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Phragmoplast expansion: the four-stroke engine that powers plant cytokinesis

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The phragmoplast is a plant-specific secretory module that partitions daughter cells during cytokinesis by constructing a cell plate from membranes and oligosaccharides. The cell plate is typically a long structure, which requires the phragmoplast to expand to complete cytokinesis. The phragmoplast expands by coordinating microtubule dynamics with membrane trafficking. Each step in phragmoplast expansion involves the establishment of anti-parallel microtubule overlaps that are enriched with the protein MAP65, which recruits cytokinetic vesicles through interaction with the tethering factor, TRAPPII. Cell plate assembly triggers dissolution of the anti-parallel overlaps and stabilization of microtubule plus ends through association with the cell plate assembly machinery. This opinion article discusses processes that drive phragmoplast expansion as well as highlights key questions that remain for better understanding its role in plant cell division.

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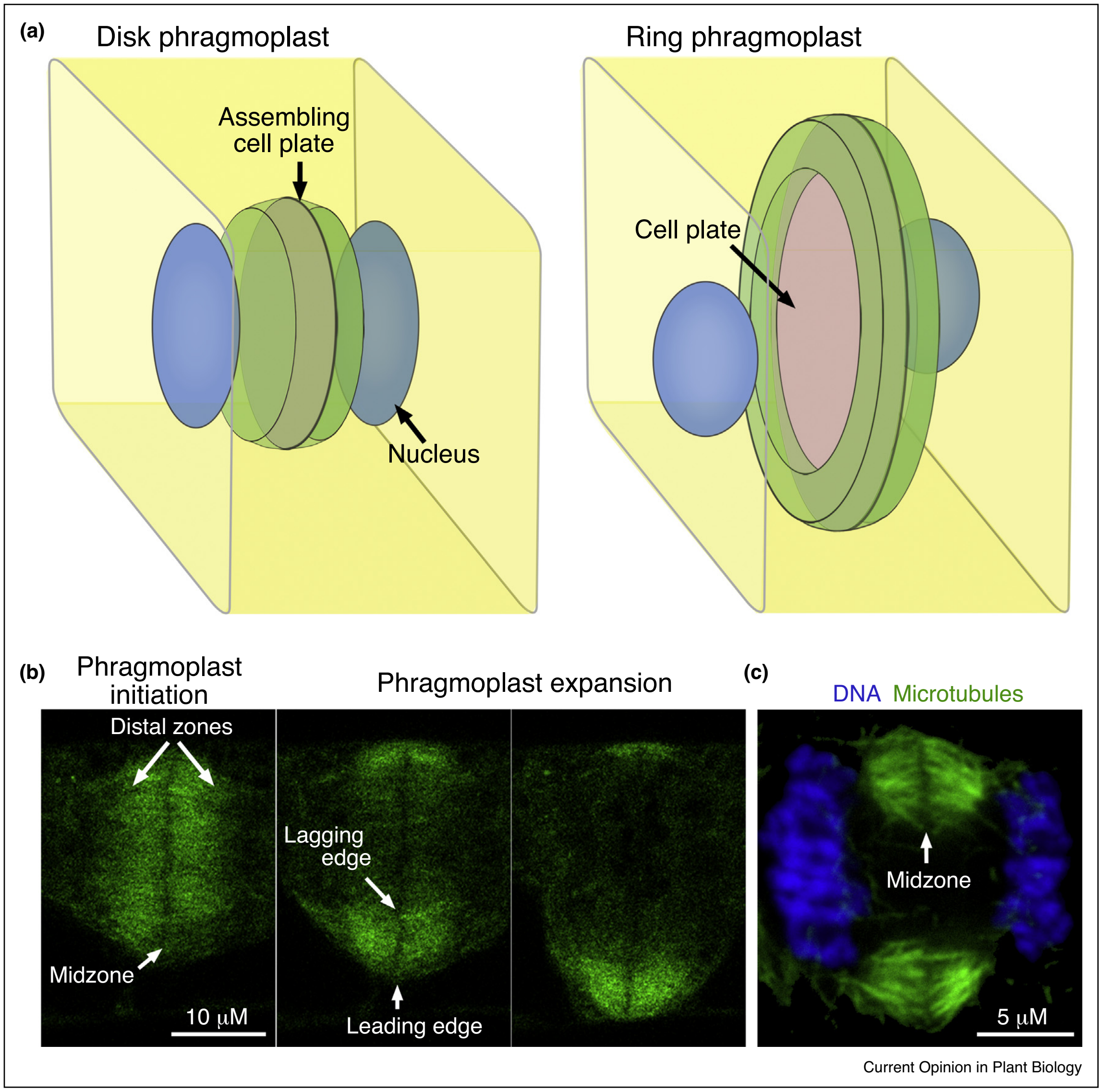
Introduction: phragmoplast expansion

The colonization of land by plants towards the end of Silurian period (444–420 mya) launched a new era in the natural history of Earth, which changed the face of our planet and ultimately resulted in the evolution of humans. The transition of plants from the marine environment to the terrestrial one would be impossible without vascular tissues, which are remarkable in that they are produced through uncommonly long cell divisions (up to 1 mm) from cambium meristem cells. What is more, these cells divide across their longest axis [1–3]. This phenomenon contrasts divisions that occur in apical meristems, which span the shortest cell axis, and breaks the rule postulated in the 19th century by Hofmeister and Léo Errera (reviewed in [4]). In addition to the vasculature, long divisions also take place during the transition from 8 to 16 cells in the embryonic stages of *Arabidopsis* [5]. These long divisions, as well as many other divisions throughout plant development, are only possible because plant-specific cytokinetic machinery, termed the *phragmoplast*, is capable of directional expansion (Figure 1).

The backbone of the phragmoplast is made of microtubules [6], which function together with microfilaments, membranes, and numerous associated proteins in cell plate assembly [7,8]. The phragmoplast is initially positioned between the daughter nuclei during the first round of cell plate construction (Figure 1a). Once the cell plate begins to mature, the microtubules depolymerize. At this point, cell plate appears as a tubular network of membrane compartments enriched with callose [8,9]. Depolymerization of the microtubules is accompanied by the loss of other phragmoplast components. New microtubules are then polymerized at the edge of the cell plate, which makes phragmoplast appear ring-like [10,11]. Subsequent recruitment of other phragmoplast components initiates the next round of cell plate assembly.

Microtubules polymerize at the cell plate edge (phragmoplast *leading zone*) and depolymerize at the sites where cell plate assembly has advanced to the stage of developing the tubular network (*lagging zone*; reviewed in [12]). Along the longitudinal axis, the phragmoplast comprises all successive stages of the nascent cell plate assembly including vesicles delivery, fusion, oligosaccharide deposition, and membrane remodelling [8]. Observationally, it is clear that the coordination of microtubule dynamics with assembly of the cell plate is a driving force for phragmoplast expansion, though the mechanism(s) behind this coordination remain unknown. Solving complexity of the phragmoplast expansion requires mathematical modelling and computer simulations, however progress toward this goal is slow due to: firstly, lack of coherent theoretical model for the phragmoplast expansion; secondly, incomplete list of phragmoplast proteins and gaps in understanding functions of known phragmoplast proteins; and finally, limited number of quantification tools and thus numerical parameters of the phragmoplast expansion. This opinion piece summarizes the available data on this topic and addresses the gaps in knowledge with speculations on the events in the midzone that comprise the 'engine' for phragmoplast expansion. The stages that precede the expansion phase, including phragmoplast establishment, are reviewed elsewhere [7,11,13].

Figure 1



Current Opinion in Plant Biology

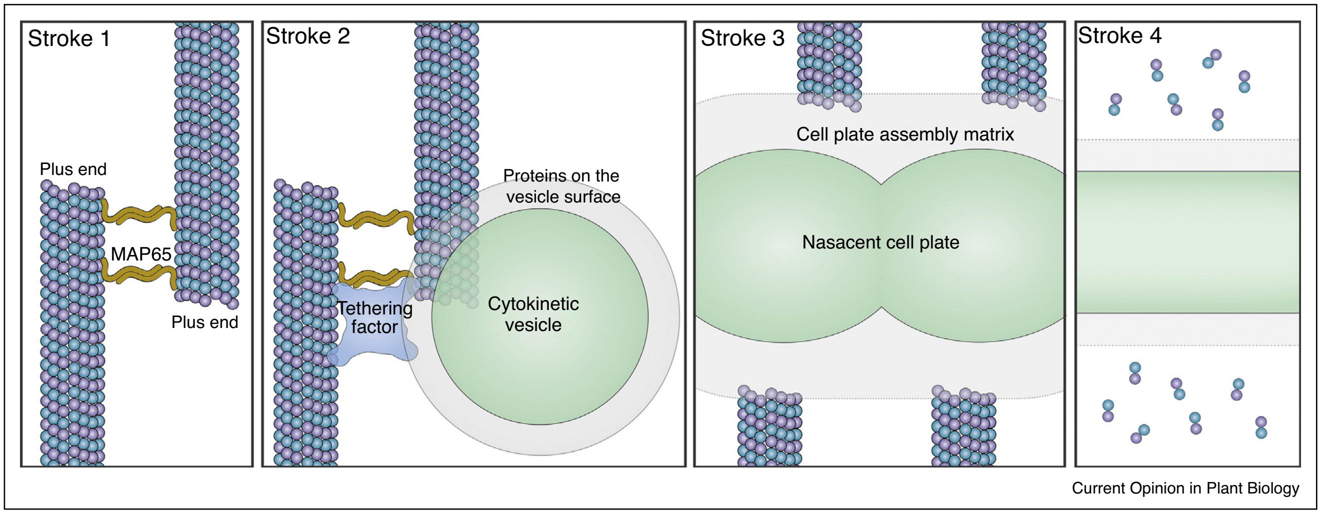
Phragmoplast expansion. **(a)** Completion of cell plate synthesis in the disk phragmoplast triggers depolymerization of microtubules and re-polymerization at the phragmoplast outer edge. Ring phragmoplast continues expansion until it reaches parental cell wall. **(b)** Phragmoplast expansion in cells expressing GFP-tubulin imaged in the single optical plane using confocal microscope. Phragmoplast vanishes after attachment to the parental cell wall. Midzone remains dark as GFP is excluded from this region. **(c)** Expanding phragmoplast stained with anti-tubulin (green) and DNA dye DAPI (blue). Phragmoplast midzone lacks staining and appears dark.

Stroke 1: establishment of anti-parallel microtubule overlaps

The majority of microtubules polymerize inward from the phragmoplast distal zones towards the midzone. Consequently, microtubules originating from opposing distal zones have reversed polarity: that is, their minus ends orient toward the distal zone, whereas their plus-ends orient toward the midzone. The establishment of such orientation is supported by both electron microscopy analysis, live-cell imaging, and *in silico* modelling [14–17]. *In vitro* experiments have determined that a microtubule-associated protein, MAP65, preferably binds anti-parallel microtubules that have come into contact with one-another in the midzone (Figure 2; [18,19]). This finding is complemented by work *in vivo*, which has shown that several members of the MAP65 gene family accumulate in the phragmoplast midzone [20–23], and can stabilize microtubules (MAP65-3; [22]) in addition to governing their spatial organization.

In animal systems, the anti-parallel microtubule overlaps can be also recognized by Group 4 kinesins, which can

Figure 2



Four stages (strokes) of the phragmoplast expansion. Anti-parallel microtubules from the opposite distal zone are cross-linked by MAP65 in the midzone during Stroke 1. In Stroke 2 MAP65 recruits cytokinetic vesicles to the midzone through interaction with vesicle-tethering factors. Cell plate biogenesis is accompanied by formation of cell plate assembly matrix around nascent cell plate in Stroke 3. At this stage the anti-parallel overlaps disappear and microtubules form attachments to the cell plate assembly matrix. Microtubules depolymerize during Stroke 4 and monomeric tubulin becomes recycled for polymerization of new microtubule in the leading zone.

reduce the length of MAP65/PRC1 microtubule overlap *in vitro* by inhibiting microtubule polymerization [24]. Knockout of MAP65 abrogates cell plate assembly resulting in multi-nucleated cells and incomplete cell pates in *Arabidopsis* (cell wall stubs; [25–27]). Conversely, deletion of two Group 4 kinesins in *Physcomitrella patens* results in longer anti-parallel microtubule overlap in the phragmoplast midzone and in thicker cell plates [28••]. In addition to Group 4, kinesins from Groups 4, 5, 7, 8, 12, 14 as well as ungrouped kinesins localized to the phragmoplast midzone (reviewed in [12]). Given ability of some kinesins to slide anti-parallel microtubules, the size of the anti-parallel microtubules overlap could also be regulated through the tug-of-war between the affinity of MAP65 for microtubules and sliding force of the kinesins. Hence, cooperation between MAP65 and kinesins establishes the position and width of the phragmoplast midzone during the ‘first stroke’.

Stroke 2: recruitment of vesicles to the anti-parallel microtubule overlap region

Recent discoveries provide novel insight into the process of recruiting cytokinetic vesicles to the phragmoplast midzone. One mechanism involves the vesicle-tethering factor TRAPPII (reviewed in [29•,30]). Yeast TRAPPII mediates intra-Golgi and post-Golgi trafficking by acting as a guanine nucleotide exchange factor (GEF) for the Rab GTPase Ypt31P. *Arabidopsis* TRAPPII is predicted to consist of ten subunits [31], whose function is seemingly conserved from yeast [30]. Knock out of individual TRAPPII subunits results in high embryonic lethality [31,32]; however, it is unclear if this phenotype is the consequence of the cytokinetic function of TRAPPII, or its additional role in establishing cell polarity [32]. Surviving TRAPPII mutant plants exhibit fragmented ‘beads-on-the-string’ cell plates in their root apical meristem [31]. Two subunits of the TRAPPII complex (TRS130/CLUB and TRS120) were shown to interact with MAP65 (isotypes MAP65-1 and MAP65-3) in immunoprecipitation and bi-molecular fluorescence complementation assays; yeast two-hybrid assay confirmed the interaction between TRS130 and MAP65-3 [33••]. Hence, binding of TRAPPII to MAP65 constitutes one mechanism for targeting cell plate vesicles to the midzone.

Genetic analyses indicate that TRAPPII is not required for the establishment of anti-parallel microtubule overlaps and for targeting of MAP65 to the midzone. This conclusion stems from findings that *TRS120* or *TRS130* knockout did not affect the localization of MAP65 to the phragmoplast [33••]. Furthermore, knockout of *TRS120* did not prevent accumulation of RabA1c-vesicles at the midzone [32]. Together, this work suggests that TRAPPII is also dispensable for the targeting of cytokinetic vesicles. In contrast, a recessive *MAP65-3* allele, *ple4*, was found to reduce the accumulation of TRS120 in the midzone [33••]. *ple4* has a single amino-acid substitution (A421V) in a conserved residue of central microtubule-binding domain [21]. As a consequence, the affinity of the interaction between MAP63-3 and the microtubule diminishes. Considering the lack of information regarding which domain of MAP65-3 interacts with TRS130/CLUB and whether/if the PLE4 protein localizes to the midzone, three scenarios could explain the reduced localization of TRS120 in the phragmoplast midzone of the *ple4* mutant. First, PLE4 fails to bind the midzone and other members of MAP65 family target TRAPPII to this region, but with lower affinity [22]. Second, PLE4 binds the midzone, but has reduced affinity for TRS130. Third, the microtubule-binding domain of MAP65-3 is not involved in the interaction with TRAPPII and PLE4 does not bind microtubules but instead titrates out TRAPPII. In whichever case, MAP65 appears to be involved in targeting or retention of TRAPPII to the phragmoplast midzone. An alternative model was recently proposed by Ravikumar and co-authors that the interaction between TRAPPII and MAP65 regulates the completion of cytokinesis by providing a link to cues that signal cell cycle progression instead of cell plate initiation [29•]. More detailed characterization of this partnership is required to determine the functions of the MAP65–TRAPPII complex in cytokinesis.

If the MAP65–TRAPPII complex functions in cell plate initiation, then double knockout of MAP65-3 and TRAPPII would display the same phenotype as what has been observed with single mutants alone. However, a double mutant between *ple2* (another MAP65-3 allele) and *trs120-4* exhibits a profound increase in the frequency of cytokinetic failure, with respect to single mutations alone, indicating that the interaction is synergistic [33••]. The underlying mechanisms for the behaviour are unknown, but it is possible that TRAPPII stabilizes the microtubule overlap by increasing the affinity of MAP65-3 for microtubules or by recruiting microtubule-regulators XMAP215/MOR1, CLASP, and TOR1/SPR2 [33••] to the midzone.

It is notable that disassembly of the phragmoplast in the lagging zone is accompanied by a loss of MAP65 [33••], however subunits of the TRAPPII complex remain associated with the cell plate [33••,34]. This finding indicates that TRAPPII also functions in the cell plate maturation after microtubule depolymerization. This role could be related to maintaining the identity of the cell plate compartment because the syntaxin KNOLLE dissociates from the cell plate after phragmoplast disassembly in *trs120*, but not in the wild type [31].

In addition to TRAPPII, targeting vesicles to the midzone can be facilitated by the tethering factor, exocyst. During interphase, exocyst subunits Sec10 and Sec15b co-sediment with microtubules [35], and EXO70A (another exocyst subunit) binds microtubules through interaction with coiled-coil proteins — vesicle tethering 1 (VETH1) and VETH2 — and the conserved oligomeric Golgi complex 2 protein (COG2; [36]). Furthermore, electron tomography identified exocyst-like L-shaped and Y-shaped structures on cytokinetic vesicles [9], and several subunits of exocyst EXO84b, SEC6, SEC8, EXO70A1, Sec15b were shown to localize to the midzone of the disk phragmoplast [37]. In the ring phragmoplast, EXO70A1 and EXO84b localize to both the midzone and the cell plate [34,37]. Knockout of *EXO84b* causes the appearance of cell wall stubs in some cells and wavy cross-walls in others [37]. This relatively mild cytokinetic phenotype indicates a functional redundancy for exocyst as a tethering factor for cytokinetic vesicles [37]. A key challenge that lies ahead is the identification of the factors that lead to targeting the exocyst to the midzone before we can further appreciate its cytokinetic role.

Recruitment of cytokinetic vesicles to the phragmoplast midzone through interaction between microtubules and tethering factors during this second 'stroke' primes the cell plate formation.

Stroke 3: dissolution of the anti-parallel microtubule overlaps and attachment of microtubules to CPAM

Fusion of cytokinetic vesicles is accompanied by the formation of the Cell Plate Assembly Matrix (CPAM), which appears on electron micrographs as a ribosome-free zone around the forming cell plate [9,38]. The average thickness of the CPAM reaches 156 nm during active vesicle delivery and then decreases to the average 56 nm after phragmoplast disassembly [9]. This correlation between CPAM thickness and vesicle delivery suggests that at least some components of the CPAM are trafficked on the vesicles. Despite the fact that ribosomes are excluded from CPAM, much larger vesicles get inside CPAM where they fuse with each other and with the nascent cell plate [7,9]. In this way CPAM forms a unique chemical environment around the cell plate. Entering this environment by the cytokinetic vesicles may require force of the kinesins. Cytokinetic vesicle fusion is described in detail in several recent reviews [13,39,40]. Therefore, this section will focus only on the impact of cell plate assembly on microtubule organization.

Cytokinetic vesicles are thought to contain pectin, which helps to shape the cell plate during initial fusion events [9]. However, the main structural component of the cell plate appears to be callose — as chemical or genetic inhibition of callose synthesis results in cytokinetic failure [41–45]. Immuno-electron microscopy shows deposition of callose to the cell plate lumen during formation of the tubulo-vesicular network [8]. At this stage, the anti-parallel microtubule overlaps mostly disappear [46], however, the reason behind this phenomenon is poorly understood. Presumably, fortification of cell plate with callose makes structural role of the overlaps obsolete. Instead, microtubules terminate proximally to the CPAM and only 0.8% of them penetrate inside [9,38]. The majority of microtubules that are proximal to the CPAM appear to be blunt-ended [46], a configuration that was predicted to correlate with microtubule stability [47]. The existence of a population of microtubules in the phragmoplast that is recalcitrant to the action of the microtubule-destabilizing drug propyzamide also indirectly suggests the ability of CPAM to stabilize microtubules [10].

Microtubule-stabilizing factors could be recruited by CPAM as it appears to be selective not just for organelles, but for proteins as well. For example, visualization of the phragmoplast with anti-tubulin antibody or with fluorescent protein tags produces a marked line in the midzone where tubulin is excluded from CPAM (Figure 1b,c; [48,49]). Microtubule binding proteins CLASP [50], TANGLED [51], TOR1/SPR2 [52,53] and AIR9 [54] are also excluded from the midzone. However, CPAM accumulates some regulators of membrane trafficking, enzymes for oligosaccharide synthesis and remodelling [13,39,40], as well as microtubule-binding proteins.

Several microtubule-binding proteins localize to the midzone including XMAP215/MOR1 [55,56], EB1 [23,57], and kinesins (reviewed in [12]). Systematic analysis of kinesin localization in *P. patens* revealed that 18 of 43 kinesins localized to the phragmoplast midzone, including members of Groups 4, 7, 8 and 12 as well as the orphan kinesins KINID1a and KINID1b [58,59]. A number of angiosperm kinesins also localize to the phragmoplast midzone [12] including a Group 7 member Kin7.3 that promotes microtubule polymerization/stabilization [60]. Furthermore, microtubule-binding protein XMAP215/MOR1 can also promote microtubule polymerization [61]. These and other yet uncharacterized midzone proteins could stabilize microtubule plus ends proximally to CPAM.

Stabilization of microtubules on the surface of CPAM is accompanied by the loss of overlapping anti-parallel microtubules. It is, therefore, likely that destabilization of anti-parallel microtubules depends on the deactivation of MAP65. The affinity of MAP65 for microtubules is modulated by its divergent C-terminal domain [22]. Several midzone-localized kinases including MAPK, CDK, and Aurora A can reduce the affinity of MAP65 for microtubules by phosphorylating this domain [12,62,63,64•]. Whether the MAP65 proteins released from overlapping microtubules contribute to microtubule stabilization by CPAM through the interaction with TRAPPII or other mechanisms remains unknown.

By the end of the third 'stroke,' CPAM takes over the role of anti-parallel microtubule overlaps in stabilizing microtubules.

Stroke 4: depolymerization of microtubules

When cell plate assembly reaches the tubular-network stage, the phragmoplast vanishes together with the majority of its associated proteins (reviewed in [7]). Microtubule polymerization behind the phragmoplast lagging zone has never been reported in somatic cells. This fact suggests that microtubule depolymerization in this zone is irreversible. Hence, there should be a quality control mechanism, which prevents microtubule depolymerization before the cell plate reaches a pre-determined stage of maturity.

What governs this stage-specific microtubule depolymerization remains the biggest unknown phenomenon in phragmoplast biology. As inhibition of cell plate assembly by caffeine or Brefeldin A prevents microtubule depolymerization in the lagging zone [65,66], microtubule depolymerization must be regulated by chemical and (or) mechanical gradients in the cell plate. Some insights as to the events in the phragmoplast lagging zone are provided in the recent review [12]. Developing tools for measuring mechanical properties of the cell plate would help to solve this riddle. Most certainly though, 'stroke 4' releases proteins, which are reused in the subsequent stages of phragmoplast expansion.

Conclusions: still much to learn

Anti-parallel microtubules play a key role in cell division in yeasts, animals, and plants. However, contrary to other systems where the overlaps are relatively static, plants can dissolve and reform them multiple times at the phragmoplast leading edge. Such dynamicity enables phragmoplast expansion, which in turn allows construction of cell plates of any size and shape in response to developmental or environmental cues. Several recent discoveries have provided key insights to early stages of the cell plate assembly. Fitting this information into the existing knowledge of microtubule organization and dynamics has enabled reconstruction of major events in phragmoplast expansion. Taking our knowledge of the phragmoplast expansion to the next level would require exploring the following areas:

1. Better understanding the TRAPPII–MAP65 complex would provide further insights into the relationship between anti-parallel microtubule overlaps and cell plate initiation. In particular, more detail is needed regarding firstly, the domains that mediate interaction between TRAPPII and MAP65 dimers; secondly, the impact of this interaction on the activity of each complex; and finally regulation of this interaction.
2. It remains unclear why anti-parallel microtubules disappear at a time when fenestrae are still abundant in the cell plate. Perhaps microtubules in the midzone interfere with the signalling processes instead of obstructing cell plate assembly.
3. Phragmoplast microtubules appear to be separated by distances that are greater than the size of known microtubule-associated proteins [9,46]. This observation raises

the question of whether stabilization of the phragmoplast during expansion requires bridging of microtubules, or if association with the cell plate itself is sufficient to maintain phragmoplast integrity.

4. If overlapping anti-parallel microtubules define the thickness of the cell plate [28••], then what defines the cell plate dimensions after dissolution of these overlaps?
5. What defines selectivity of the CPAM for proteins and organelles?
6. Developing tools for quantification of microtubule dynamics in the phragmoplast.

Understanding phragmoplast expansion is an important keystone for answering fundamental questions of plant cytokinesis [67••]. Furthermore, considering the pivotal role of the coordination between vesicles trafficking, the cytoskeleton and cell wall synthesis for cellular architecture, research on phragmoplast expansion will undoubtedly uncover novel insights into the rules of plant development.

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An updated cytokinesis model proposed in this article unifies live-cell imaging data with results of high-resolution electron tomography. In this model anti-parallel microtubule overlaps cooperate with cell plate assembly matrix in supporting phragmoplast structural integrity.