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Spotlight

Phosphoinositides Break Microtubule Dynamics Symmetry in the Phragmoplast

Andrei Smertenko [1,*]

Plant cytokinesis rely on asymmetric behavior of microtubules: bulk polymerization at the phragmoplast leading zone and bulk depolymerization at the phragmoplast lagging zone. Recent findings demonstrate that phosphatidylinositol 4-kinase beta (PI4Kβ) plays an essential role in this asymmetry by facilitating the establishment of the phragmoplast lagging zone.

Plant cytokinesis depends on the centrifugal expansion of a specialized microtubule structure, the phragmoplast (Figure 1A,B). The expansion is driven by the asymmetry of microtubule dynamics whereby bulk polymerization at the phragmoplast leading zone and bulk depolymerization at the lagging zone occur. Microtubule behavior in the phragmoplast correlates with cell-plate assembly stages (Figure 1A,B,D). The initial delivery and fusion of cytokinetic vesicles (Stage I) is accompanied by the establishment of the antiparallel microtubule overlap. During Stage II (the tubulovesicular network), the overlaps disappear and microtubule ends terminate at the ribosome-free cell-plate assembly matrix. Consolidation of membranes into the tubular network during Stage III triggers microtubule depolymerization. The subsequent two stages, formation of the fenestrated sheet (Stage IV) and cell-plate maturation that results in a functional cross-wall (Stage V), occur without microtubules [1,2].

Phragmoplast expansion necessitates coordination between the processes of microtubule dynamics (polymerization–depolymerization), membrane trafficking, and the deposition of oligosaccharides in the phragmoplast midzone [1]. However, the mechanism by which microtubule organization perceives transitions between cell-plate assembly stages remains a mystery.

Experimental data suggest the existence of a 'quality control mechanism' in the cell plate that prevents premature microtubule depolymerization (reviewed in [3]). For example, cell-plate assembly failure following treatment with inhibitors of exocytosis (brefeldin A) or vesicle fusion (caffeine) prevents the depolymerization of microtubules, resulting in larger, disk-shaped phragmoplasts (reviewed in [3,4]). By contrast, ectopic stabilization of microtubules with Taxol reduces the rate of phragmoplast expansion but does not prevent microtubule depolymerization in the distal zone [5]. Therefore, the asymmetry of microtubule dynamics and organization could be governed by the chemical composition of the cell plate.

Lipid composition in the cell plate may act as a potential regulator of microtubules in the phragmoplast. For example, mutations in enzymes that are responsible for sterol biosynthesis impair cell-plate assembly, such as sterol methyltransferase, *smt1/cph* [6], and cyclopropylsterol isomerase1-1, *cpi1-1* [7]. Inhibition of the synthesis of long-chain sphingolipids with fumonisin B1 also prevents the fusion of cytokinetic vesicles [8].

Recent work by Lin *et al.* [9] demonstrates the importance of phosphatidylinositol phosphates in phragmoplast asymmetry. A double knockout of PI4Kβ, *pi4kβ1* pi4k*β2*, results in cytokinetic failure and the appearance of multinucleated cells (*ca* 10%) in the root apical meristem. Electron microscopy revealed an accumulation of vesicles in the midzone that apparently failed to fuse. These results indicate that the product of PI4Kβ, phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P2], plays a role in establishing the tubulovesicular network during Stage II of cell-plate assembly.

According to the current cytokinesis model (Figure 1D), inhibition of cytokinetic vesicle fusion in the leading zone would slow cytokinesis and prevent the dissolution of the antiparallel microtubule overlaps. Consistent with this prediction, Lin

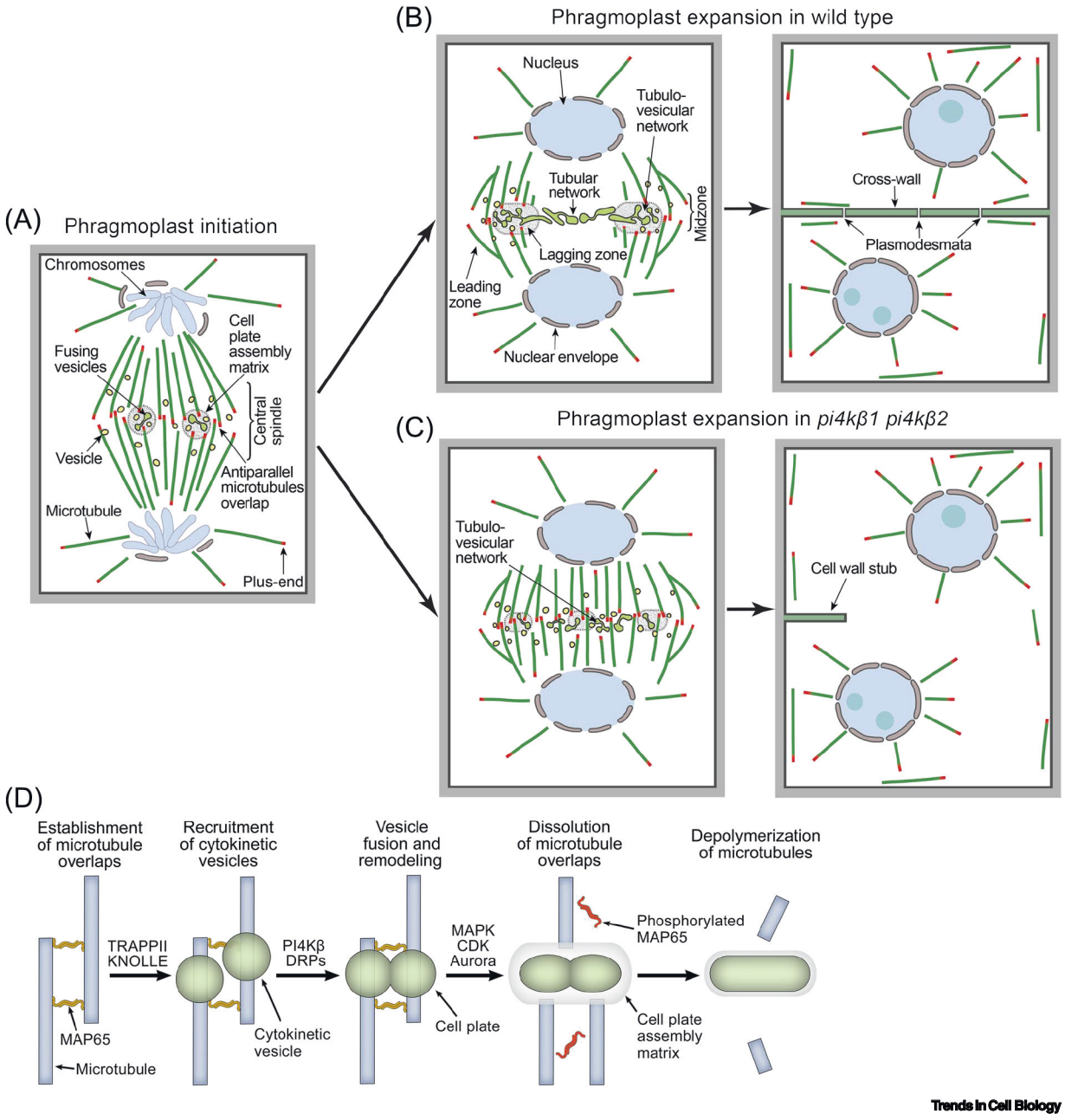

Figure 1. Phragmoplast Expansion in Control and *pi4kβ1* pi4k*β2* Mutant Cells. (A) During phragmoplast initiation, antiparallel microtubule overlaps form in all parts of the central spindle. Cytokinetic vesicles accumulate and fuse in this region, giving rise to the partition between daughter cells, the cell plate. The central spindle gives rise to the phragmoplast midzone. (B) During phragmoplast expansion, cytokinetic vesicles coalesce in the phragmoplast leading zone. Some microtubules form antiparallel overlaps. Vesicle fusion results in a tubulovesicular network surrounded by the cell-plate assembly matrix. Next, cell-plate assembly progresses to the tubular network stage, which triggers bulk microtubule depolymerization. (C) The tubulovesicular network fails to form in the expanding phragmoplast of *pi4kβ1* pi4k*β2* and the lagging zone is not established. The phragmoplast expands, but the antiparallel microtubule overlaps persist throughout the midzone. Consequently, cytokinesis in the mutant culminates with the cell-wall stubs instead of the cross-wall. (D) MAP65 stabilizes antiparallel microtubule overlaps, which recruit cytokinetic vesicles with the aid of the tethering complex TRAPPII and the syntaxin KNOLLE. Vesicles fuse and undergo remodeling through the activity of dynamin-related proteins (DRPs); PI4Kβ2 could facilitate vesicle fusion and remodeling by a PtdIns(4,5)P2-dependent increase of membrane rigidity. PI4Kβ2 acts upstream of the signal that causes dissolution of the antiparallel overlaps. Vesicle fusion promotes MAP65 phosphorylation by the MAPK, CDK, and Aurora kinases, phosphorylated MAP65 dissociates from the microtubules, and microtubules are stabilized through interaction with the cell-plate assembly matrix. The establishment of the tubular network and the deposition of oligosaccharides in the cell-plate lumen (mostly callose) trigger the depolymerization of microtubules.

et al. report slower phragmoplast expansion in *pi4kβ1* pi4kβ2 and persistence of the antiparallel microtubule marker MAP65-3 through the midzone of the expanding phragmoplast. These findings mean that: (i) the cell plate in *pi4kβ1* pi4kβ2 fails to produce the signal for dissolution of the overlaps; and (ii) PtdIns(4,5)P2-dependent processes in the phragmoplast act upstream of CDK, MAPK, and Aurora kinase, which collectively control the binding of MAP65 to microtubules (Figure 1D). Furthermore, these findings provide further experimental support for the existence of checkpoints in cell-plate assembly that prevent premature microtubule reorganization in the midzone.

In addition to slowed expansion, phragmoplast microtubule depolymerization was inhibited in *pi4kβ1* pi4kβ2 cells resulting in large, disk-shaped phragmoplasts that resembled those induced by treatment with brefeldin A or caffeine (reviewed in [3,4]). This phenotype indicates a failure to establish the phragmoplast lagging zone. Hence, inhibition of the assembly of the tubulovesicular network prevents destabilization of the antiparallel microtubule overlaps and all downstream cell-plate assembly stages (Figure 1C,D). Interestingly, however, even with failure to remodel the cell plate, delivery of the cytokinetic vesicles is not disrupted and the phragmoplast continues to expand (Figure 1C). These findings indicate that: (i) the production of the cytokinetic vesicles is independent of the cell-plate assembly rate; (ii) the rate of phragmoplast expansion is limited by membrane fusion and remodeling rather than by the availability of the cytokinetic vesicles; and (iii) PtdIns(4,5)P2 is not essential for the delivery of vesicles to the midzone.

The key question remains: how does PtdIns(4,5)P2 contribute to the assembly of the cell plate? Phosphoinositides could rigidify the plasma membrane and in doing so provide a counterforce for the conformational changes of dynamin that facilitate clathrin-dependent endocytosis [10]. Dynamin-related proteins decorate dumbbell-shaped membrane compartments at the phragmoplast leading edge [1]. Plausibly, PtdIns(4,5)P2 facilitates vesicle fusion and the subsequent dynamin-dependent remodeling during assembly of the tubulovesicular network.

In conclusion, PI4Kβ2 and its product PtdIns(4,5)P2 appear to play a role in the tubulovesicular network assembly. However, careful structural analysis of the cell plate in *pi4kβ1* pi4kβ2 is necessary to test this hypothesis. Future work on the *pi4kβ1* pi4kβ2 mutant would facilitate understanding the role of crosstalk between cell-plate assembly and microtubule organization in the generation and maintenance of phragmoplast asymmetry.

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