DIVISION PLANE CONTROL IN PLANTS: NEW PLAYERS IN THE BAND

Sabine Müller[1], Amanda J. Wright[2], and Laurie G. Smith[2]

[1]School of Biological Sciences, University of Auckland, Auckland 1142, New Zealand

[2]Section of Cell and Developmental Biology, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0116, USA

ABSTRACT

Unique mechanisms are used to orient cell division planes in plants. A cortical ring of cytoskeletal filaments called the preprophase band (PPB) predicts the future division plane during G2 and is disassembled as the mitotic spindle forms, leaving behind a "cortical division site" that guides the placement of the new cell wall (cell plate) during cytokinesis. The molecular features of the cortical division site have remained elusive for decades. Recently, a few proteins have at last been identified that are specifically localized to or excluded from the cortical division site and participate in the orientation, attachment or maturation of cell plates. Significant progress has also been made in identifying proteins needed for PPB formation and thus for division plane establishment.

INTRODUCTION

The positions of cells within plant tissues are fixed by cell walls. Consequently, plant cells must be formed in the positions where they are needed, requiring exquisite spatial regulation of the cell cycle and cell division planes. Mechanisms governing the orientation of division planes in plants appear to be different in many respects from those in other eukaryotes (see Box 1 for comparison, pp. 10-11). In plant cells, division planes are determined before mitosis. During G2, a band of cortical microtubules and actin filaments called the preprophase band (PPB) forms at the future division plane as the nucleus migrates into this plane (Figure 1), but the PPB is disassembled at the transition from prophase to prometaphase. Following chromosome segregation, cytokinesis is accomplished through the action of the phragmoplast (Figure 1), a microtubule and actin-based structure with structural and functional similarities to the mammalian spindle midzone/midbody [1]. Golgi derived vesicles deliver membranes and non-cellulosic polysaccharides along phragmoplast microtubules to the phragmoplast midzone, where they fuse to form the cell plate [2]. In somatic cells, the phragmoplast is initiated between daughter nuclei and expands laterally, guiding the growing cell plate to the former location of the PPB (the cortical division site), where it attaches to the parental cell wall (Figure 1).
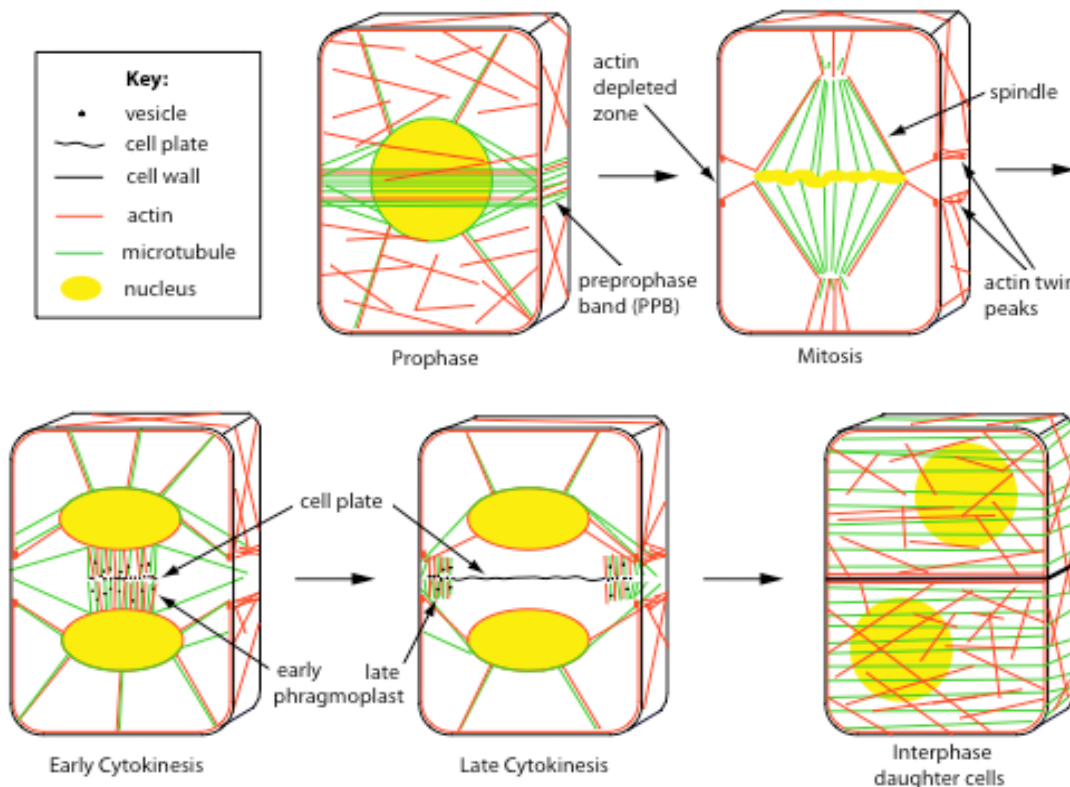

Figure 1: Cytoskeletal organization in dividing plant cells. Microtubules (green) and actin filaments (red) are illustrated at successive cell cycle stages in relation to nuclei/chromosomes (yellow) and the cell plate (black). Prophase and interphase are illustrated as whole cell views, while other stages are shown as mid plane cut-away views for clarity. The PPB forms during G2 at the future division plane and persists throughout prophase, disassembling as the

mitotic spindle forms at the transition to metaphase. Concomitantly, actin filaments are depleted from the PPB zone to create an "actin-depleted- zone" (ADZ), which persists until the conclusion of cytokinesis and is flanked by actin enriched regions of the cortex termed "actin twin peaks". The dividing nucleus is positioned within the division plane from prophase through the conclusion of cytokinesis and is connected to the cortex by microtubules and/or actin filaments depending on the cell cycle stage (filament types making these connections appear to vary somewhat among cell types, but the arrangements illustrated have been widely observed and most are discussed with references cited in the text). The phragmoplast is composed of opposing arrays of microtubules and actin filaments along with endoplasmic reticulum (ER), Golgi-derived vesicles and the evolving cell plate, but for simplicity the ER component is not shown. At the conclusion of cytokinesis, the cell plate becomes attached at the former site of the PPB.

Relatively little is known about mechanisms governing the establishment of the division plane or the guidance of expanding phragmoplasts to the cortical division site during cytokinesis. However, a variety of proteins have recently been implicated in these processes by their localization at (or exclusion from) the division plane and/or by functional studies. Recent progress has also been made in understanding mechanisms of cell plate formation along with phragmoplast assembly and dynamics. These topics have been addressed in other, recent reviews [3-5] and will not be discussed. Here, we focus on recent studies that have had an impact on our understanding the orientation of division planes in plant cells; readers may also wish to consult other recent reviews on this topic for discussion of aspects not covered here [6-8].

PPB FORMATION

Since its discovery over forty years ago, the PPB has been thought to play an essential role in division plane establishment [9,10] and recent studies have also established a role for the PPB in spindle assembly and orientation [11,12] (although the spindle does not determine the division plane in plant cells, proper spindle orientation facilitates subsequent orientation of the phragmoplast). Understanding how PPBs are formed and how their positions are determined is therefore central to understanding division plane control in plant cells. Most studies on PPB formation have focused on the microtubule component. As discussed in more detail in Box 2 (pp. 12-13), plant cells lack central microtubule nucleators like the centrosomes found in animal cells and their microtubules are nucleated at a variety of surfaces including the nuclear envelope and the cell cortex. During interphase, microtubules are distributed throughout the cell cortex, but at preprophase, they become restricted to the future plane of division via selective depolymerization of non-PPB microtubules [13] and/or selective stabilization of microtubules in the PPB zone [14]. A variety of proteins have now been identified that appear to participate in PPB formation by differentially regulating microtubule nucleation, dynamics and/or stability in the PPB zone.

Although plant cells lack centrosomes (see Box 2, pp. 12-13), plant proteins related to two animal centrosome proteins are essential for PPB formation. Plant cells lacking *Arabidopsis* FASS (also known as TON2) [15], or its maize homologues DCD1 and ADD1 [16], do not make PPBs and divide in abnormal orientations. These proteins are putative regulatory B'' subunits of the PP2A phosphatase complex, which are thought to target the complex to particular sub-cellular locations. The *C. elegans* homolog of FASS/DCD1/ADD1, RSA-1, localizes to the centrosome where it interacts with proteins that mediate microtubule outgrowth and stability [17]. DCD1 and ADD1 localize to the PPB (Figure 2) suggesting that PP2A-mediated protein dephosphorylation promotes the local assembly and/or stabilization of microtubules in the PPB zone of the cortex [16]. Unexpectedly, DCD1 and ADD1 persist at the cortical division site after PPB disassembly at least through metaphase (Figure 2), suggesting that these proteins may have other functions in addition to promoting PPB assembly [16]. Targets of
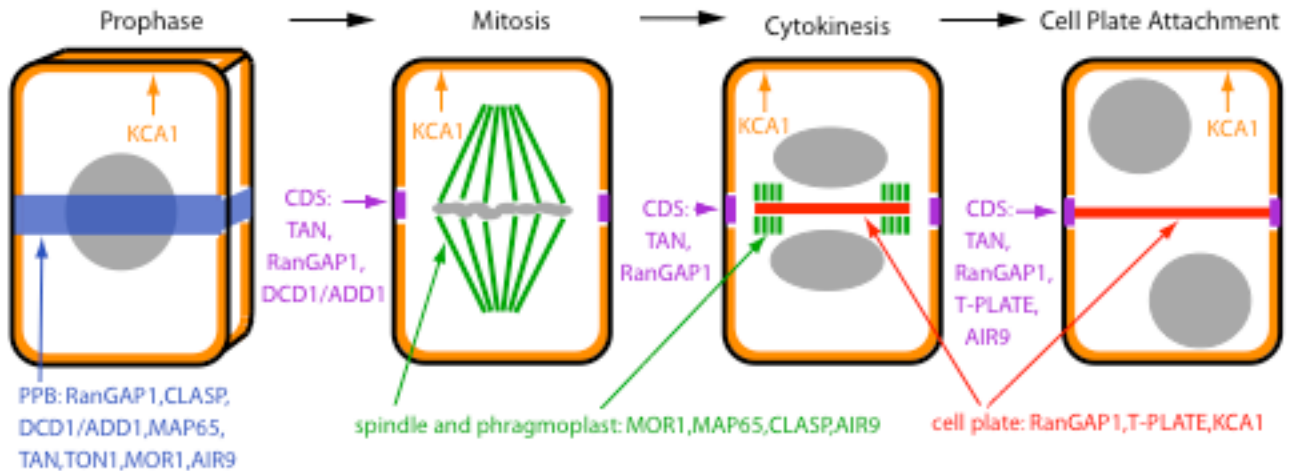
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Figure 2: Schematic illustration of PPB and/or cortical division site-localized proteins discussed in this review (not all proteins ever demonstrated to associate with mitotic microtubule arrays are shown). Prophase is illustrated as a 3-D projection, while other stages are shown as mid-plane cross sectional views for clarity. Each feature is color-coded and labeled to indicate the combination of proteins localized to that feature at the illustrated stages of cell division. Eight protein families (RanGAP1, CLASP, DCD1/ADD1, multiple MAP65s, TAN, TON1, MOR1 and AIR9) co-localize with PPBs (blue) during prophase while KCA1 (orange) is locally depleted in the PPB zone of the cortex. Four of the PPB-localized proteins (MOR1, MAP65s, CLASP and AIR9) are also associated with the spindle and phragmoplast (green), and three others remain at the cortical division site (CDS, purple): DCD1 and ADD1 are maintained there through metaphase whereas TAN and RanGAP1 are maintained there through the conclusion of cytokinesis. AIR9 and TPLATE become localized at the cortical division site just as the cell plate (red) is attaching there. DNA/nuclei are shown in gray.

FASS/DCD1/ADD1-dependent dephosphorylation are currently unknown, but the *Arabidopsis* proteins TON1a and TON1b are candidates to act in the same pathway since they are also required for PPB formation and co-localize with PPBs [18]. TON1a/b contain domains related to the human centrosomal proteins FOP and OFD1 and interact with the *Arabidopsis* homologues of another animal centrosomal protein, centrin [18]. Thus, plant relatives of animal proteins localized at discrete microtubule organizing centers function within a broad zone of the plant cell cortex to support PPB formation.

Several highly conserved microtubule-binding proteins have also been implicated in PPB formation. MOR1 is the plant homologue of animal XMAP215 [19]. Upon shifting a temperature-sensitive *mor1* mutant allele to restrictive temperature, half of the dividing cells fail to form PPBs and those that do form are often disorganized, indicating an important role for MOR1 in PPB formation [20]. Consistent with this observation, MOR1 localizes to PPBs along with other mitotic microtubule arrays in both *Arabidopsis* and tobacco cells (Figure 2) [20-22]. XMAP215 accelerates both microtubule elongation and shortening *in vitro* [23,24]. Consistent with these findings, analysis of microtubule dynamics in *mor1* mutants showed that MOR1 accelerates microtubule growth and shortening rates *in vivo*, but suggested that its most important role in promoting the formation of cortical microtubule arrays is to suppress pauses in microtubule dynamics – that is, to lengthen the time spent continuously growing or shrinking [25].

Another microtubule-binding protein implicated in PPB formation is the *Arabidopsis* homolog of animal CLASP, a regulator of microtubule dynamics [26]. *Arabidopsis* CLASP binds to microtubule plus ends as well as to discrete spots along microtubule walls, localizing to PPBs along with other

mitotic microtubule arrays (Figure 2) [27,28]. Demonstrating a role for CLASP in PPB formation/maturation, PPBs in *clasp* mutants tend to be disorganized and fail to narrow as wild-type PPBs do during prophase [27]. CLASP localized to the sidewalls of microtubules appears to mediate their interactions with the cell cortex [29]. Thus, CLASP could promote PPB organization and narrowing via modulation of microtubule dynamics in the PPB zone and/or by mediating microtubule-cortex interactions.

MAP65 is a third microtubule-binding protein implicated in PPB formation. Nine different MAP65 proteins have been identified in *Arabidopsis* and some have been shown to localize to the PPB and other mitotic microtubule arrays (Figure 2) [30-33]. MAP65s bundle microtubules by forming cross bridges between overlapping microtubules and thus could potentially stabilize PPB microtubules via bundling [30,32,34,35]. Not surprisingly in view of the potential for functional redundancy among members of the MAP65 family, no function for a MAP65 in PPB formation has yet been demonstrated genetically.

Animal XMAP215 [36] and CLASP [37,38] are regulated by phosphorylation so their plant homologs are potential targets of FASS/DCD1/ADD1-dependent phosphatase activity. Moreover, phosphorylation of plant MAP65-1 downregulates its microtubule bundling activity and is required for timely progression through mitosis and cytokinesis [39-41]. Although modulation of PPB-associated MAP65 bundling activity by phosphorylation has not been demonstrated, phosphatase activity in the PPB zone could potentially contribute to PPB assembly by upregulating the microtubule bundling activity of MAP65. Further studies will be needed to determine whether any of these microtubule-binding proteins are regulated by FASS/DCD1/ADD1-dependent phosphatase activity.

HOW DO DIVIDING CELLS 'REMEMBER' THE CDS DURING MITOSIS AND CYTOKINESIS?

A variety of observations have indicated that after the PPB is disassembled, some type of "memory" of its location remains throughout mitosis and cytokinesis. For example, if the spindle or early phragmoplast is displaced from the plane of the former PPB either by experimental manipulation or by spontaneous spindle rotation, phragmoplasts bend, rotate or migrate as they expand so that the cell plate becomes attached at the former PPB site [9,10]. Recent work has added significantly to our knowledge about the nature of the cortical division site that persists after PPB disassembly.

Negative Markers of the Cortical Division Site

For many years, the only known marker of the cortical division site during mitosis and cytokinesis was the "actin depleted zone" (ADZ) of the cell cortex created when both actin and microtubule components of the PPB are disassembled while cortical actin remains elsewhere (Figure 1) [42-44]. More recently, tobacco BY-2 cells expressing a fimbrin actin binding domain (ABD2) fused to GFP were described as having cortical actin "twin peaks" – bands of high actin density flanking the cortical division site (Figure 1) [45]. A recent review stresses the point that the ADZ should be viewed as a zone of low actin abundance rather than complete loss of the filaments [46]. The significance of the ADZ for division plane control has been difficult to analyze. Treatment of dividing cells with actin depolymerizing drugs causes cell plates to be misoriented, but as there are many potential roles for F-actin in division plane orientation, these studies have not definitively established a function for the ADZ. When semi-synchronized tobacco BY-2 cells were treated with actin-depolymerizing drugs at different points in the cell cycle, maximal disruption of cell plate orientation was observed when the drugs were present during prophase/metaphase and then washed out before cytokinesis. Application of the drug only

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during cytokinesis had almost no effect on cell plate orientations [44,45]. These studies suggest that the presence of an ADZ during cytokinesis is not critical for phragmoplast guidance, but that the ADZ and/or PPB F-actin plays an important role in the establishment of the cortical division site.

The *Arabidopsis* kinesin KCA1 is as a second negative marker of the cortical division site. In tobacco BY-2 cells, GFP-KCA1 localizes to the plasma membrane and cell plate. Like cortical F-actin, it is locally depleted at the cortical division site during mitosis and cytokinesis, creating a "KCA1 depleted zone" or KDZ (Figure 2) [47]. KDZs and ADZs coincide, but the KDZ appears to form earlier, as it is already present in cells with PPBs. Maintenance of the KDZ is not affected by microtubule or actin depolymerizing drugs applied during mitosis or cytokinesis. However, KDZs were no longer seen when early PPB microtubules were depolymerized. After drug washout, most cells reformed both a PPB and a KDZ, but some failed to reconstitute a PPB or a KDZ, suggesting that KDZ formation depends on the microtubule PPB [47]. Given that the cortex is devoid of microtubules during mitosis and cytokinesis, a plausible role for cortically localized KCA1 is to mediate interaction with microtubules that link the dividing nucleus to the cortex during cytokinesis, described later.

Recent work has established that endocytic vesicles form more frequently in the PPB zone than in other areas of the cell cortex, suggesting that endocytosis could be important for establishment of the division plane [48,49]. It would be interesting to know whether creation of ADZs or KDZs depends on selective depletion of actin and KCA1 from the PPB zone via endocytosis.

Positive Markers of the Cortical Division Site

More recently, two proteins, TAN and RanGAP1, have been identified as positive markers of the division plane, continuously localizing there from preprophase through the completion of cytokinesis. Analysis of *tan* mutants of maize demonstrated an important role for TAN in guidance of expanding phragmoplasts to former PPB sites [50]. TAN is distantly related to the basic, microtubule-binding domain of vertebrate adenomatous polyposis coli (APC) proteins and binds microtubules *in vitro* [51]. *Arabidopsis* TAN-YFP co-localizes with PPBs in preprophase/prophase cells (Figure 2) [52]. After disintegration of the PPB, TAN-YFP rings remain at the division site through the completion of cytokinesis and then rapidly disappear. Initial recruitment of TAN-YFP requires microtubules, but maintenance of already formed rings does not. TAN-YFP fails to form cortical rings in *fass* mutants, which do not form PPBs. This may simply reflect the dependence of TAN localization on PPBs. Alternatively, TAN might depend more directly on FASS for its proper localization, perhaps requiring FASS-dependent dephosphorylation for its localization at the cortical division site. Consistent with this possibility, one TAN phosphorylation site was recently identified in a survey of *Arabidopsis* phosphoproteins [53] and many other potential phosphorylation sites are found throughout the TAN protein sequence, but the impact of TAN phosphorylation on its localization has not yet been analyzed. As described earlier for maize *tan* mutants, *Arabidopsis tan* mutants form normal PPBs that are correctly oriented, but some phragmoplasts are not guided back to former PPB sites, resulting in misoriented cell divisions [52]. Together, these results clearly implicate TAN as a functional component of the cortical division site, which may interact directly or indirectly with cytoskeletal filaments linking the expanding phragmoplast to the cortex.

Arabidopsis RanGAP1 also positively marks the cortical division site [54]. RanGAP1 is a negative regulator of the small GTPase Ran, whose functions in nucleocytoplasmic transport during interphase and in several aspects of mitosis are well documented in animal cells [55]. Like TAN, RanGAP1 is recruited to the division plane in a FASS-dependent manner, colocalizing with the PPB and remaining at the cortical division site throughout mitosis and cytokinesis (Figure 2) [54]. Unlike TAN,

RanGAP1 is also localized elsewhere in dividing cells including the cell plate (Figure 2). Inducible disruption of RanGAP1 and its close relative RanGAP2 results in occasional misoriented and incomplete divisions revealing function(s) for RanGAPs during cytokinesis, but these mutants were not analyzed for structural or positional defects in cytoskeletal arrays in dividing cells [54]. Thus it is not yet clear whether RanGAPs are required for PPB assembly or disassembly (a role that would be consistent with known functions for RanGTPases in microtubule nucleation during mitosis in animal cells), or for phragmoplast guidance during cytokinesis (a role that would fit with RanGAP1 localization at the cortical division site throughout mitosis and cytokinesis).

A closely related pair of *Arabidopsis* kinesins, POK1 and POK2 (one of which, POK1, interacts with TAN and RanGAP1 in yeast), are required for the correct localization of TAN and RanGAP1. Although neither *pok1* nor *pok2* single mutants have obvious defects, *pok1;pok2* double mutants exhibit a high frequency of misoriented (but complete) cell divisions in all tissues analyzed including embryos and root tips [56]. Like *tan* mutants of maize and *Arabidopsis*, PPBs are formed in *pok1;pok2* double mutants, but phragmoplasts are not consistently guided back to former PPB sites. Whether POK1 and POK2 interact directly or act in redundant pathways is not known. Based on the double mutant phenotype, it seems that POK1 and 2 together are needed for localization of TAN to the PPB and cortical division site, suggesting that TAN becomes associated with the division plane as cargo of POK1 and POK2 [52]. In contrast, RanGAP1 does not require POK1 and/or POK2 for co-localization with PPBs, but does require these kinesins for its maintenance at the cortical division site after PPB disassembly [54]. To explain this, the authors propose that POK1 and POK2 might be responsible for the deposition of factors at the PPB site during prophase that later maintain RanGAP1. However, maintenance of RanGAP1 rings does not appear to require TAN, the only other known POK1 and POK2-dependent cortical component [54]. Moreover, the yeast two-hybrid interaction observed between RanGAP1 and POK1 [54] would seem to suggest a more direct role for POK1 and POK2 in maintenance of RanGAP1 at the cortical division site after PPB disassembly. This intriguing notion challenges the view of the cortical division site as something that is built through the action of the PPB during prophase and simply held in place after the PPB breakdown, suggesting instead that cortical division site components may be continually delivered to the site after PPB disassembly via a POK1/2-dependent mechanism.

A ROLE FOR MICROTUBULES IN PHRAGMOPLAST GUIDANCE

The expanding phragmoplast interacts with the mother cell cortex during cytokinesis to attach the cell plate at the cortical division site. Most studies investigating this interaction have focused on actin filaments that link the phragmoplast to the cell cortex (Figure 1) [45,57,58]. However, as discussed previously, selective application of actin depolymerizing drugs at different cell cycle stages has suggested that the most important contribution of F-actin to the spatial regulation of cytokinesis occurs prior to cytokinesis [44,45]. With this in mind, it is particularly interesting that recent work has suggested a previously unsuspected role for microtubules in phragmoplast guidance. In living preprophase/prophase cells, microtubules labeled at their plus ends with EB1::GFP grow out from the nuclear surface in all directions, contacting the PPB and other areas of the cortex (Figure 1) [11,48]. Pharmacological studies support the view that these microtubules position the nucleus in the plane of the PPB [8]. Spindle-radiating microtubules are short and few in number during metaphase, but become longer and increasingly abundant as cells progress through anaphase (Figure 1). During telophase, microtubules were observed to connect daughter nuclei to the cortex mainly at the cell poles in *Arabidopsis* tissue culture cells [11] but made frequent contacts at the cortical division site as well in tobacco BY-2 cells [48] (Figure 1).

Microtubules linking the dividing nucleus to the cortex in plant cells have been likened to the astral microtubules that interact with the cortex to position the spindle in dividing animal cells [59], and could play an important role in orienting the expanding phragmoplast during cytokinesis [11,48]. This hypothesis is difficult to test by a pharmacological approach since microtubules are essential for other aspects of mitosis and cytokinesis, although studies using low doses of microtubule-depolymerizing drugs to selectively destabilize astral microtubules in animal cells [60,61] suggest that this might be a successful approach to investigate the significance of plant astral-like microtubules in dividing plant cells. Identification of proteins that participate in microtubule-dependent aspects of phragmoplast guidance would also provide a handle on understanding the role of this microtubule population. As discussed earlier, the *Arabidopsis* kinesins POK1 and 2 in combination are required for the proper localization of two cortical division site components (TAN and RanGAP1), but POK1 and/or POK2 could also mediate microtubule-dependent interactions between the dividing nucleus and cell cortex during cytokinesis, perhaps via direct interaction with TAN and/or RanGAP1. Astral-like microtubules might also interact with KCA1 in the cell cortex during cytokinesis to help guide phragmoplast expansion to the cortical division site.

THE FINAL STEP: CELL PLATE ATTACHMENT

Classic experiments demonstrated that when the expanding cell plate was forced experimentally to attach to the mother cell surface somewhere other than the cortical division site, the new cell wall failed to mature normally, suggesting that cell plate interaction with the cortical division site or adjacent cell wall promotes proper wall maturation [62,63]. A recently identified microtubule-associated *Arabidopsis* protein, AIR9, has been implicated in this interaction [64]. In tobacco BY-2 cells, GFP-AIR9 localizes to cortical microtubules in interphase and to the PPB in prophase (Figure 2). A weak GFP-AIR9 signal is associated with the spindle during mitosis, and a stronger signal with the phragmoplast during cytokinesis. GFP-AIR9 disappears from the cell cortex when the PPB is disassembled, but reappears at the cortical division site upon contact of the phragmoplast/cell plate with this site (Figure 2). Shortly thereafter, GFP-AIR9 becomes distributed diffusely across the cell plate, and as microtubules start populating the adjacent cell cortex, GFP-AIR9 assumes a filamentous appearance, resembling microtubules. Drug treatment of dividing cells that did not interfere with PPB formation but caused the phragmoplast/cell plate to branch as it expanded showed that branches contacting the cortical division site elicited GFP-AIR9 accumulation while those encountering other areas of the cortex did not [64]. Thus, AIR9 is recruited specifically to the cortical division site when the cell plate comes into contact with it and its subsequent dispersal across the cell plate may promote wall maturation, perhaps via a microtubule-dependent mechanism.

Recent work has also implicated the *Arabidopsis* protein TPLATE in cell plate attachment [65]. TPLATE contains domains shared with adaptins and β-COP coat proteins, which are involved in vesicle formation [66]. Knock down of TPLATE function via RNAi in *Arabidopsis* resulted in cytokinesis defects including misoriented and incomplete cell walls. In BY2 cells, TPLATE knock down caused cell plates to have diffuse edges that did not attach efficiently to the mother wall. Consistent with a role in cell plate attachment, TPLATE-GFP localizes to the cell plate and accumulates at the cortical division site immediately prior to cell plate attachment (Figure 2). Based on these observations the authors suggest a role for TPLATE in vesicle trafficking events leading to site-specific cell wall modifications needed for cell plate anchoring [65]. With this hypothesis in mind, it is striking that the localization pattern and loss of function phenotype observed for TPLATE are similar to those reported earlier for RSH, a hydroxyproline-rich glycoprotein of *Arabidopsis* [67]. Thus, TPLATE might facilitate cell plate attachment by promoting the localized deposition of RSH into the cell wall.

CONCLUDING REMARKS

In the past few years, a variety of proteins have been implicated by localization and/or functional studies as participants in the spatial regulation of cytokinesis in plant cells. FASS and its maize homologs DCD1 and ADD1 along with MOR1, CLASP and MAP65s appear to participate in PPB formation via regulation of microtubule nucleation, dynamics and/or stability within the PPB zone of the cortex. Proteins defining the cortical division site after PPB disassembly by their presence (TAN and RanGAP1) or absence (KCA1) are implicated as components of the pathway(s) mediating microtubule- and/or actin-dependent interactions between the expanding phragmoplast and the cortex that guide the phragmoplast to the former PPB site. AIR9, TPLATE and RSH are implicated in cell plate attachment and/or maturation at the conclusion of cytokinesis. As exciting as it is to have in hand a few of the players in division plane control, scores of others undoubtedly remain to be identified. Indeed, the paucity of proteins implicated in this process to date suggests that many such regulators might be needed for viability or fertility, or that functional redundancy impedes their discovery via forward genetics. Thus, other approaches such as proteomics and creative genetic strategies that circumvent the problems of redundancy and pleiotropy may be needed to expand the inventory of players and to determine their functions in somatic cell division. In addition to further advancing our understanding of the roles played by the division plane regulators already identified, another important challenge for future research will be to understand their functional relationships.

ACKNOWLEDGEMENTS

Box 1: Division Plane Determination: A Comparison Among Eukaryotes

Placement of the division plane involves interactions between the nucleus and cortex in all eukaryotes. Although the timing of the interactions and the proteins involved vary, certain mechanistic themes can be identified including an important role for microtubules [68,69].

In animal cells, cortical cues [59] and/or cell shape [70] orient the premitotic nucleus. During mitosis, the spindle in turn communicates its position to the cortex to dictate the location of the contractile ring (Figure I). The mechanism by which this communication occurs is still being actively investigated, but spindle microtubule-dependent local regulation of the activity of a cortically-localized Rho GTPase appears to play a central role [69,71].

In the fission yeast *S. pombe*, the division plane is determined during prophase by the position of the nucleus (Figure I), which becomes centered by a microtubule-dependent mechanism [68]. The anillin-like protein Mid1p is then delivered from the nucleus to the cortex, becoming localized to the future division plane through the action of multiple polarity-promoting proteins [72-74] and subsequently recruiting other contractile ring components [75].

In the budding yeast *S. cerevisiae*, the division plane is determined at the beginning of the cell cycle by landmark proteins left behind at the previous site of cytokinesis (in the axial budding pattern illustrated in Figure I, the next bud will be initiated adjacent to this "bud scar") [68]. During mitosis, astral microtubules interact with actin cables reaching into the bud and cortical proteins within the bud to center the spindle at the mother-bud neck, where the contractile ring is assembled [76,77].

As described in more detail in the Introduction, cytokinesis in plant cells does not involve membrane contraction, but instead is achieved via construction of a new cell wall (cell plate) between daughter nuclei (Figure I). The position where the new cell plate will become attached to the parental wall is predicted during G2 by a cortical preprophase band (PPB) encircling the nucleus [9]. Nuclear position has been shown in some but not all cases to influence the placement of the PPB [78-81]. Other factors including cell polarity, cell geometry and extracellular cues can also play a role, but the mechanisms by which nuclear position and other factors impact division plane selection are completely unknown [8]. The spindle forms with its axis perpendicular to the plane of the PPB and usually remains in this orientation and centered at the division plane throughout mitosis, but the spindle does not determine the division plane. As discussed in the text, the expanding cell plate is guided to the former PPB site even if the spindle is displaced from the division plane.
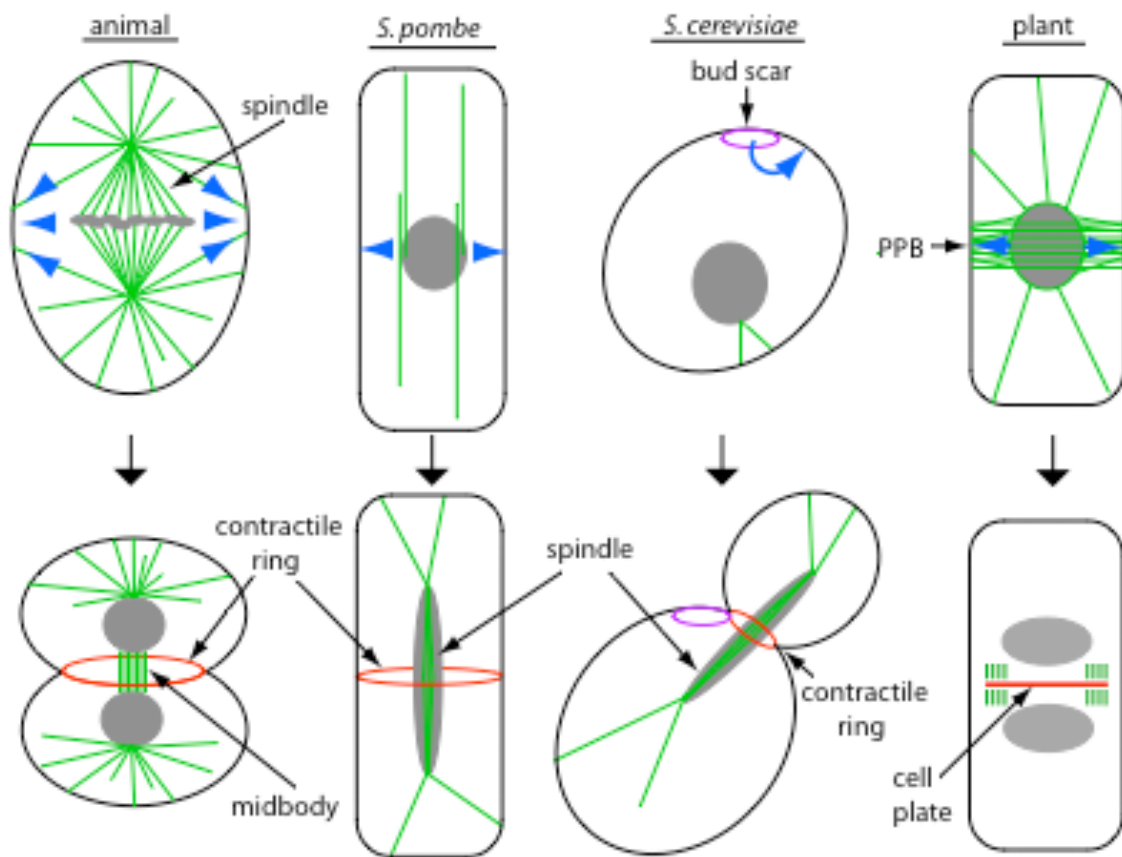
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Figure I: Schematic illustration of animal, yeast (*S. pombe* and *S. cerevisiae*) and plant cells at the cell cycle stage when the division plane is determined (top line), with blue arrows/arrowheads connecting the source of determining factors to the future site of the division plane (note that in plant cells, the nucleus is not the sole source of such determinants). The bottom line depicts cells initiating or undergoing cytokinesis with the contractile ring or cell plate shown in red. Green, microtubules; gray, DNA or nucleus; purple, bud scar.

Box 2: Plant Microtubules: Dynamics, Nucleation and Organization

Microtubules are polar, linear polymers of alpha/beta tubulin heterodimers, and are key elements in cellular processes such as intracellular transport and cell division. As in animal cells, microtubules in living plant cells exhibit "dynamic instability" characterized by periods of rapid elongation alternating with periods of rapid shortening at their plus ends [82] (Figure II, A). Plant microtubules also exhibit "treadmilling" characterized by sustained growth at the plus end accompanied by depolymerization at the minus end [82] (Figure II, B). Investigations of microtubule dynamics in living plant cells have focused mainly on interphase cortical microtubules (illustrated in FigureII, D) in part because the high density of microtubules in mitotic arrays (PPBs, spindles, and phragmoplasts) makes their dynamic behavior more difficult to analyze.

In animal cells, microtubules are nucleated at discrete microtubule organizing centers called centrosomes. Plant cells lack centrosomes, and their microtubules are nucleated at a variety of sites within the cell. In interphase, most microtubules are nucleated at widely dispersed sites within the cell cortex, which are frequently associated with the side walls of pre-existing microtubules [83], but are also found in vacant cortical regions [82,84] (Figure II, C). The nuclear surface becomes a prominent site for microtubule nucleation in premitotic and prophase plant cells (e.g. [48]) while microtubule nucleation sites in mitotic and cytokinetic cells are less well characterized. Gamma tubulin, which appears to play a key role in microtubule nucleation in plant cells as it does in animal and yeast cells [85,86], is focused initially at spindle poles but later becomes more broadly distributed within the spindle, and is also broadly distributed within phragmoplasts, suggesting dispersed microtubule nucleation sites within these mitotic arrays [87]. Interestingly, non-centrosomal microtubules also exist in non-plant eukaryotes but have received little attention until recently [88].

Organization of microtubules into higher order arrays within plant cells is accomplished by their self-organizing capability and regulated by a number of associated proteins that stabilize, destabilize, sever or crosslink microtubules [89]. In interphase plant cells microtubules are generally arranged in cortical bundles that are predominantly aligned perpendicular to the growth axis of the cell (Figure II, D). Encounter of a growing microtubule plus end with an existing microtubule at an angle below 40° promotes bundling, while encounters at larger angles commonly result in local severing and disassembly of the resulting shorter microtubules [90] (Figure II, E). Assembly and disassembly of the diverse microtubule arrays found in plant cell undergoing mitosis and cytokinesis (Figure 1) is only beginning to be understood.
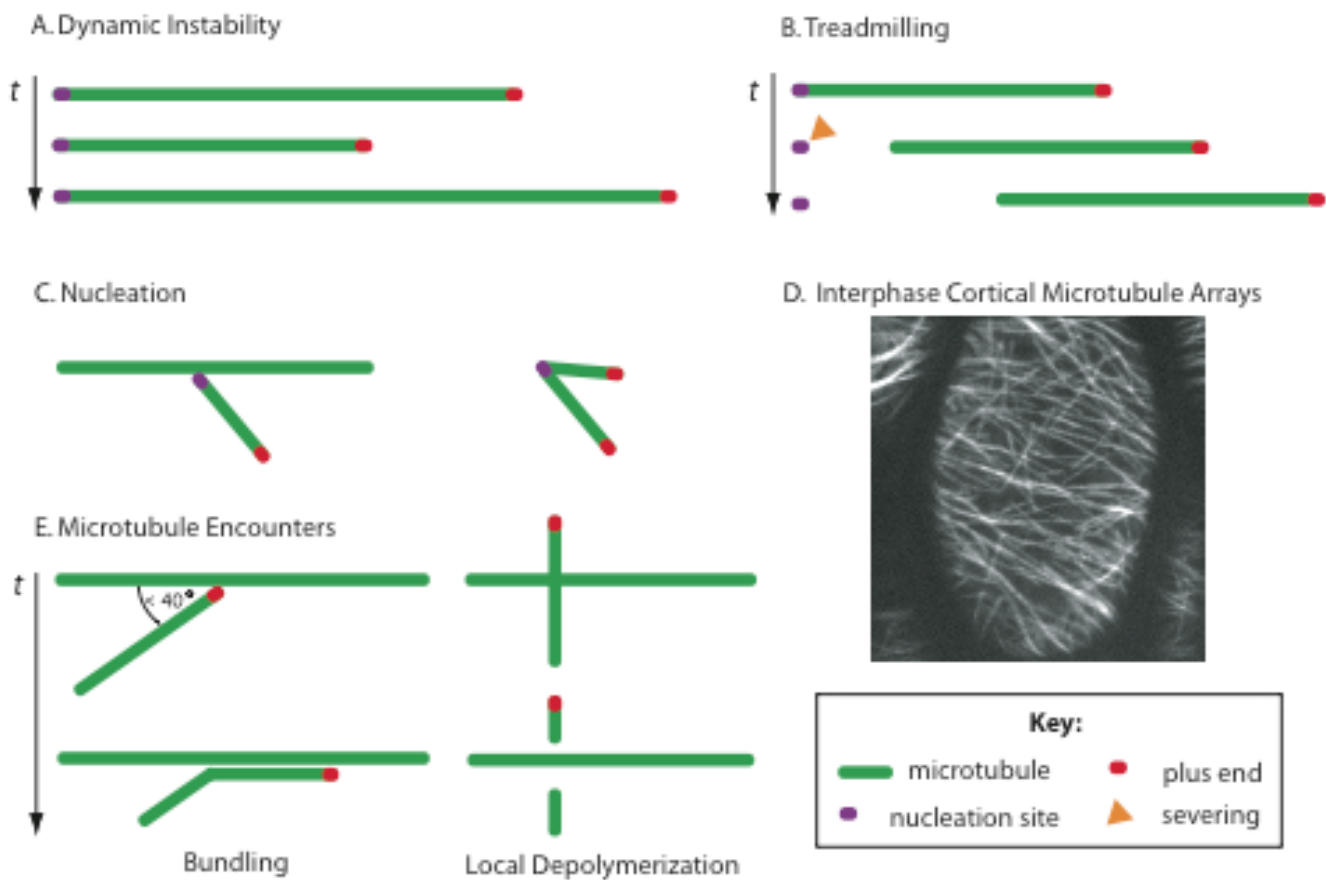
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Figure II: Dynamics and origin of non-centrosomal plant microtubules.
Common observations of microtubule behavior and rules of microtubule encounters are depicted.
Microtubules (green) exhibit intrinsic polarity; the fast-growing end (red) is defined as the plus end and
the depolymerizing or slow-growing end is defined as the minus end. *t* time, blue, nucleation site;
yellow, severing event. Adapted from [88].

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