

# Expansion of the phragmoplast during plant cytokinesis: a MAPK pathway may MAP it out

Ryuichi Nishihama\* and Yasunori Machida†

Plant cytokinesis involves the formation of a cell plate. This is accomplished with the help of the phragmoplast, a plant-specific cytokinetic apparatus that consists of microtubules and microfilaments. During centrifugal growth of the cell plate, the phragmoplast expands to keep its microtubules at the leading edge of the cell plate. Recent studies have revealed potential regulators of phragmoplast microtubule dynamics and the involvement of a mitogen-activated protein kinase cascade in the control of phragmoplast expansion. These studies provide new insights into the molecular mechanisms of plant cytokinesis.

## Addresses

\*Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599-3280, USA

†Developmental Biology Group, Division of Biological Science, Graduate School of Science, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8602, Japan; e-mail: yas@biol1.bio.nagoya-u.ac.jp  
Correspondence: Yasunori Machida

Current Opinion in Plant Biology 2001, 4:507–512

1369-5266/01/\$ – see front matter

© 2001 Elsevier Science Ltd. All rights reserved.

## Abbreviations

|                   |   |
|-------------------|---|
| <b>Ase1</b>       | Anaphase spindle elongation1  |
| <b>AtPAKRP1</b>   | <i>Arabidopsis thaliana</i> phragmoplast-associated kinesin-related protein 1 |
| <b>BFA</b>        | brefeldin A   |
| <b>BY-2</b>       | bright yellow-2   |
| <b>CDK</b>        | cyclin-dependent kinase   |
| <b>DcKRP120-2</b> | <i>Daucus carota</i> kinesin-related protein of 120 kDa-2                     |
| <b>FTN</b>        | fusion-tube-generated membrane network  |
| <b>KLP</b>        | kinesin-like MT-based motor protein   |
| <b>MAP</b>        | MT-associated protein   |
| <b>MAPK</b>       | mitogen-activated protein kinase  |
| <b>MAPKK</b>      | MAPK kinase   |
| <b>MAPKKK</b>     | MAPKK kinase  |
| <b>MT</b>         | microtubule   |
| <b>NPK1</b>       | nucleus- and phragmoplast-localized protein kinase1                           |
| <b>Ntf6</b>       | <i>Nicotiana tabacum</i> MAPK 6   |
| <b>NtMAP65-1</b>  | <i>Nicotiana tabacum</i> MAP65-1  |
| <b>PRC1</b>       | Protein Regulator of Cytokinesis1   |
| <b>TKRP125</b>    | tobacco kinesin-related polypeptide of 125 kDa                                |
| <b>TN</b>         | tubular network   |
| <b>TVN</b>        | tubulo-vesicular network  |

## Introduction

Cytokinesis is a physical process that distributes genetic information and cytoplasm from a parent cell into two daughter cells. In higher plants, cytokinesis is achieved through the formation of a new cross-wall, called the cell plate, which stretches from the interior to the periphery of the cell [1]. Materials for the construction of the cell plate are supplied from the Golgi complex through the vesicle trafficking system [2,3,4]. Membrane vesicles that have budded from the Golgi fuse with one another to form an immature cell plate between the two daughter nuclei. The

details of cell-plate maturation have been revealed through the observation of cryofixed tobacco cells by Samuels *et al.* [5] (Figure 1a). Vesicles fuse via long thin (i.e. 20 nm in diameter) curving fusion tubes to produce a fusion-tube-generated membrane network (FTN). This network undergoes a series of morphological and biochemical changes, including the formation of a tubulo-vesicular network (TVN), a tubular network (TN), and a fenestrated membrane sheet. The cell plate grows centrifugally, through the continuous addition of vesicles to its edge, until it reaches the parental cell walls. Therefore, the center of the growing cell plate is the most mature part.

The apparatus responsible for localization and fusion of vesicles is the phragmoplast, whose formation is initiated during late anaphase [1]. A phragmoplast complex is composed of two bundles of anti-parallel microtubules (MTs) and actin filaments. MTs are, in general, orientated and consist of a plus end and a minus end. The MTs overlap at their plus ends in the center of the phragmoplast. The initial shape of the phragmoplast is a cylinder- or barrel-like structure. Golgi-derived vesicles are transported to the equator of the phragmoplast by the MTs, whose action is probably assisted by MT motor proteins that have yet to be identified [6]. Once cell-plate formation begins in its equatorial zone, the phragmoplast changes into a ring-like structure and centrifugally expands, maintaining localization of the MTs at the leading edge of the cell plate. This change in phragmoplast shape is crucial for the lateral growth of the cell plate. This review focuses on the mechanism of lateral expansion of the phragmoplast and encompasses recent findings that shed light on the regulation of this process. For aspects of cytokinesis not covered in this review, such as mechanisms of vesicle fusion, roles of actin filaments, and control of the division plane, the reader is referred to other recent review articles [7–10].

## Phragmoplast expansion driven by MT turnovers

How do phragmoplast MTs move centrifugally toward the cortex as the cell plate grows? A clue came from an experiment performed by Yasuhara *et al.* [6] who used taxol, a chemical that blocks depolymerization of MTs, to examine the mechanism of cell-plate expansion. Taxol treatment of tobacco bright yellow-2 (BY-2) cultured cells during telophase inhibits the centrifugal expansion of their phragmoplasts, producing cells with abnormally thick cell plates that apparently result from the increased accumulation of vesicles (Figure 1d). The requirement for MT depolymerization during phragmoplast expansion indicates that neither the pushing force of the cell plate nor the pulling force of the parental cell walls is responsible for the

Figure 1

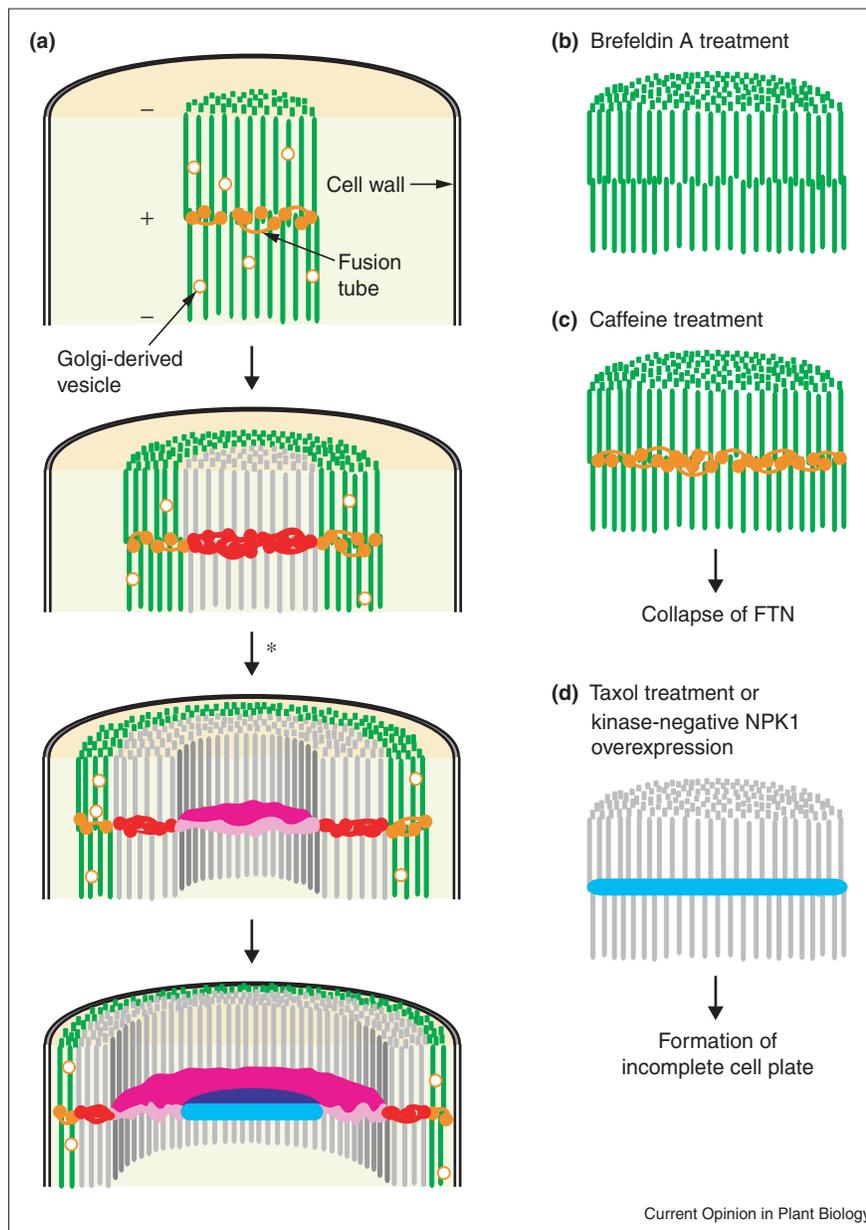


Diagram of phragmoplast expansion and cell-plate formation in normal, drug-treated, or genetically modified plant cells. **(a)** In a normal plant cell, a phragmoplast is assembled with anti-parallel MTs overlapping in the center at their plus ends (+). Fusion of Golgi-derived vesicles (open circles), through fusion tubes in the equatorial zone of the phragmoplast, gives rise to a FTN (orange), which successively matures into a TVN (red), a TN (pink), and a fenestrated sheet/cell plate (blue). In (a), green bars represent MTs that have recently been polymerized, or in **(b,c)**, that are not yet destined for depolymerization, whereas gray bars represent MTs that are destined for depolymerization during the next stage. Note that the shape of the phragmoplast changes during the transition from a cylinder to a ring, which is marked by an asterisk. **(b)** Brefeldin A blocks the supply of vesicles by disrupting the Golgi, resulting in a lack of cell-plate materials in the phragmoplast equator. **(c)** Caffeine blocks the maturation of the FTN into the TVN, which eventually results in the collapse of the FTN. **(d)** Taxol blocks the depolymerization of MTs, which inhibits phragmoplast expansion but allows the formation of an incomplete cell plate. Overexpression of a kinase-negative mutant of the NPK1 MAPKKK results in a similar phenotype. Note that, in **(b-d)**, phragmoplasts are never transformed into ring shapes.

mechanical enlargement of the phragmoplast. Rather, it seems more likely that the supply of free tubulins, which have been depolymerized from preexisting MTs on the inner side of the phragmoplast, forces the lateral expansion of the phragmoplast by constructing new MT arrays at its outer edge. Thus, the phragmoplast appears to possess an activity that can initiate MT polymerization at its outer edge.

The incorporation of free tubulins into the phragmoplast is known to occur at the plus ends of MTs located in the equatorial zone [11,12]. On the basis of the fluorescence redistribution after photobleaching (FRAP) analysis performed by Hush *et al.* [13], phragmoplast MTs seem to exhibit dynamic instability [14]. Following the photobleaching of phragmoplast MTs that had incorporated

fluorescein-labeled tubulins, Hush *et al.* [13] observed a rapid and uniform recovery of fluorescence. This observation is more consistent with a mechanism of dynamic instability and exchange of tubulin dimers than with treadmilling. Therefore, depolymerization of MTs might also occur at the plus end of MTs in the phragmoplast. If this were the case, then MTs on the inner side of the phragmoplast would be more unstable than those on the outer side.

### Phragmoplast MT-associated proteins (MAPs)

Recently, two classes of MT-associated proteins (MAPs), which are possible regulators of MT dynamics, have been shown to localize to the phragmoplast. One of these classes is comprised of the kinesin-like MT-based motor proteins

(KLPs). Plus-end-directed KLPs, including *Xenopus* kinesin central motor1 (XKCM1) [15] and *Xenopus* kinesin superfamily2 (XKIF2) [16], and the minus end-directed KLP KAR3 [17] are known to depolymerize MTs at the plus and minus end, respectively (reviewed in [18]). *Arabidopsis thaliana* phragmoplast-associated kinesin-related protein1 (AtPAKRP1) is localized to the center of the phragmoplast, and microinjection of anti-AtPAKRP1 antibodies or truncated AtPAKRP1 proteins into tobacco BY-2 cells leads to the disorganization of phragmoplast MTs [19••]. Carrot DcKRP120-2 (*Daucus carota* kinesin-related protein of 120 kDa-2) is a KLP homologue of tobacco TKRP125 (tobacco kinesin-related polypeptide of 125 kDa) [20•], which possesses an activity that translocates phragmoplast MTs toward their minus ends. TKRP125 is localized to whole arrays of phragmoplast MTs except for their plus ends [21]. Unlike TKRP125, DcKRP120-2 is also localized to the MT-interdigitating zone of the phragmoplast [20•]. The minus end-directed KLPs, KCBP (kinesin-like calmodulin-binding protein) [22] and KatA (kinesin-like protein in *Arabidopsis thaliana* A) [23], localize along the length of phragmoplast MTs. It will be intriguing to test the effects of these KLPs on MT disassembly. The roles of these KLPs in the progression of M phase or the expansion of phragmoplasts remain unknown.

Another MAP family is comprised of the structural MAPs recently identified by Smertenko *et al.* [24••]. They identified a member of the tobacco NtMAP65-1 (*Nicotiana tabacum* MAP65-1) family that is able to bind to taxol-stabilized MTs. This MAP promotes polymerization of MTs *in vitro* but does not promote the bundling of MTs [24••]. This polymerizing activity is sufficient to classify the protein as a MT-dynamics regulator. Antiserum that recognizes multiple members of the NtMAP65-1 family does not stain all MT arrays. At metaphase, the antiserum stains an area of spindle MTs proximal to the metaphase plate but not those at the pole [24••]. The antibody also stains the spindle midzone and the phragmoplast equatorial zone [24••]. These patterns of localization suggest a role for NtMAP65-1 proteins in the regulation of MT dynamics in specific areas of the spindle and the phragmoplast where MTs overlap at their plus ends.

It is intriguing to note that NtMAP65-1 proteins have significant amino-acid-sequence homology to two other MAPs: human PRC1 (Protein Regulator of Cytokinesis1), which is required for cytokinesis [25], and yeast Ase1 (Anaphase spindle elongation1), which is required for spindle assembly, elongation and disassembly [26]. PRC1 and Ase1 share two notable sequence features: a consensus cyclin-dependent kinase (CDK) phosphorylation site and a sequence that is similar to a mitotic cyclin destruction box. It has been proven both *in vivo* and *in vitro* that PRC1 is phosphorylated at the consensus CDK phosphorylation sites by CDK2 [25]. It has also been proven that Ase1 is a target of proteolysis mediated by the anaphase-promoting complex [27]. NtMAP65-1 proteins also exhibit these

characteristic sequences. Our computer-based analysis also predicted that NtMAP65-1 proteins may form a highly  $\alpha$ -helical structure similar to that of PRC1 (R Nishihama, Y Machida, unpublished data).

After anaphase, PRC1 is restricted to plus-end MT-overlapping regions [25], a pattern that is reminiscent of NtMAP65-1 localization in plant cells. Remarkably, the microinjection of anti-PRC1 antibodies into HeLa cells blocks the completion of cytokinesis [25]. Although it is still unclear what PRC1 actually does to MTs, this study on PRC1 raises the possibility that NtMAP65-1 proteins also control the progression of cytokinesis in plants by regulating phragmoplast-MT dynamics. Whether PRC1 and Ase1 actively promote MT polymerization is now an open question.

Recent studies have revealed the existence of plant proteins in addition to the NtMAP65-1 family that have homologies to animal MAPs [28•,29•]. Furthermore, our brief search of the complete *Arabidopsis* genome sequence revealed two putative MAPs with similarity to human EB1 protein and MAP1A/B light chain 3 (R Nishihama, Y Machida, unpublished data). Interestingly, EB1 is known to localize to MT plus ends and is involved in the regional control of MT dynamics and capture (see [30,31] for reviews). Further research should be performed to test the involvement of various MAPs in the regulation of phragmoplast MT dynamics.

### Co-ordination of phragmoplast expansion with cell-plate formation

As described above, expansion of the phragmoplast is essential for the lateral growth of the cell plate. Cell-plate formation and phragmoplast expansion are co-dependent: without formation of the cell plate the phragmoplast never expands. For example, the use of brefeldin A (BFA), a chemical that disrupts the Golgi apparatus, to block the supply of vesicles carrying cell-plate materials prevents the formation of the cell plate and results in the generation of binucleate cells [2], but does not disturb phragmoplast formation [32]. Interestingly, under appropriate conditions, BFA treatment completely arrests the lateral expansion of phragmoplasts at the cylindrical stage after only an initial slight enlargement [32] (Figure 1b). These phragmoplasts no longer become ring-shaped and no MT disassembly takes place in their central region. Thus, the process of phragmoplast expansion is tightly linked to cell-plate formation. This finding suggests that there may be a mechanism that induces MT depolymerization in response to cell-plate formation.

What is the cue for MT depolymerization? Caffeine is known to disrupt cell-plate formation in plants ([33] and references therein). In *Tradescantia* stamen-hair cells treated with caffeine, cell-plate formation is initiated but the immature cell plate ceases to grow and is ultimately degraded after it has expanded to about three-quarters

of the cell's width [34]. Although the initiation of the phragmoplast is not inhibited in this system, the phragmoplast fails to adopt a ring structure and remains fixed as a cylindrical structure (Figure 1c). Electron microscopy of cryofixed BY-2 cells shows that caffeine disrupts the conversion of the FTN into the TVN during the development of the cell plate [33,35]. This implies that a minimal level of TVN formation is required for the induction of MT disassembly. Consistent with this finding, it has been observed that phragmoplast MTs are associated with the TVN but not with the TN [5] (Figure 1a). Taken together, these results suggest that MT depolymerization is induced during the TVN→TN transition. An ultrastructural study has also revealed several features of the TVN, such as assembly of the fuzzy membrane coat, widening of the connecting tubules, formation of clathrin-coated buds, and initiation of callose synthesis [5]. These features are good candidates for events that constitute 'MT-disassembly signals'. In conclusion, cell-plate expansion is driven by successive rounds of the following events: vesicle transport to the phragmoplast equator, formation of the FTN by vesicle fusion, maturation of the FTN into the TVN, and MT disassembly on the inside and its reassembly on the outside of phragmoplast.

### **A mitogen-activated protein kinase (MAPK) cascade as a regulator of phragmoplast expansion**

A coupling mechanism should exist between the events involved in cell-plate formation and the disassembly of phragmoplast MTs. Recent studies have suggested the involvement of a mitogen-activated protein kinase (MAPK) cascade in the regulation of such a coupling mechanism [36–38,39••]. The MAPK cascade is a signaling pathway that is conserved in all eukaryotes and that consists of members of three protein kinase families. In their respective order in the signaling cascade, they are the MAPK kinase kinase (MAPKKK) family, the MAPK kinase (MAPKK) family and the MAPK family [40].

On the basis of the sites of its transcript accumulation, a tobacco MAPKKK, Nucleus- and Phragmoplast-localized Protein kinase1 (NPK1) [41], has been suggested to play a role in cell division [42]. The accumulation of NPK1 protein is cell-cycle dependent; proteins are present in the cell from S to M phase, with a peak in accumulation during M phase, after which NPK1 is degraded [39••]. NPK1 is confined to the nucleus from S phase to prophase [39••]. NPK1 kinase activity is transiently increased late in M phase [39••]. Upon nuclear envelope breakdown, NPK1 proteins are dispersed into the cytoplasm and localize in patches until early anaphase. By late anaphase, NPK1 begins to concentrate at the spindle midzone before becoming localized to the equatorial zone of the cylindrical phragmoplast. During cytokinesis, NPK1 is consistently found at the equator of the ring-shaped phragmoplast, but not at the cell plate. Some NPK1 proteins relocate back to the reforming daughter nuclei.

These findings suggest a role for NPK1 in cytokinesis. Indeed, inhibition of NPK1 signaling has been shown to result in a defect in cytokinesis [39••]. Overexpression of a kinase-negative mutant of NPK1 (NPK1KW) induces the formation of multinucleate cells that contain incomplete disc-shaped cell plates. This mutant protein still localizes to the equator of the phragmoplast. The phragmoplast does not expand, however, in NPK1KW-overexpressing plants (Figure 1d). Thus, NPK1 regulates the expansion of both the cell plate and the phragmoplast.

It should be noted that NPK1KW-induced incomplete cell plates that are formed in NPK1KW-expressing cells contain callose [39••], a polysaccharide whose synthesis is initiated at the TVN stage [5] (see above). Thus, it would appear that NPK1 is primarily involved in a step that takes place after the formation of the TVN, that is, in the outward redistribution of phragmoplast MTs. Consistent with this, the effect provoked by blocking NPK1 signaling is similar to that of taxol treatment (see above; Figure 1d). NPK1 might regulate MT depolymerization at plus ends in response to MT-disassembly signals generated in the TVN/TN. There is, however, still a possibility that NPK1 might be involved in some of the earlier steps if inhibition by the dominant-negative mutant were not complete.

The identification of downstream targets of NPK1 will be decisive in completing our understanding of the molecular mechanism of phragmoplast expansion. It will be intriguing to examine whether NPK1 regulates the MAPs that are mentioned above and, if so, whether this regulation is mediated directly by NPK1 or indirectly through a putative MAPK cascade initiated by NPK1. In light of this, it should be noted that the p43<sup>Nt6</sup> MAPK from tobacco [36] and alfalfa MMK3 (*Medicago* MAPK3) [37] are activated during cytokinesis. Both of these proteins are localized to the equatorial zone of the phragmoplast, and MMK3 is also localized to the cell plate. Most recently, a tobacco MAPKK, *Nicotiana tabacum* MAPK/extracellular signal-regulated protein kinase kinase (NtMEK1), that activates p43<sup>Nt6</sup> has been identified as a possible component of a MAPK cascade involved in phragmoplast expansion [38], although its sub-cellular localization remains to be determined. Thus, phragmoplast expansion in tobacco may be regulated by a MAPK cascade, involving NPK1 MAPKKK, NtMEK1 MAPKK and p43<sup>Nt6</sup> MAPK, although evidence for the involvement of NtMEK1 and p43<sup>Nt6</sup> remains to be found.

In animal cells, active MAPKs have been shown to be localized to the spindle midzone and midbody, a cytokinetic bridge composed of MTs [43,44]. Although direct evidence for the involvement of MAPKs in cytokinesis is still lacking, we speculate that animal MAPKs might also regulate MT dynamics. In animal cells, cytokinesis proceeds in an outside-in mode by the constriction of the cortex. Therefore, spindle MTs must change their positions to move inwardly during cortex constriction because the width

of the spindle is limited by the cortex. This redistribution may involve MT turnovers. In addition, midbody MTs should disassemble upon cell separation. Thus, the mechanism that regulates MT dynamics during cytokinesis may be conserved in plants and animals.

## Conclusions

In this review, we highlight the molecular mechanism for the expansion of the phragmoplast during plant cytokinesis. Phragmoplast expansion is a process of repeated depolymerization and repolymerization, during which MT disassembly is linked to the progression of cell-plate formation. Changes in MT stability may be mediated by MAPs, such as various KLPs and NtMAP65-1 proteins that localize to the equator of the phragmoplast. The NPK1 MAPKKK, and probably a MAPK cascade initiated by NPK1, is involved in phragmoplast expansion. One of the possible functions of this pathway would be to regulate MT depolymerization in response to signals generated by the forming cell plate. Further research in this field will aim to elucidate the MT-disassembly signals, identify the target proteins of the MAPK pathway, and examine whether MAP activities are spatially regulated at the inner and outer edges of the phragmoplast.

## Acknowledgements

We thank Hiroki Yasuhara, Tetsuhiro Asada, and members of the Machida laboratory for helpful discussion. Work performed by the authors was supported by Grants-in-Aid for Scientific Research on Priority Areas (Award numbers 0678101-3 and 10182101-2) from the Japanese Ministry of Education, Science, Culture and Sports; and by a grant from the Research for the Future Program of the Japan Society for the Promotion of Science. RN was supported by Research Fellowships from the Japan Society for the Promotion of Science for Young Scientists.

## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Gunning BES: **The cytokinetic apparatus: its development and spatial regulation.** In *The Cytoskeleton in Plant Growth and Development*. Edited by Lloyd CW. London: Academic Press; 1982:229-292.
  2. Yasuhara H, Sonobe S, Shibaoka H: **Effects of brefeldin-A on the formation of the cell plate in tobacco BY-2 cells.** *Eur J Cell Biol* 1995, **66**:274-281.
  3. Sonobe S, Nakayama N, Shimmen T, Sone Y: **Intracellular distribution of subcellular organelles revealed by antibody against xyloglucan during cell cycle in tobacco BY-2 cells.** *Protoplasma* 2000, **213**:218-227.
- The authors of this paper report that xyloglucan, a polysaccharide that is used for the construction of the cell wall matrix, is transported from the Golgi to the cell plate via the phragmoplast.
4. Yokoyama R, Nishitani K: **Endoxyloglucan transferase is localized both in the cell plate and in the secretory pathway destined for the apoplast in tobacco cells.** *Plant Cell Physiol* 2001, **42**:292-300.
- Endoxyloglucan transferase, an enzyme responsible for forming and rearranging the cellulose-xyloglucan network of the cell wall, is shown to be transported from the Golgi to the cell plate via the phragmoplast.
5. Samuels AL, Giddings TH Jr, Staehelin LA: **Cytokinesis in tobacco BY-2 and root tip cells: a new model of cell plate formation in higher plants.** *J Cell Biol* 1995, **130**:1345-1357.
  6. Yasuhara H, Sonobe S, Shibaoka H: **Effects of taxol on the development of the cell plate and of the phragmoplast in tobacco BY-2 cells.** *Plant Cell Physiol* 1993, **34**:21-29.
  7. Heese M, Mayer U, Jürgens G: **Cytokinesis in flowering plants: cellular process and developmental integration.** *Curr Opin Plant Biol* 1998, **1**:486-491.
  8. Smith LG: **Divide and conquer: cytokinesis in plant cells.** *Curr Opin Plant Biol* 1999, **2**:447-453.
  9. Nacry P, Mayer U, Jürgens G: **Genetic dissection of cytokinesis.** *Plant Mol Biol* 2000, **43**:719-733.
  10. Verma DPS: **Cytokinesis and building of the cell plate in plants.** *Annu Rev Plant Physiol Plant Mol Biol* 2001, **52**:751-784.
  11. Vantard M, Levilliers N, Hill AM, Adoutte A, Lambert AM: **Incorporation of *Paramecium* axonemal tubulin into higher plant cells reveals functional sites of microtubule assembly.** *Proc Natl Acad Sci USA* 1990, **87**:8825-8829.
  12. Asada T, Sonobe S, Shibaoka H: **Microtubule translocation in the cytokinetic apparatus of cultured tobacco cells.** *Nature* 1991, **350**:238-241.
  13. Hush JM, Wadsworth P, Callahan DA, Hepler PK: **Quantification of microtubule dynamics in living plant cells using fluorescence redistribution after photobleaching.** *J Cell Sci* 1994, **107**:775-784.
  14. Mitchison T, Kirschner M: **Dynamic instability of microtubule growth.** *Nature* 1984, **312**:237-242.
  15. Walczak CE, Mitchison TJ, Desai A: **XKCM1: a *Xenopus* kinesin-related protein that regulates microtubule dynamics during mitotic spindle assembly.** *Cell* 1996, **84**:37-47.
  16. Desai A, Verma S, Mitchison TJ, Walczak CE: **Kin I kinesins are microtubule-destabilizing enzymes.** *Cell* 1999, **96**:69-78.
  17. Endow SA, Kang SJ, Satterwhite LL, Rose MD, Skeen VP, Salmon ED: **Yeast Kar3 is a minus-end microtubule motor protein that destabilizes microtubules preferentially at the minus ends.** *EMBO J* 1994, **13**:2708-2713.
  18. Hunter AW, Wordeman L: **How motor proteins influence microtubule polymerization dynamics.** *J Cell Sci* 2000, **113**:4379-4389.
  19. Lee YR, Liu B: **Identification of a phragmoplast-associated kinesin-related protein in higher plants.** *Curr Biol* 2000, **10**:797-800.
- The cloning of *AtPAKRP1* cDNA is reported. *AtPAKRP1* is the first kinesin-like protein shown to be localized to the phragmoplast equator. Microinjection of anti-*AtPAKRP1* antibodies into BY-2 cells results in the disorganization of the phragmoplast, implicating *AtPAKRP1* involvement in phragmoplast MT assembly.
20. Barroso C, Chan J, Allan V, Doonan J, Hussey P, Lloyd C: **Two kinesin-related proteins associated with the cold-stable cytoskeleton of carrot cells: characterization of a novel kinesin, DcKRP120-2.** *Plant J* 2000, **24**:859-868.
- The authors report the biochemical purification of two proteins with kinesin-like properties from carrot, and the identification of one of them as DcKRP120-2. The other purified protein is recognized by an anti-TKRP125 antibody. DcKRP120-2 is homologous to tobacco TKRP125 but, unlike TKRP125, it is localized to the phragmoplast equator in some cells.
21. Asada T, Kuriyama R, Shibaoka H: **TKRP125, a kinesin-related protein involved in the centrosome-independent organization of the cytokinetic apparatus in tobacco BY-2 cells.** *J Cell Sci* 1997, **110**:179-189.
  22. Bowser J, Reddy AS: **Localization of a kinesin-like calmodulin-binding protein in dividing cells of *Arabidopsis* and tobacco.** *Plant J* 1997, **12**:1429-1437.
  23. Liu B, Cyr RJ, Palevitz BA: **A kinesin-like protein, KatAp, in the cells of *Arabidopsis* and other plants.** *Plant Cell* 1996, **8**:119-132.
  24. Smertenko A, Saleh N, Igarashi H, Mori H, Hauser-Hahn I, Jiang CJ, Sonobe S, Lloyd CW, Hussey PJ: **A new class of microtubule-associated proteins in plants.** *Nat Cell Biol* 2000, **2**:750-753.
- The first cloning of the plant structural MAP genes NtMAP65-1a-c is reported. Recombinant NtMAP65-1a proteins stimulate MT polymerization *in vitro*. The localization of NtMAP65-1 proteins to the phragmoplast equator suggests that they are involved in the regulation of phragmoplast MT dynamics at the plus end.
25. Jiang W, Jimenez G, Wells NJ, Hope TJ, Wahl GM, Hunter T, Fukunaga R: **PRC1: a human mitotic spindle-associated CDK substrate protein required for cytokinesis.** *Mol Cell* 1998, **2**:877-885.

26. Pellman D, Bagget M, Tu YH, Fink GR, Tu H: **Two microtubule-associated proteins required for anaphase spindle movement in *Saccharomyces cerevisiae***. *J Cell Biol* 1995, **130**:1373-1385.
27. Juang YL, Huang J, Peters JM, McLaughlin ME, Tai CY, Pellman D: **APC-mediated proteolysis of Ase1 and the morphogenesis of the mitotic spindle**. *Science* 1997, **275**:1311-1314.
28. Smith LG, Gerttula SM, Han S, Levy J: **Tangled1: a microtubule binding protein required for the spatial control of cytokinesis in maize**. *J Cell Biol* 2001, **152**:231-236.  
The *tangled1* gene is involved in the orientation of cytoskeletal structures. The authors show that it encodes a protein that has a basic region that is similar to those of vertebrate APC proteins, which bind to MTs. Tangled1 protein binds to MTs *in vitro* and to mitotic and cytokinetic MT structures *in vivo*.
29. Whittington AT, Vugrek O, Wei KJ, Hasenbein NG, Sugimoto K, Rashbrooke MC, Wasteneys GO: **MOR1 is essential for organizing cortical microtubules in plants**. *Nature* 2001, **411**:610-613.  
*Temperature-sensitive (ts)* mutations in the *MICROTUBULE ORGANIZATION 1 (MOR1)* gene result in the disruption of cortical MTs at the restrictive temperature. Cloning of *MOR1* revealed it to encode a HEAT-domain-containing protein that is homologous to animal MAPs including *Xenopus* XMAP215 and human TOGp, which regulate MT dynamics during mitosis. It would be interesting to test MOR1 involvement in the regulation of phragmoplast MT dynamics, although *ts* mutations do not affect mitotic and cytokinetic MT arrays.
30. Tirnauer JS, Bierer BE: **EB1 proteins regulate microtubule dynamics, cell polarity, and chromosome stability**. *J Cell Biol* 2000, **149**:761-766.
31. Schuyler SC, Pellman D: **Microtubule 'plus-end-tracking proteins': the end is just the beginning**. *Cell* 2001, **105**:421-424.
32. Yasuhara H, Shibaoka H: **Inhibition of cell-plate formation by brefeldin A inhibited the depolymerization of microtubules in the central region of the phragmoplast**. *Plant Cell Physiol* 2000, **41**:300-310.
33. Samuels AL, Staehelin LA: **Caffeine inhibits cell plate formation by disrupting membrane reorganization just after the vesicle fusion step**. *Protoplasma* 1996, **195**:144-155.
34. Valster AH, Hepler PK: **Caffeine inhibition of cytokinesis: effect on the phragmoplast cytoskeleton in living *Tradescantia* stamen hair cells**. *Protoplasma* 1997, **196**:155-166.
35. Staehelin LA, Hepler PK: **Cytokinesis in higher plants**. *Cell* 1996, **84**:821-824.
36. Calderini O, Bögre L, Vicente O, Binarova P, Heberle-Bors E, Wilson C: **A cell cycle regulated MAP kinase with a possible role in cytokinesis in tobacco cells**. *J Cell Sci* 1998, **111**:3091-3100.
37. Bögre L, Calderini O, Binarova P, Mattauch M, Till S, Kiegerl S, Jonak C, Pollaschek C, Barker P, Huskisson NS *et al.*: **A MAP kinase is activated late in plant mitosis and becomes localized to the plane of cell division**. *Plant Cell* 1999, **11**:101-113.
38. Calderini O, Glab N, Bergounioux C, Heberle-Bors E, Wilson C: **A novel tobacco mitogen-activated protein (MAP) kinase kinase, NtMEK1, activates the cell cycle-regulated p43<sup>Nt6</sup> MAP kinase**. *J Biol Chem* 2001, **276**:18139-18145.
39. Nishihama R, Ishikawa M, Araki S, Soyano T, Asada T, Machida Y: **The NPK1 mitogen-activated protein kinase kinase kinase is a regulator of cell-plate formation in plant cytokinesis**. *Genes Dev* 2001, **15**:352-363.  
Cell-biological and reverse-genetic analyses reveal the involvement of the NPK1 MAPKKK in cell-plate formation. NPK1 is localized to the phragmoplast equator. Overexpression of a kinase-negative NPK1 inhibits phragmoplast expansion, resulting in the formation of incomplete cell plates.
40. English J, Pearson G, Wilsbacher J, Swantek J, Karandikar M, Xu S, Cobb MH: **New insights into the control of MAP kinase pathways**. *Exp Cell Res* 1999, **253**:255-270.
41. Banno H, Hirano K, Nakamura T, Irie K, Nomoto S, Matsumoto K, Machida Y: **NPK1, a tobacco gene that encodes a protein with a domain homologous to yeast BCK1, STE11, and Byr2 protein kinases**. *Mol Cell Biol* 1993, **13**:4745-4752.
42. Nakashima M, Hirano K, Nakashima S, Banno H, Nishihama R, Machida Y: **The expression pattern of the gene for NPK1 protein kinase related to mitogen-activated protein kinase kinase kinase (MAPKKK) in a tobacco plant: correlation with cell proliferation**. *Plant Cell Physiol* 1998, **39**:690-700.
43. Shapiro PS, Vaisberg E, Hunt AJ, Tolwinski NS, Whalen AM, McIntosh JR, Ahn NG: **Activation of the MKK/ERK pathway during somatic cell mitosis: direct interactions of active ERK with kinetochores and regulation of the mitotic 3F3/2 phosphoantigen**. *J Cell Biol* 1998, **142**:1533-1545.
44. Zecevic M, Catling AD, Eblen ST, Renzi L, Hittle JC, Yen TJ, Gorbsky GJ, Weber MJ: **Active MAP kinase in mitosis: localization at kinetochores and association with the motor protein CENP-E**. *J Cell Biol* 1998, **142**:1547-1558.