

## Assembly of the phragmoplast microtubule array in plant cells

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The cytokinetic apparatus phragmoplast is an evolutionary landmark that is shared by advanced green algae and plants. It contains a bipolar microtubule framework with microtubule plus ends facing the cell division site. The phragmoplast microtubule array expands centrifugally toward the cell periphery while the cell plate is built by fusions of Golgi-derived vesicles delivered toward microtubule plus ends by microtubule-based motors. Upon the completion of anaphase, microtubules in the spindle midzone have their polarities sorted out to assume a bipolar configuration. Segments near their plus ends are cross-linked by proteins like MAP65-3. Once cytokinesis takes off, new microtubules are polymerized at the peripheral edge of the phragmoplast. As critical regulators of microtubule nucleation and organization, the  $\gamma$ -tubulin complex, the WD40 repeat protein NEDD1, and the augmin complex play critical roles in the nucleation of new microtubules and organization of the array. Newly polymerized microtubules are captured by MAP65-3 toward their plus ends near the division site in order to be stabilized. The motor Kinesin-12 ensures that microtubule plus ends are correctly positioned near the division site. Based on results obtained us and others, we present a modular model depicting the assembly of the phragmoplast microtubule array by mini-phragmoplasts units.

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## Acentrosomal cell division in the moss *Physcomitrella patens*

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The centrosome is the major microtubule nucleation site in animal somatic cells. In contrast, land plant cells nucleate microtubules and assemble spindles and phragmoplasts in the absence of centrosomes during mitosis. Molecular mechanisms underlying mitosis are more poorly characterised in plants, partly due to the lack of an appropriate model cell system in which loss-of-function analysis can be easily combined with high-resolution microscopy. In this talk, I will present our development of an inducible RNAi system and 3D time-lapse confocal microscopy in the moss *Physcomitrella patens* that allow in-depth phenotype characterisation of mitotic genes. On the basis of our findings, the common and specialised mechanisms of microtubule formation and cell division in plant and animal cells will be discussed.

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## Cell plate formation through microtubule turnover controlled by the MAPK pathway

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Cytokinesis in plant cells occurs through the formation of cell plates including cell membranes and cell walls, from the interior to the periphery of the cell. These dynamic events are supported by a microtubule (MT)-based structure, which is known as a phragmoplast. The phragmoplast is centrifugally expanded, which appears to be mediated by MT turnover involving the depolymerization of MTs and polymerization of tubulins around the midzone of the phragmoplast. In tobacco and Arabidopsis, we have shown that the pathway including the MAP kinase cascade, which is designated the NACK-PQR pathway, positively controls the expansion of phragmoplast (1, 2, 3, 4). We have sought for substrate proteins of the MAP kinase in the pathway and identified microtubule-associated protein 65 (MAP65) as one of targets in tobacco and Arabidopsis: the phosphorylation by the MAP kinase reduces activity of MAP65 to bundle MTs and phosphorylated MAP65 localizes to the mid zone of the phragmoplast where MT turnover occurs (5). Overexpression of MAPK-non-phosphorylatable MAP65 in tobacco cells delays the expansion of the phragmoplast (5). Although plant MAP65s are phosphorylated by CDKs like those of animals, overexpression of CDK-non-phosphorylatable mutants of MAP65 does not affect progression of the cell cycle during any phase, or MT bundling activity. These results suggest that the MAP kinase is crucial for controlling the function of MAP65 in the cell plate formation. Recently, we have also identified two proteins in addition to MAP65 that were phosphorylated by the MAPK and will report recent progresses of our investigation (6).

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## Microtubule dynamics in plant cytokinesis as revealed by quantitative live imaging

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In land plants, the cell plate separates the parental cell into two. The cell plate initially appears as a disc between daughter nuclei and then expands towards the cell periphery until it fuses with parental cell walls. Our goal is to understand mechanism of the cell plate expansion.

The periphery of expanding cell plate is surrounded by a doughnut-shape microtubule structure, called phragmoplast, which works for transportation of cell wall precursors to the leading edge of the cell plate. Previous studies have shown that translocation of the phragmoplast towards the cell periphery is the driving force of the cell plate expansion, and microtubule polymerization/depolymerization is involved in the translocation. However, how microtubules are polymerized and depolymerized in the phragmoplast is unknown.

Using quantitative analyses of fluorescent protein-expressing tobacco suspension culture cells, we found microtubules on the leading edge of the cell plate are more stable than inside of the phragmoplast. We also found that microtubules are mainly nucleated on existing microtubules in the phragmoplast. We propose that microtubule-dependent microtubule formation coupled with biased stabilization/destabilization leads net movement of phragmoplast microtubules, which in turn causes redistribution of cell plate deposition towards the cell periphery.

## Organization of cortical microtubule arrays in Arabidopsis

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Microtubules are nucleated from dispersed cortical regions in interphase plant cells, where the majority of nucleation events occur from the  $\gamma$ -tubulin-containing sites on the pre-existing microtubules as branching patterns. The minus-ends of newly formed daughter microtubules are usually released from sites of nucleation by the action of the microtubule severing complex katanin, and the free microtubules are then transported on the cortex by polymer treadmilling. Subsequent microtubule-microtubule interactions promote microtubule bundling and ordering, which establishes particular patterns of interphase cortical arrays. With special interests in helical microtubule arrays, we have been studying possible mechanisms and particular molecules that underlie organization of cortical microtubule arrays.

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