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An overview of plant division-plane orientation

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Summary

Plants, a significant source of planet-wide biomass, have an unique type of cell division in which a new cell wall is constructed de novo inside the cell and guided towards the cell edge to complete division. The elegant control over positioning this new cell wall is essential for proper patterning and development. Plant cells, lacking migration, tightly coordinate division orientation and directed expansion to generate organized shapes. Several emerging lines of evidence suggest that the proteins required for division-plane establishment are distinct from those required for division-plane maintenance. We discuss recent shape-based computational models and mutant analyses that raise questions about, and identify unexpected connections between, the roles of well-known proteins and structures during division-plane orientation.

I. Introduction

Cell division, a fundamental requirement for life, is carefully regulated in both space and time. Symmetrical proliferative divisions are essential for growth and account for the vast majority of plant cell divisions. When and where proliferative divisions occur along with expansion and differentiation allows formation of the entire plant body. Formative (asymmetrical) divisions are critical for the development of new cell-types. Due to their precise role in development, asymmetrical cell divisions tend to be initiated by specific transcription factors and signaling pathways: readers are directed to recent reviews on asymmetrical division-plane specification and polarization (Kajala et al., 2014; Shao & Dong, 2016). Here, we discuss computational division-plane modeling approaches and our perspective on division-plane establishment and maintenance in plants, whereas recent reviews provide insight into phragmoplast organization and new cell wall formation (Lee & Liu, 2013; Jürgens et al., 2015; Smertenko et al., 2017).

II. Models of plant cell division

Basic patterns of plant cell divisions were originally described in the late 1800s (Hofmeister, 1863; Sachs, 1878; Errera, 1888), and recently revisited (Besson & Dumais, 2014). Divisions typically occur perpendicular to the cell’s long axis at positions that minimize the final surface area of the new cell wall, similar to soap-films (Errera, 1888; Flanders et al., 1990). For each cell, multiple division planes represent possible minimal final surface areas (Besson & Dumais, 2014).

Within the past few years, computational modeling was used to predict plant cell division-plane orientation. For simplicity, most models used 2D instead of 3D plant cells. Predicting divisions along the shortest plane through the center of mass accounts for many features of tissues composed of symmetrically dividing cells (Sahlin & Jönsson, 2010; Shapiro et al., 2015). Empirically determined ‘stochasticity factors’ added variability to shape-based division-plane predictions notably improving their ability to match...
in vivo division planes (Dupuy et al., 2010; Besson & Dumais, 2011). More elongated cells tend to divide along the shortest plane, whereas less elongated cells have more division-plane variability (Besson & Dumais, 2011). Importantly, these models emphasize division-plane orientation variability, an idea underappreciated for more than a century (Besson & Dumais, 2014).

Recently, the 3D shape of plant cells has been used to predict division-plane orientations (Yoshida et al., 2014; Martinez et al., 2017a). One method identifies only the shortest division plane through the cell’s center-of-mass (Yoshida et al., 2014). Another method generates multiple soap-film minima division predictions (Martinez et al., 2017a) to directly test the hypothesis that division planes mimic soap-film minima (Errera, 1888). Simple 3D geometric properties are sufficient to generate probabilistic division predictions that are often consistent with in vivo division planes of epidermal maize cells (Martinez et al., 2017a). It has still not been demonstrated how divisions are specified, but microtubule organization and nuclear positioning contribute: mutants with division-plane specification defects are discussed in the next section.

Cell geometry may account for many in vivo division-plane orientations, but other factors, such as local (Asada, 2013; Martinez et al., 2017a) or tissue-level mechanical stresses (Lintilhac & Vesecky, 1981; Louveaux et al., 2016) and developmental cues (Van Damme et al., 2011; Kajala et al., 2014; Yoshida et al., 2014; Walbot & Egger, 2016) can alter division-plane orientation. Indeed, factors that override geometry-based cell divisions are of great interest. Local mechanical stresses likely alter the division plane during avoidance of four-way junctions (Fig. 1a), when the location of the preprophase band (PPB), a structure that indicates the future division plane (discussed in the next section, Fig. 2a), shifts to avoid an adjacent, perpendicular cell wall or a neighboring PPB (Gunning et al., 1978; Flanders et al., 1990; Martinez et al., 2017a). PPB repositioning suggests cell–cell communication potentially mediated by mechanical cues, but this remains to be experimentally addressed. Another example occurs in shoot apical meristem boundary cells (Fig. 1b). These long, thin cells divide more slowly than adjacent cells. Boundary cell divisions occur along the long division plane in higher frequencies than expected based on the 2D Besson–Dumais shape-based model, reflecting division-plane alignment parallel to maximal stress (Louveaux et al., 2016). The observation is consistent with imposed mechanical stresses, such as laser ablation or wounding of adjacent cells causing microtubule arrays and corresponding division planes to realign parallel with maximal stress (Hush et al., 1990; Sampathkumar et al., 2014). In addition, when tobacco cells are plasmolyzed, they divide with higher frequencies along the long plane than would be predicted by the Besson–Dumais model, likely parallel to maximal stress (Asada, 2013). These exceptions indicate an urgent need to compare cell-shape and mechanical models to determine their relative contribution in division-plane selection in different tissues. Although little has been done yet to compare mutants with division-plane defects to model-based predictions (Yoshida et al., 2014), together their feedback will inform both future experiments and model refinements.

III. Establishing the division plane

Before the cell divides, several requirements must be met. The cell reaches a minimal size (Jones et al., 2017) and the nucleus migrates toward the center of the cell during symmetrical divisions (Wada, 2017) or to another location in asymmetrical divisions (Rasmussen et al., 2011a; Facette & Smith, 2012; Kimata et al., 2016). Interactions between cell-cycle regulators and proteins required for division-plane establishment (below) have been identified (Hush et al., 1996; Boruc et al., 2010; Spinnet et al., 2013; Costa, 2017). In the next sections, we focus on PPB form and function, but note that not all plant cells require a PPB for division-plane orientation. Examples of PPB-independent divisions include meiocytes (Otegui & Staehelin, 2004), endosperm (Brown & Lemmon, 2001) and some moss cells (Doonan et al., 1987; Kosetsu et al., 2017). Many PPB-independent divisions occur in invariant locations suggesting strong positioning cues. Discovering yet unknown positioning mechanisms may identify highly conserved features of plant cell division orientation.

The PPB is a microtubule and actin filament structure that assembles in G2 and aligns with the future division site (Fig. 2a, top left), (Rasmussen et al., 2013). PPB orientation often matches that of interphase microtubules (Gunning & Sammut, 1990). Multiple microtubule-associated proteins co-localize with the PPB (Li et al., 2015). This PPB subtends the cortical division zone (CDZ), a local region of the membrane (Smertenko et al., 2017; Van Damme et al., 2007). The CDZ is characterized by increased accumulation of clathrin-coated endocytotic vesicles (Karahara et al., 2009). As the PPB forms, increased interactions occur between actin filaments and microtubules (Takeuchi et al., 2016). Indeed, actin filament disruption by drugs or mutants induces both PPB widening and

![Fig. 1](image-url)
defects in division-plane orientation (Mineyuki & Palevitz, 1990; McDowell et al., 1996; Sano et al., 2005; Rasmussen et al., 2011a; Väskelä et al., 2017). PPB actin-microtubule interactions are possibly mediated by actin and microtubule-binding proteins that localize to the PPB, such as formins (Li et al., 2010), Myosin VIII (Wu & Bezanilla, 2014), or kinesins (Buschmann et al., 2011; Klitz & Nick, 2012; Schneider & Persson, 2015; Tian et al., 2015; Walter et al., 2015; Tseng et al., 2017; Yamada et al., 2017). The potential role of microtubule-actin crosslinking proteins in refining division-plane orientation or PPB narrowing is still unknown.

Proper PPB assembly and division-plane establishment requires a complex of conserved type 2A protein phosphatase subunits (PP2A), plant-specific proteins, and those similar to centrosomal proteins, called the TON1/TRM/PP2A (TTP) complex (Figs 3, 4) (Spinner et al., 2013). Key components of the TTP complex are identified by mutants with short, thick ‘barrel’ stature called tonneau (ton) (Camilleri et al., 2002; Azimzadeh et al., 2008) and fass (Torres-Ruiz & Jürgens, 1994). These mutants have cell elongation defects due to aberrant interphase microtubule array organization (Azimzadeh et al., 2008; Spinner et al., 2010; Kirik et al., 2012). In addition, cells do not form PPBs and have division-plane defects (Camilleri et al., 2002; Azimzadeh et al., 2008). fass is allelic to ton2, encoding a B’ regulatory subunit of the PP2A (Camilleri et al., 2002). Similar to fass, maize fass homologs discordia1 and alternative discordia1 together are required for PPB formation and their proteins localize to the division site until metaphase, potentially to promote specific protein dephosphorylation (Wright et al., 2009; Spinner et al., 2013). Other TTP components have conserved domains common to centrosomal proteins encoded by two highly similar genes tonneau1a (ton1a) and ton1b which together are required for PPB formation and interphase microtubule array organization. TON1 colocalizes with interphase microtubules and PPBs (Azimzadeh et al., 2008). Recently, an interaction between TON1 and many of a 34-member protein family containing a conserved motif named the TON1-recruiting motif (TRM) was identified (Drevensek et al., 2012). Several, but not all, TRM proteins bind microtubules and different TRM proteins interact with TTP proteins (Fig. 3) (Spinner et al., 2013). Specificity may be controlled by TRMs with different binding affinity for TTP members or microtubules. It is still unclear what proteins are de-phosphorylated and how that leads to proper interphase microtubule array organization and PPB formation.

One difficult question is whether interphase microtubule array organization can be functionally separated from PPB formation. Important insight has come from recent analysis of partial-loss-of-function mutants with more severe defects in PPB formation than apparent interphase microtubule array organization. These mutants display almost normal growth and mild division-plane orientation defects (Zhang et al., 2016; Schaefer et al., 2017). The ton1a single mutant lacks proper PPBs, yet many divisions were still properly oriented, especially in root cortex cells (Zhang et al., 2016). The triple trm6,7,8 mutant lacks proper PPBs but grows well (Fig. 2b, left panel, Schaefer et al., 2017). These three TRMs compose a small subfamily and encode about a quarter of TRMs with a probable microtubule-binding motif (Drevensek et al., 2012). Although the PPB does not form normally, Phragmoplast orienting kinesin1 (POK1, discussed in the next section) still localizes at the division site, although less often than in wild-type (WT) cells (Schaefer et al., 2017). The trm mutants lacking proper PPBs had aberrant spindle rotation and division-plane defects.

The PPB is thought to promote spindle bipolarity and prevent spindle rotation (Ambrose & Cyr, 2008). When the PPB forms, microtubules accumulate around the nucleus perpendicular to the PPB before metaphase. If the PPB does not form, microtubules accumulate nonspecifically around the nucleus (Camilleri et al., 2002; Chan et al., 2003; Azimzadeh et al., 2008; Schaefer et al., 2017), which delays spindle formation (Chan et al., 2003).
Establishment

Maintenance

Fig. 3 Accumulation of division site localized proteins required for establishment and maintenance of symmetrical plant cell divisions. This schematic representation of the cell cycle indicates key transitions, not the timing of the transitions. The position of cortical microtubule arrays (black) and DNA (gray) of plant cells is shown together with phases of the cell cycle. The localization of proteins that promote proper formation of the preprophase band (PPB) are listed under Establishment. TON1a (red) localizes to the interphase microtubule array, then the division site during prophase and part of metaphase (Azimzadeh et al., 2008). FASS/TON2/DCD1/ADD1 and TRM7 (orange) localize to the division site from prophase to metaphase (Wright et al., 2009; Spinner et al., 2013; Schaefer et al., 2017). TRM1 and TRM8 (green) localize to the interphase cortical array and the PPB (Drevensek et al., 2012; Schaefer et al., 2017), similar to many microtubule-binding proteins (Li et al., 2015). TAN1, POK1, POK2, KCBP, RAN-GAP and MAP65-4 (blue) localize to the division site from prophase through cytokinesis (Walker et al., 2007; Xu et al., 2008; Lipka et al., 2014; Buschmann et al., 2015; Li et al., 2017; Martinez et al., 2017b). PHGAP1 and PHGAP2 (indigo) localize to the division site from metaphase through cytokinesis (Stöckle et al., 2016). AIR9 (violet) localizes to the division site along the violet track, co-localizing with the interphase microtubule array, then co-localizing with the PPB. AIR9 localizes to the division site when the phragmoplast reaches the cortex (Buschmann et al., 2006).
Interestingly, in early gametophytic moss cells that do not make PPBs, spindle bipolarity is still anticipated by bipolar accumulation of cytoplasmic microtubule organizing centers to promote proper division-plane orientation (Kosetsu et al., 2017), similar to cytoplasmic microtubule organizing centers that accumulate before PPB formation in Marchantia polymorpha (Buschmann et al., 2016). Although altered spindle positioning may lead to division-plane defects, spindle rotation and other defects occur in many cells without division-plane defects (Rasmussen et al., 2013).

**IV. Maintaining the division plane during mitosis and cytokinesis**

Once a division plane has been established, information about its location must be maintained until the phragmoplast, a structure that helps direct assembly of the new cell wall (Lee & Liu, 2013; Smertenko et al., 2017), reaches the division site. Defects in division-plane maintenance are identified by comparing the division plane specified by the PPB to the placement of the new cell wall (Fig. 2b, right panel) (Rasmussen, 2016). When the final division occurs outside the PPB location, division-plane maintenance is defective. If the cell naturally does not form a PPB, division occurs outside the PPB location, division-plane maintenance defects and delays in mitotic progression may lead to division-plane defects (Wu & Bezanilla, 2014).

Two class XII kinesins, POK1 and POK2 localize to the division site throughout mitosis and cytokinesis (Lipka et al., 2014). The *pok1* *pok2* double mutant has short stature and misplaced cell walls (Müller et al., 2006). Time-lapse indicates that the phragmoplast does not return to the division site specified by the PPB (Lipka et al., 2014). A number of division-site localized proteins required for division-plane maintenance interact directly with POK1 (Fig. 3, discussed below).

A mutant identified in maize, *tan1*, has short stature and aberrant cell wall placement indicative of division-plane defects (Smith et al., 1996). *TAN1*, a microtubule-binding protein (Smith et al., 2001), localizes to the division site throughout mitosis and cytokinesis, making it the first identified positive division-site marker (Walker et al., 2007). In maize, *TAN1*-YFP also localizes to mitotic microtubule arrays (Martinez et al., 2017b). *TAN1* interacts with POK1 and its division-site accumulation is FASS-, PO2K1- and PPB-dependent (Walker et al., 2007; Rasmussen et al., 2011b; Martinez et al., 2017b). The maize *tan1* mutant has both division-plane maintenance defects and delays in mitotic progression (Fig. 2b) (Martinez et al., 2017b). A partially rescued *TAN1*-YFP line, which no longer localized to mitotic microtubule arrays, had significant mitotic progression delays but only minor defects in division-plane orientation. These plants grew normally, suggesting that division-plane orientation is critical for proper growth (Martinez et al., 2017b). *TAN1* may have separate functions in microtubule organization and division-plane orientation. It is
unclear whether defects in microtubule organization per se lead to
division-plane maintenance defects because many mutants with
general microtubule organization defects produce abnormal PPBs
(Rasmussen et al., 2013).
Auxin-induced-in-root-cultures 9 (AIR9), a microtubule-binding protein that colocalizes with the PPB, disappears from
the division site before metaphase later accumulating at the division site as the phragmoplast touches the cortex (Buschmann et al.,
2006). AIR9’s contribution to division-plane orientation remained elusive because air9 mutants have no obvious division-plane defects
(Buschmann et al., 2015) similar to very minor division-plane defects in Arabidopsis thaliana tan1 mutants (Walker et al., 2007).
Recently, a function in division-plane orientation was revealed for AIR9 using a tan1air9 double mutant (Mir et al., 2018). The
double mutant displays a synthetic phenotype: short plants with
division-plane defects, hypersensitivity to microtubule-depolymerizing
drugs, and root cell-file rotation (Mir et al., 2018). Around half of the divisions completed in a location different than the PPB, indicating a significant defect in division-plane maintenance. Surprisingly, tan1air9 double mutants have
unexpected interphase microtubule array organization defects leading to defects in cell elongation and aberrant root cell-file rotation. Although full-length TAN1-YFP rescued the double mutant, a TAN1-YFP protein lacking a domain required for its localization to the PPB (Rasmussen et al., 2011b) rescued every-thing but the cell-file rotation defect, potentially highlighting this domain’s function in interphase microtubule array organization
(Mir et al., 2018). TAN1 and AIR9 likely act in parallel pathways to promote division-plane maintenance and organize cortical microtubule arrays but the mechanisms are still unknown.
A pair of putative Rho-of-plants (ROP) GTase-activating-proteins (GAPs, ROP-GAPs) with pleckstrin homology (PH)
domains (PHGAPs) were identified via interaction with POK1. These proteins localize during interphase to the plasma membrane and appear at the division site during metaphase. Double phgap1 phgap2 mutants have minor defects in division-plane orientation (Stöckle et al., 2016). It is tempting to speculate that ROP proteins generally participate in division-plane orientation, in addition to their role in polarization during asymmetrical divisions (Humphries et al., 2011).
Several other proteins localize to the division site from prophase through cytokinesis (Xu et al., 2008; Buschmann et al., 2015; Li et al., 2017), but obvious roles in division-plane orientation cannot be assigned because mutants do not have division-plane defects. Kinesin-like calmodulin-binding protein (KCBP), a kinesin-14 with microtubule minus-end directed motility (Yamada et al., 2017), localizes to the division site (Buschmann et al., 2015). Another plant-specific microtubule-associated protein, MAP65-4, also localizes to the division site. MAP65-4 plays a semi-redundant function with MAP65-3 in phragmoplast assembly, possibly by crosslinking antiparallel microtubules, but its role in division-plane orientation is unknown (Li et al., 2017). Newly identified proteins with division-site localization suggests that we still have much to learn about the proteins required for division-plane maintenance.
The past few years have led to new insights. Computational
modeling approaches can be used to clarify relative contributions of
mechanics with geometry in division-plane orientation as well as the
nature of defects in known division-plane orientation mutants. New
players in division-plane establishment and maintenance (Figs 2–4), in addition to unanticipated connections between known proteins, lead to the hypothesis that establishment and maintenance are regulated by different protein modules. Many more proteins are likely required for division-plane establishment and maintenance, making this an exciting area for future research. Considering the tremendous recent progress, we expect to identify both new players and their interconnections to clarify this fundamental cellular process.

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