, BRASSINOSTEROID-INSENSITIVE 2 (BIN2) kinase, YDA/MAPK kinases, MAPK SUBSTRATES IN THE STOMATAL LINEAGE (MASS) proteins, and BSU1-LIKE (BSL) family phosphatases (Dong et al., 2009; Pillitteri et al., 2011; Zhang et al., 2015; Houbaert et al., 2018; Rowe et al., 2019; Xue et al., 2020; Guo et al., 2021b). These factors undergo dynamic interactions during amplifying and spacing divisions and target the fate determinant SPEECHLESS (SPCH) to control cell differentiation (Zhang et al., 2015; Guo et al., 2021a). The disruption of polarity establishment also induces a reduced difference in daughter cell size, suggesting a tight correlation between division asymmetry and fate determination (Dong et al., 2009; Rowe et al., 2019). How does the polarity module control division site selection? Before division, the nucleus is positioned far from the polarity crescent, indicating that polarity proteins have a negative effect on division plane selection (Zhao and Sack, 1999). A recent study showed that MTs are necessary for propelling the pre-mitotic nucleus toward the opposing direction (Muroyama et al., 2020). Interestingly, the nucleus in the large daughter cell moves toward the inherited polarity crescent after division, which also depends on the same

polarity module. What causes the two opposite responses is unknown, but actin and Myosin-Xi rather than MTs are required for post-mitotic nuclear migration (Muroyama et al., 2020). Presumably, MT-associated motors are responsible for pre-mitotic nuclear migration.

Besides nuclear positioning, PPB assembly is thought to be controlled by the BASL/YDA/MAPK module (Shao and Dong, 2016). MICROTUBULE ORGANIZATION 1 (MOR1), an MT-binding protein required for PPB assembly, contains canonical MAPK phosphorylation sites and could be a direct target of YDA/MAPK (Kawamura et al., 2006). Other PPB-associated factors might also be regulated by phosphorylation, such as CLIP-ASSOCIATING PROTEIN (CLASP) and MICROTUBULE END BINDING PROTEIN 1c (EB1c) (Vavrdova et al., 2019). The BASL/YDA/MAPK module is thought to create a gradient of post-translationally modified proteins to influence PPB positioning (Shao and Dong, 2016). In support of this view, loss-of-function of the PPB-localized kinesin ARMADILLO REPEAT KINESIN (ARK3) results in phenotypes similar to those observed in the Arabidopsis *basl* mutants, implying a functional interaction between the polarity module and PPB (Malcos and Cyr, 2011; Lau et al., 2014).

The BSL phosphatase family was recently identified as a component of the polarity complex (Guo et al., 2021b). BSL1 is recruited to the polarity crescent later than BASL, and its appearance is closely correlated with PPB assembly. A checkpoint is thought to enable spatiotemporally controlled PPB assembly and localization. How the polarity complex controls or coordinates PPB formation as well as nuclear positioning is still unknown. Additionally, BASL may regulate cell growth to indirectly affect division site selection. The ectopic expression of BASL is sufficient to establish a polarizing domain to induce cell outgrowth, which is reminiscent of the function of ROP GTPases (Dong et al., 2009). BASL-induced cell growth is abrogated in ROP mutants, further supporting genetic interactions between BASL and ROPs. Whether the BASL complex plays additional roles in regulating cell morphology to control stomatal division remains obscure.

Nevertheless, the membrane fraction of BASL, rather than its nuclear localization, is necessary and sufficient to establish division asymmetry (Dong et al., 2009).

In the stomatal lineage, hormone signaling also plays a crucial role in oriented cell division (Lee and Bergmann, 2019; Herrmann and Torii, 2021). One prominent pathway involves the EPIDERMAL PATTERNING FACTOR (EPF) peptides and their membrane receptors (Geisler et al., 2000; Nadeau and Sack, 2002; Shpak et al., 2005; Hara et al., 2007; Hunt and Gray, 2009; Lee et al., 2012). These factors are linked to the division regulators by interacting with the YDA/MAPK cascade (Meng et al., 2015). The auxin and cytokinin pathways were recently shown to regulate orientated cell division. Through analyzing the targets of SPCH, cytokinin signaling was shown to promote asymmetric division and form a feedback loop with SPCH (Vaten et al., 2018). Auxin may also promote asymmetric division. Using auxin reporters, Le et al. found a high level of auxin activity in meristemoids primed for asymmetric division and a significantly lower level in GMCs, suggesting that auxin positively regulates stemness but inhibits differentiation (Le et al., 2014). This notion is supported by two other studies showing negative effects of auxin on stomatal development (Balcerowicz et al., 2014; Zhang et al., 2014). How the developmental signals are intertwined with the polarity pathway to control division asymmetry remains unresolved. It appears that there are complex interactions, as revealed by genetic analyses (Dong et al., 2009; Balcerowicz et al., 2014; Herrmann and Torii, 2021). Nevertheless, signaling cues, polarity regulators, and cytoskeleton-dependent nuclear positioning and PPB assembly may act successively to control asymmetric divisions in the stomatal lineage.

THE DIVISION OF SUBSIDIARY MOTHER CELLS IN GRASSES

In contrast to Arabidopsis cells, subsidiary mother cells (SMCs) in grasses (e.g., maize [*Zea mays*] and wheat [*Triticum aestivum*]) exhibit a unique type of asymmetric division (Facette and Smith, 2012; Apostolakos et al., 2018) (Figure 4). Before division, SMCs are polarized at a site in contact

with the neighboring guard mother cells (GMCs) (Galatis and Apostolakos, 2004). Subsequently, their nuclei migrate toward the polarized cell cortex and undergo an asymmetric division (Panteris et al., 2006). As SMC division is highly asymmetric, the nucleus, as well as the mitotic spindle, must be anchored toward the GMC, which is critical for the establishment of the curved division plane (Panteris et al., 2006). The PPB is assembled at the edge of the SMC-GMC contact site following nuclear positioning, where it guides cell plate expansion (Cho and Wick, 1989). Under experimental conditions, the formation of a PPB can be induced when the nucleus is not properly anchored, pointing to the self-organizing capacity of the PPB, as mentioned earlier (Galatis et al., 1983; Galatis et al., 1984). Therefore, in cereal species, nuclear positioning, PPB formation, and spindle anchoring are coordinated to govern the orientation of the unique division plane. Among these processes, nuclear migration is an early hallmark event that correlates with and predicts the asymmetric division of SMCs. The mechanism of nuclear migration is not yet fully understood, but cytoskeletal arrays play an indispensable role in this process. In maize, MTs are essential for polar nuclear migration and anchoring, while actin influences the initial migration of the nucleus but not the maintenance of its position (Panteris et al., 2006). By contrast, actin rather than MTs plays a more important role in this process in Virginia spiderwort (*Tradescantia virginiana*) (Kennard and Cleary, 1997). Other factors such as MT- or actin-dependent motors have not been shown to be involved in polar nuclear migration and anchoring.

In addition to the cytoskeleton, cell polarization acts as an initiating factor for nuclear positioning. Before nuclear migration, SMCs are polarized, with an actin patch at the GMC contact site (Cho and Wick, 1990). This process depends on two polarly localized membrane receptor-like kinases: PAN1 and PAN2 (Cartwright et al., 2009; Zhang et al., 2012). PAN1 acts genetically downstream of PAN2 and functions to recruit ROP2/9 (Humphries et al., 2011; Zhang et al., 2012). ROPs are small GTPases that activate WAVE/WASP complexes to stimulate actin polymerization (Feiguelman et al., 2018), making them promising candidates linking polarity establishment and

actin patch formation. Indeed, the SCAR/WAVE subunits BRICK1 (BRK1) and BRICK3 (BRK3) display polar localization and control SMC division (Gallagher and Smith, 2000; Frank and Smith, 2002; Frank et al., 2003; Facette et al., 2015). However, the polar recruitment of BRK1 precedes that of PAN1 and PAN2, suggesting that BRK1 and BRK3 function upstream rather than downstream of ROPs. This notion is supported by the observation that the localization of PAN1, PAN2, and ROP2 is dependent on BRK1 and BRK3 but not vice versa (Facette et al., 2015). Although the SCAR/WAVE complex coimmunoprecipitates with ROP2, and a feedback regulatory loop has been proposed (Facette et al., 2015), it appears that ROPs might not activate SCAR/WAVE to promote actin patch formation and nuclear positioning.

Polarity signaling is not the sole factor regulating nuclear positioning, since a significant number of nuclei can be polarized in the absence of an actin patch (Cartwright et al., 2009; Humphries et al., 2011; Zhang et al., 2012; Facette et al., 2015). The division of SMCs is preceded by local cell expansion at the GMC contact site, which is correlated with the modification of cell wall composition in this region (Giannoutsou et al., 2016). Cell wall remodeling and altered SMC morphology may provide additional signals to influence nuclear dynamics and division plane positioning. Indeed, the failure of nuclear migration can be partly induced by the decreased size of SMCs (Apostolakos et al., 2018). The relationship between cell expansion and polarity establishment is not fully understood. These processes might act partly in the same pathway to control nuclear migration, since cell protrusion and actin patch formation are closely correlated (Panteris et al., 2007). In Arabidopsis, auxin can stimulate cell elongation by modulating cell wall composition (Majda and Robert, 2018). Auxin signaling induces SMC polarization and nuclear migration (Livanos et al., 2015). Therefore, auxin signaling may play a role in regulating cell wall remodeling and coordinating cell expansion and polarity establishment. In addition, the involvement of receptor-like kinases suggests a role for uncharacterized signals upstream of polarity proteins. These findings together indicate that development signals, cell morphology,

polarity, and cytoskeletons act cooperatively to control pre-mitotic nuclear positioning and SMC division.

GAMETOPHYTIC DIVISIONS IN THE MOSS *PHYSCOMITRIUM PATENS*

The bryophyte moss *P. patens* is an emerging basal land plant model for studying plant cell division and pattern formation (Rensing et al., 2020; de Keijzer et al., 2021; Naramoto et al., 2022). During the lifecycle of *P. patens*, many types of gametophytic cells undergo asymmetric divisions (Kofuji and Hasebe, 2014; de Keijzer et al., 2021) (Figure 5). These include protonema tip cells (apical cells), gametophore initials, side-branch precursor cells (subapical cells), and shoot initials. The PPB is not formed during the division of protonema cells and gametophore initials but is present in the leafy shoots of *P. patens* (Doonan et al., 1987; Kosetsu et al., 2017). These observations support the notion that the PPB has evolved as an innovation in complex tissues. Accordingly, the division pattern in leafy shoots resembles those of meristems in seed plants and involves conserved signaling pathways (Cammarata et al., 2019; Veron et al., 2021).

Moss protonemata consist of two types of cells, the chloronema and caulonema cells, whose tip cells undergo polarized growth (Menand et al., 2007; Vidali and Bezanilla, 2012). Similar to pollen tubes and root hairs in flowering plants, tip growth in these cells is predominantly controlled by polar F-actins and ROP GTPases (Doonan et al., 1988; Finka et al., 2007; Vidali and Bezanilla, 2012; Burkart et al., 2015; Wu and Bezanilla, 2018; Cheng et al., 2020; Yi and Goshima, 2020). As tip cells elongate, their nuclei are maintained at the cell center by concomitant migration along the growth direction (Pressel et al., 2008). Meanwhile, a large vacuole is associated with the nucleus at the basal site and expands as the nucleus moves forward (Pressel et al., 2008). Although nuclear division occurs at the cell center, the division of apical cells gives rise to daughter cells with distinct fates: one contains more chloroplasts and continues to undergo tip growth; the other inherits the large vacuole and becomes a subapical cell (Figure 5A).

Whether the asymmetric location of the vacuole is important for nuclear positioning in these cells is unknown, but MTs are crucial for nuclear migration (Doonan et al., 1985). In support of this notion, the minus-end-directed kinesin KINESIN WITH CALPONIN HOMOLOGY DOMAIN (KCH) is required to place the interphase nucleus at the cell center. Loss of KCH results in an apical shift of the nucleus and the division plane (Yamada and Goshima, 2018). Moreover, MTs are predominantly oriented toward the apical end, further supporting the involvement of MT-dependent retrograde transport by KCH (Hiwatashi et al., 2014). The function of KCH is evolutionarily conserved. When overexpressed in tobacco BY-2 cells, the rice OsKCH1, which localizes to the leading edge of the migrating nucleus, can influence pre-mitotic nuclear migration (Frey et al., 2010). The motor for anterograde transport of the pre-mitotic nucleus has not been identified. Surprisingly, the MT destabilizing factor Kinesin-13 controls anterograde nuclear migration, suggesting that the regulation of MT dynamics also directly or indirectly affects nuclear positioning (Leong et al., 2020).

As cell growth is closely correlated with nuclear migration, the growth-associated molecular machinery might interact with structures such as the cytoskeleton to influence nuclear positioning. The growth of apical cells requires polarized F-actins as well as subcortical MT-actin foci (Vidali and Bezanilla, 2012; Hiwatashi et al., 2014; Wu and Bezanilla, 2018; Yamada and Goshima, 2018). Since KCH is required for the coalescence of MT-actin foci and can potentially bind MTs and actin, it may directly link directed cell growth to nuclear positioning (Yamada and Goshima, 2018). However, the putative actin-binding domain of KCH is not functional in either process. Instead, its C-terminus is essential for apical growth but is not required for nuclear migration (Yamada and Goshima, 2018). Therefore, the two functions of KCH are separate, but it remains enigmatic how they are coordinated.

Subapical cells that generate side branches represent another model of asymmetric division in mosses (Figure 5B). Before division, subapical cells form a polarized bulge at the apical site. Subsequently, the nucleus migrates into the bulge and divides asymmetrically to generate a side-branch initial (Schmiedel and Schnepf, 1979; Doonan et al., 1986). Polarization of subapical cells depends on F-actins and ROP GTPases (Quader and Schnepf, 1989; Cheng et al., 2020; Yi and Goshima, 2020). This process is required for nuclear migration. As MT organization is altered during bulge formation, it was proposed that ROP-actin-dependent cell polarity and morphological alteration guide nuclear migration by regulating MT remodeling (Yi and Goshima, 2020). Indeed, loss-of-function of ROPs affects MT dynamics in non-dividing cells (Burkart et al., 2015). Additionally, plus-end-directed kinesins may be involved in nuclear transport, since MTs in subapical cells are predominantly oriented toward the apical site, similar to those in apical cells (Hiwatashi et al., 2014; Yi and Goshima, 2020). The identification of such kinesins and their interaction with ROP-actin will be of interest toward understanding the mechanisms underlying pre-mitotic nuclear migration. In addition to regulating cell polarization, ROPs may affect division plane orientation in bulge-forming subapical cells (Yi and Goshima, 2020). PpROP4, which was observed on the assembling cell plate (Figure 2B), could potentially interact with the CDZ-localized PpREN, the moss homolog of PHGAPs, which regulate division orientation in Arabidopsis (Stockle et al., 2016; Yi and Goshima, 2020) (Figure 5C). However, whether ROP signaling is directly involved in division orientation remains unknown.

Besides side-branch initials, caulonema subapical cells also generate gametophore initials, but at a lower frequency (Brandes and Kende, 1968). Gametophore initials are indistinguishable from side-branch initials at the early stage of development. However, they gradually become bulbous and undergo an oblique instead of transverse division, a characteristic distinct from side-branch cells (Brandes and Kende, 1968; Harrison et al., 2009; Tang et al., 2020). Nuclear movement before the first division of the gametophore initial is not obvious, but the nucleus is placed around the

apical cytoplasm, with a large vacuole occupying the basal cytoplasm (Kosetsu et al., 2017). Upon entering mitosis, gametophore initials assemble a tilted spindle and rotate the division plane at an angle of ~42 degrees (Figure 5D). Interestingly, the nucleus of the basal daughter cells moves to the bottom and progressively migrates to the apical site before the second round of asymmetric division. Subsequent divisions of the apical and basal cells are also oblique, generating a group of four cells, which later produce a tetrahedral shoot. The mechanism of nuclear positioning and its contribution to division plane selection in gametophore initials have not been investigated. However, spindle orientation and phragmoplast guidance are important. A cytoplasmic MTOC termed the gametosome was found to regulate the orientation of the spindle and division plane (Kosetsu et al., 2017). Gametosomes are functionally analogous to polar caps in angiosperms and centrosomes in animals. Together with other cytoplasmic MTOCs, they are thought to represent another conserved mechanism of division plane regulation in plants (Kosetsu et al., 2017; Yi and Goshima, 2018).

As in other plants, hormone signaling also influences division patterns in *P. patens* (Thelander et al., 2018; Cammarata et al., 2019). For example, the cytokinin pathway is a well-known positive regulator of the induction of gametophore initials (Brandes and Kende, 1968; von Schwartzenberg et al., 2016). The CLAVATA3 (CLV3)/EMBRYO SURROUNDING REGION-related (CLE) pathway was recently shown to antagonize cytokinin signaling (Cammarata et al., 2019; Cammarata et al., 2021). Disruption of CLE signaling results in an increased number of gametophores, ectopic shoot apical meristem formation, and mis-oriented divisions in gametophore initials (Whitewoods et al., 2018; Cammarata et al., 2021). Although the involvement of cytokinin in division plane selection is unclear, the crosstalk between cytokinin and CLE signaling implies a potential role of the cytokinin pathway in this process. In addition to cytokinin, auxin may also regulate asymmetric division. However, it displays opposite effects on the induction of gametophore initials and side branches (Ashton et al., 1979; Thelander et al., 2018).

A direct role of cytokinin and auxin signaling in division plane selection and orientation has not been revealed. Interestingly, the auxin efflux carriers exhibit polar localization at the apical membrane, similar to ROPs, implying a potential link between cell polarity and locally controlled auxin activity (Bennett et al., 2014; Viaene et al., 2014). Consistent with this observation, a ratiometric reporter of the primary auxin response shows minimal auxin levels in apical cells and other gametophytic stem cells, indicating a negative role for auxin in regulating stemness and likely stem cell division (Thelander et al., 2019). How developmental signals genetically interact with polarity proteins to control cell expansion, nuclear migration, and division site determination remains an open question.

CONCLUDING REMARKS

How the division site is determined is one of the most fundamental questions in plant cell biology (Roeder et al., 2022). With advances in genetic manipulation, computational modeling, and live-cell imaging, our knowledge of this process has greatly improved. Several mechanistic models, such as geometry-based surface minimization and phragmoplast guidance (Livanos and Muller, 2019), have been put forth as universal rules guiding division plane determination (Figure 1). However, how these mechanisms are modified for asymmetric division is poorly understood. In particular, the underlying mechanism for asymmetric division varies from the canonical rules and appears to be species-, cell type-, and/or developmental context-dependent (De Smet and Beeckman, 2011; Rasmussen et al., 2011; Livanos and Muller, 2019). Therefore, whether a broadly conserved theme exists in plants is a thought-provoking issue.

Recent and earlier findings have revealed similarities in the ACD process in cells with distinct shapes and fates (Table 1). First, these cells are polarized in preparation for asymmetric division. The polarization process exploits membrane-localized polarity proteins, which are reminiscent of the Par proteins in animals (Shao and Dong, 2016). Notably, these molecules are usually

responsible for the asymmetric division of cells that generate daughter cells of significantly different sizes. Although many of these proteins are species-specific, conserved proteins, such as ROP GTPases, are emerging. In animals, Cdc42, a functional homolog of ROPs, also controls physically asymmetric division (Gotta et al., 2001; Knoblich, 2010). Hence, polarity-induced asymmetric division is an evolutionarily conserved mechanism in animals and plants and exhibits similarities at both the cellular and molecular level. Additionally, during organogenesis, many cells undergo asymmetric divisions without generating significant physical asymmetry. In these cells, a tissue-wide polarity gradient is important. Several proteins that display globally polar localization and regulate division pattern formation have been identified, such as PINs (Muroyama and Bergmann, 2019), DEFECTIVE KERNEL 1 (DEK1) protease (Perroud et al., 2014; Perroud et al., 2020), and SOSEKI (SOK) proteins (Yoshida et al., 2019; van Dop et al., 2020; Ramalho et al., 2022). The DIX domain of SOKs is functionally conserved in Dishevelled proteins in animals, which are core components of the Wnt pathway that regulate tissue patterning (Yoshida et al., 2019; van Dop et al., 2020). Although novel regulators of plant division plane determination continue to emerge (Moody et al., 2018; Moody et al., 2021), animals and plants seem to take similar strategies by exploiting polarity proteins at different developmental stages.

Second, cell polarity is closely associated with cell morphology and nuclear positioning. As plant cells are encased in rigid cell walls, the formation of a new tissue layer strongly relies on directed cell growth and oriented cell division. Polarity proteins are the best choice to fulfill these requirements. How polarity controls off-center nuclear positioning is an important question for exploring physically asymmetric division. One possibility is that polarity-guided changes in cell shape induce a geometric signal to influence cytoskeleton dynamics and nuclear movement. This model applies to some cells, such as founder cells and zygotes in Arabidopsis and moss protonema cells, but perhaps not others. For instance, cell expansion is not observed in maize or rice zygotes (Khanday and Sundaresan, 2021). Another possibility is that polarity proteins directly target

cytoskeletal elements to regulate nuclear positioning and/or division orientation. Although the downstream effectors, as well as the polarity proteins themself, can be cell type-dependent, this model explains most if not all physically asymmetric divisions. Intriguingly, since the spindle positioning mechanism in animals appears to be absent in plants, polarity-triggered nuclear positioning could represent an alternative choice during plant evolution (Figure 6A). If this model is correct, cytoskeletal effectors that link polarity proteins and the nucleus would be expected to play an important role in this process. For example, polarity proteins may directly or indirectly activate kinesin or myosin motors to transport the nucleus and/or modulate MT/actin organization to facilitate this process. Indeed, molecular pathways involving ROPs, cytoskeletal dynamics, and cytoskeleton-dependent intracellular trafficking are known to control plant morphogenesis and physiology (Feiguelman et al., 2018). A similar mechanism might be functional during asymmetric division.

Third, nuclear positioning, geometry and mechanical stress sensing, and phragmoplast guidance jointly control division site determination during plant ACD (Figure 6B). In Arabidopsis, changes in tissue-wide mechanical forces can influence polarization site selection and subsequent division orientation (Bringmann and Bergmann, 2017). When polarity-induced nuclear positioning is defective, division asymmetry can still be generated, likely via the geometric rules (Muroyama et al., 2020). Hence, nuclear positioning and geometry sensing are intertwined but not strictly correlated. Similarly, nuclear positioning and phragmoplast guidance are partly independent but coordinated, as discussed earlier. In animals, the spindle positioning and myosin-mediated furrowing mechanisms also possess the ability to regulate division asymmetry separately (Sunchu and Cabernard, 2020). Both animals and plants have taken advantage of multiple coordinated pathways for ACD. Since plants lack the spindle displacement mechanism, they have evolved elaborate mechanisms to position the nucleus asymmetrically for ACD, thus enabling the generation of tissue patterns properly and efficiently (Figure 6A). Although the mechanisms

underlying nuclear positioning differ depending on cell type, polarity proteins and the cytoskeleton are commonly involved in this process. We propose that polarity-cytoskeleton-nuclear positioning is an evolutionarily conserved strategy for generating physical division asymmetry in plants. This mechanism is integrated with the default rules involving geometry sensing and phragmoplast guidance and is modulated by developmental contexts (Figure 6B). How the nucleus responds to signals derived from polarity proteins, geometric input, and developmental cues and how nuclear positioning is coordinated with phragmoplast guidance to determine an asymmetric division plane require further investigation. Future studies addressing these questions in a broad range of developmental contexts and species, especially for cell divisions that do not involve cortical MTs, will shed light on the conceptual framework for ACD in plants.

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AUTHOR CONTRIBUTIONS

P. Y. and G. G. wrote the manuscript. P. Y. prepared the figures and tables.

Conflict of interest

The authors declare no conflict of interest.

Table 1. Cellular events, structures, and regulators in asymmetrically dividing cell models.

Species	Cell type	Developmental cues and signaling	Polarization	Cell expansion	Nuclear migration or positioning	PPB	CDZ
Arabidopsis	Zygote	+, auxin (Friml et al., 2003), membrane receptors (Bayer et al., 2009; Yu et al., 2016), and YDA/MAPK kinase cascade (Lukowitz et al., 2004; Zhang et al., 2017)	+, ROP3 (Huang et al., 2014)	+, MT and actin (Kimata et al., 2016)	+, actin (Kimata et al., 2016)	+, not determined	+, not determined
Arabidopsis	Lateral root founder cell	+, auxin (Dubrovsky et al., 2008; De Rybel et al., 2010; Goh et al., 2012; Marhavy et al., 2013) and GLV signaling (Fernandez et al., 2015; Fernandez et al., 2020)	+, ROP2/6* (Li et al., 2001; Poraty-Gavra et al., 2013)	+, MT, actin (Vilches Barro et al., 2019), Expansin (Ramakrishna et al., 2019), and cell wall composition (Vilches Barro et al., 2019; Wachsman et al., 2020)	+, actin (Vilches Barro et al., 2019)	+, not determined	+, not determined
Arabidopsis	Stomatal lineage	+, auxin (Balcerowicz et al., 2014; Le et al., 2014;	+, BASL/YDA/MAPK module (Dong et al., 2009; Pillitteri	unclear	+, MT (pre-mitotic), actin, and	+, ARK3 (Lau et al., 2014)	+, not determined

		Zhang et al., 2014), cytokinin (Vaten et al., 2018), and EPF signaling (Geisler et al., 2000; Nadeau and Sack, 2002; Shpak et al., 2005; Hara et al., 2007; Hunt and Gray, 2009; Lee et al., 2012; Meng et al., 2015)	et al., 2011; Zhang et al., 2015; Houbaert et al., 2018; Rowe et al., 2019; Xue et al., 2020; Guo et al., 2021b)		myosin XI-i (post-mitotic) (Muroyama et al., 2020)		
Z. mays	Subsidiary mother cell	+, auxin (Livanos et al., 2015)	+, PAN1/2 (Cartwright et al., 2009; Zhang et al., 2012), ROP2/9 (Humphries et al., 2011), SCAR/WAVE (Facette et al., 2015), actin (Galatis and Apostolakos, 2004)	+, cell wall composition (Giannoutsou et al., 2016)	+, MT and actin (Panteris et al., 2006)	+, not determined	+, not determined
P. patens	Protonema cell	+, auxin* (Bennett et al., 2014; Thelander et al., 2018, 2019)	+, ROP (Burkart et al., 2015; Cheng et al., 2020; Yi and Goshima, 2020)	MTs and actin (Doonan et al., 1988; Finka et al., 2007; Wu and Bezanilla, 2018; Yi and Goshima, 2020)	+, MT (Doonan et al., 1986; Yi and Goshima, 2020), KCH (Yamada and Goshima, 2018), and Kinesin-13	–	+, Myosin VIII (Wu and Bezanilla, 2014), REN (Yi and Goshima, 2020), and PHRAGMOPLAST ORIENTING KINESINs (Miki et al., 2014)

					(Leong et al., 2020)		
P. patens	Gametophore initial	+, cytokinin* (Brandes and Kende, 1968; Ashton et al., 1979; von Schwartzenberg et al., 2016; Cammarata et al., 2021), auxin* (Ashton et al., 1979; Thelander et al., 2018), CLE signaling (Whitewoods et al., 2018; Cammarata et al., 2021), and novel regulators (Perroud et al., 2014; Moody et al., 2018; Moody et al., 2021)	+, not determined	+, not determined	+, not determined	–	+, spindle rotation and anchoring (Kosetsu et al., 2017; Kozgunova et al., 2021)

Key regulators of asymmetric division in plant cell models are listed for comparison. Although some of the regulators are cell type- and/or species-specific (e.g., BASL and PAN1/2), it appears that asymmetric division in plants generally involves signaling cues, cell polarity, cell morphology, the cytoskeleton, nuclear positioning, and phragmoplast guidance governed by the PPB and CDZ. Cellular structures, events, and regulators that are involved or not involved are marked with "+" and "–", respectively. Stars indicate a putative function in division site selection that has not been experimentally examined.

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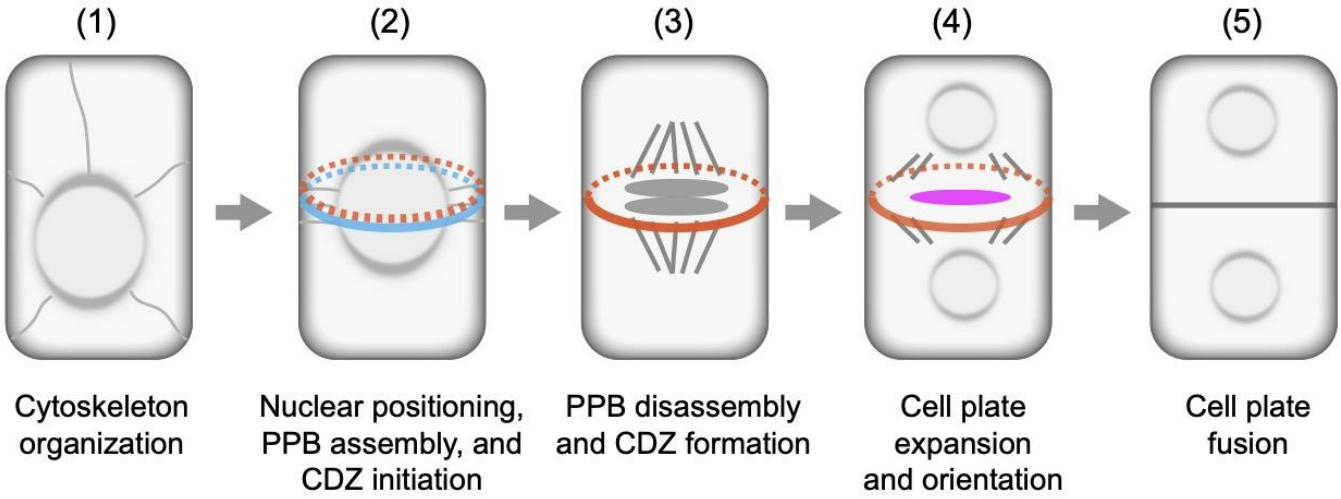

Figure 1. The default mechanism of division site determination during symmetric cell division.

In most plant cells, the nucleus occupies a large cytoplasmic area at the cell center. In vacuolate cells, the nucleus is off-center and has to migrate to the cell center before mitosis. This process is mediated by microtubules (MTs) (gray lines) that connect the nuclear membrane and the cell cortex (1). At preprophase, a ring-shaped preprophase band (PPB, blue circle) is assembled by the realignment of cortical MTs (2). At prometaphase, the PPB is disassembled (3). A series of MT-associated proteins that have been recruited to the cortical ring following PPB assembly are maintained. These proteins collectively mark the cortical division zone (CDZ, orange circle) (3). At anaphase, with the aid of the mitotic spindle, the assembly of the cell plate (magenta disk) is initiated at the cell center. Later on, the mitotic spindle is transformed into a phragmoplast. The assembling cell plate expands toward the CDZ under the guidance of the phragmoplast (4). Eventually, the cell plate fuses with the parental cell membrane at the place labeled by the CDZ (5).

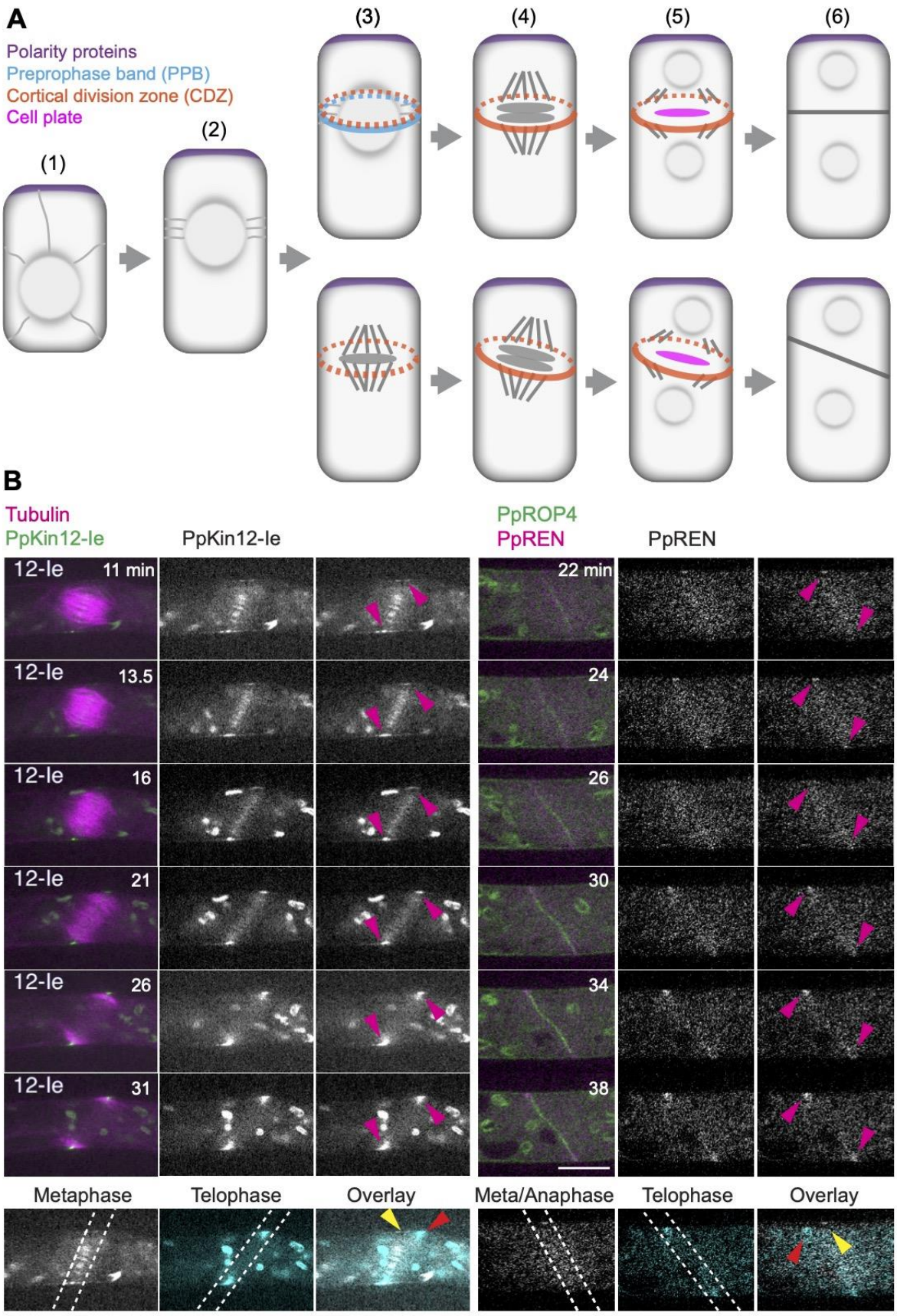

Figure 2. Division site determination during asymmetric cell division.

(A) In many plant cells, cell polarization is a prerequisite for initiating asymmetric division (1). Once polarized, the cell undergoes polar cell expansion and migrates its nucleus to an off-center position (2). This process relies on polarity proteins (purple) and cytoskeletal elements (gray lines). However, the responsible molecules can be different depending on the cell type. In the majority of cells in flowering plants, the preprophase band (PPB, blue circle) is subsequently assembled around the nucleus and facilitates the establishment of the cortical division zone (CDZ, orange circle), which also occurs during symmetric divisions (3, upper). The CDZ then guides phragmoplast-mediated cell plate (magenta disk) expansion (4-6, upper). In some types of cells such as moss protonema cells and gametophore initials, the PPB is not formed. Instead, the CDZ itself is established after nuclear envelope breakdown (3, lower). In addition, both the mitotic spindle and the CDZ can rotate, thus generating an oriented division plane (4-6, lower). Note that CDZ assembly occurs earlier in Arabidopsis than in mosses and that the rotation of the CDZ has only been reported in moss caulonema tip cells, whose functional significance has not been addressed (see Figure 2B).

(B) Dynamic establishment of the CDZ in *P. patens* caulonema tip cells. Time-lapse images show the localization of PpKin12-Ie (a homolog of POKs, left) and PpREN (the homolog of PHGAPs, right). Magenta arrowheads indicate the position of the CDZ. The CDZ localization of PpKin12-Ie and PpREN at metaphase or meta/anaphase (yellow arrowheads) is overlaid with their localization at telophase (red arrowheads) for comparison. Dashed lines show the orientation of the CDZ. Time is shown following nuclear envelope breakdown (0 min). Images are reproduced from Movie S6 in (Miki et al., 2014) and Video S5 in (Yi and Goshima, 2020). Scale bars: 10 μm.

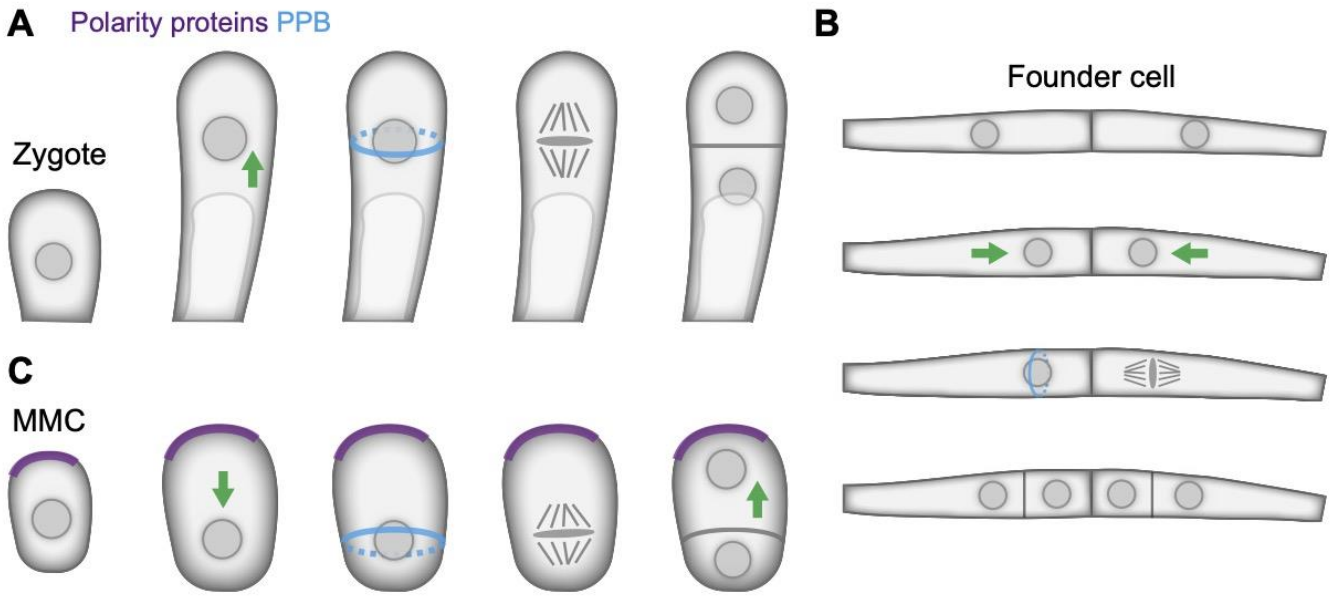

Figure 3. Cell models for studying asymmetric cell division in Arabidopsis.

(A) Zygotic division. After fertilization, the zygote exhibits rapid polar growth. Concurrently, its nucleus migrates toward the apical cytoplasm (green arrow) and an expanding vacuole occupies the basal cytoplasm. The PPB is then assembled around the nucleus (blue circle). As the position of the nucleus is asymmetric, the subsequent division produces a small apical cell and a large basal cell, which will develop into the proembryo and suspensor, respectively.

(B) Founder cell division. Lateral roots are initiated by the division of pairs of founder cells. Before division, two abutting founder cells expand radially. The expansion near the common wall (central domain) is faster than that at the periphery domain. During cell expansion, their nuclei migrate toward the common wall (green arrows) and divide asymmetrically to produce two central cells and two peripheral cells that differ significantly in shape.

(C) The division of meristemoid mother cells (MMCs). The pre-mitotic MMC is polarized with the BASL-associated protein complex on the membrane (purple). This polarity crescent instructs the opposing movement of the nucleus (green arrow). The PPB is formed around the nucleus (blue line) and marks the asymmetric division site. The polarity crescent is inherited by the large daughter cell. After division, the nucleus in the large daughter cell migrates toward the polarity axis. This process is also controlled by the polarity signal.

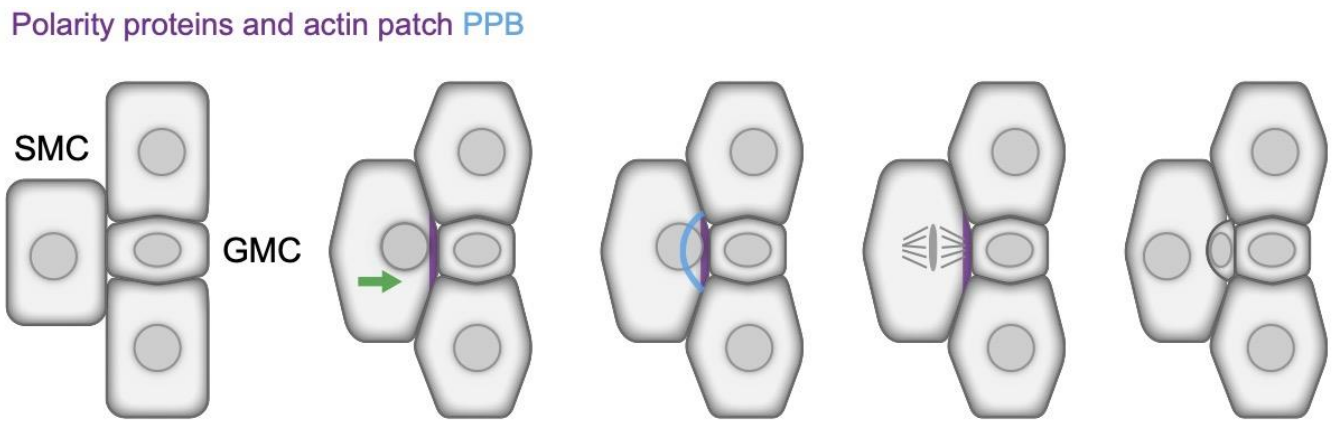

Figure 4. The division of stomatal subsidiary mother cells in maize.

Before division, the maize stomatal subsidiary mother cells (SMCs) are polarized by signals from the closely associated guard mother cells (GMCs). The SMCs undergo local cell expansion and form a polarized domain at the GMC contact site. The polarization process depends on polarity proteins (PAN1/2 and ROP2/9) and an actin patch (purple). Subsequently, their nuclei migrate toward the polarized cortex (green arrow). As the protrusion of the SMCs is limited in space, the PPB is assembled in a curved shape (blue line). After nuclear envelope breakdown, the mitotic spindle is anchored to the polarized cortex. Cytokinesis proceeds under the guidance of the phragmoplast and generates a lens-shaped small subsidiary cell.

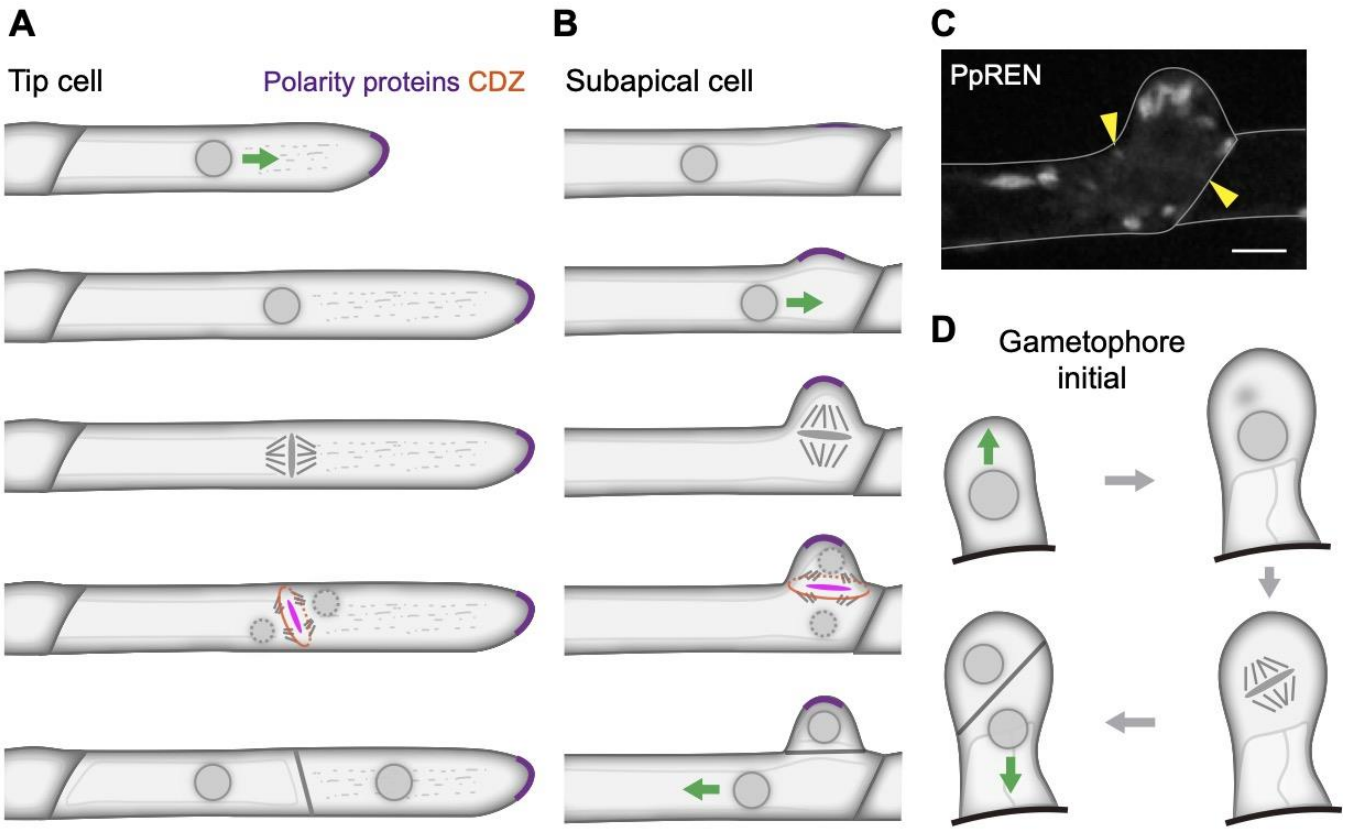

Figure 5. Cell models for studying asymmetric cell division in *P. patens*.

(A) The division of caulonema tip cells. The tip cell exhibits polarized growth under the control of the polarity proteins ROP GTPases (purple). During growth, the tip cell nucleus moves to the cell center (green arrow) and then undergoes mitosis when the cell length reaches approximately twice the length of subapical cells. The PPB is not formed. Instead, the mitotic spindle and CDZ (orange) can rotate to generate an oblique division plane. Although the resulting daughter cells are equal in size, they show remarkable differences in cellular contents and cell fates.

(B) The division of subapical cells. Subapical cells first initiate a polarized bulge at the apical end (purple), a process that depends on ROP GTPases. During bulging, the nucleus undergoes directed migration (green arrow) and is eventually located in the bulge. Subsequently, the cell divides asymmetrically to produce a small side-branch initial cell. After division, the nucleus in the large daughter cell moves back to the cell center (green arrow). The PPB is absent during subapical cell division, but the CDZ (orange) is retained.

(C) Localization of the CDZ component PpREN (the moss homolog of PHGAPs) in a subapical cell (yellow arrows) during cytokinesis. Scale bar: 10 μm.

(D) Division of gametophore initials. Gametophore initials are produced in a similar manner to side-branch initials. At the early stage, these two types of cells are indistinguishable. With the progression of cell growth, gametophore initials gradually adopt a bulbous shape and position the nucleus at the apical cytoplasm (green arrow). Before nuclear envelope breakdown, an MT cloud termed the gametosome forms at the apical cytoplasm. The gametosome merges into the subsequently assembled mitotic spindle and plays an important role in guiding the rotation of the spindle and division

plane. The PPB is absent in gametophore initials and is thought to be functionally replaced by the gametosome. After division, the nucleus in the basal daughter cell migrates down to the basal cytoplasmic region (green arrow).

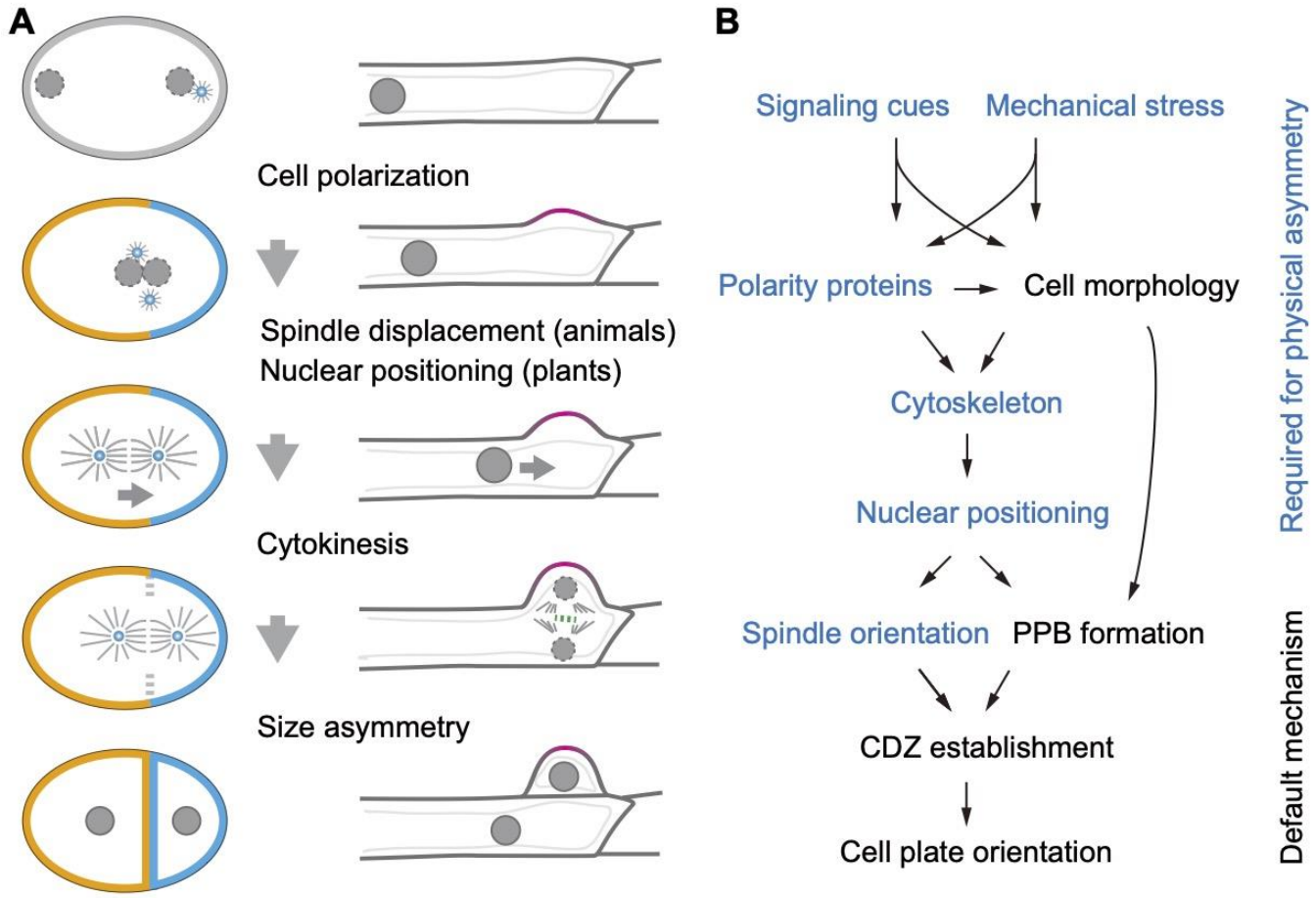

Figure 6. A general model for asymmetric cell division in plants.

(A) Strategies for physically asymmetric cell division in animals and plants. The one-cell stage embryo in *Caenorhabditis elegans* (*C. elegans*) (left) and the subapical cell in *P. patens* (right) are shown as examples to compare the mechanistic models. In general, both cells are polarized by asymmetrically localized membrane proteins (orange and blue in the *C. elegans* embryo; magenta in the *P. patens* subapical cell). These polarity proteins act on cytoskeletal elements to position cellular structures (spindle in animals and nucleus in plants), thus determining the asymmetric location of a division site.

(B) A generalized pathway for division site determination in plants. The default mechanism involves cell morphology, PPB formation, CDZ establishment, and cell plate orientation and is employed for both symmetric and asymmetric divisions. Additional factors are required to execute physically asymmetric division. These include extracellular signals, such as developmental cues and tissue-level mechanical stress, and intracellular pathways comprising polarity proteins and cytoskeletons that affect nuclear positioning and/or spindle orientation.