

IN THIS ISSUE

High-Resolution Imaging of Cortical Microtubule Arrays

Plant cortical microtubules (CMTs) form highly organized arrays in the cell cortex adjacent to the plasma membrane. In the early 1960s, it was discovered that glutaraldehyde fixation could preserve the fine structure of microtubules for electron microscopy (Ledbetter and Porter, 1963; Hepler and Newcomb, 1964). Ledbetter and Porter (1963) asked whether there might be any organization within the cytoplasm that “anticipates and influences the deposition of specific patterns of thickenings in secondary walls of plant cells.” They studied the fine structure of CMTs from the grass *Phleum pratense* and thought it could be of significance that microtubules lying beneath the surface of the protoplast mirrored the orientation of cellulose microfibrils in the adjacent cell walls. Hepler and Newcomb (1964), working with *Coleus blumei*, showed that groupings of CMTs were aligned parallel to underlying bands of cellulose in the secondary wall of differentiating xylem elements, providing more solid support for a functional connection between CMTs and cellulose deposition.

Since that time, evidence has accumulated that CMT arrays play a fundamental role in controlling plant morphogenesis, as they form patterns or tracks that guide cellulose microfibril deposition within the cell wall on the outer surface of the plasma membrane, thereby influencing cell shape (reviewed in Lucas and Shaw, 2008). Although a great deal of progress has been made in understanding the finer details of CMT array assembly, organization, and function, there is much that remains to be discovered to determine in what ways CMT arrays influence cell morphology during development and in response to environmental signals.

Microtubules are made up of α - and β -tubulin dimers, which polymerize to form protofilaments that fuse together and curl into cylindrical filaments. Hardham and Gunning (1978) presented an important study describing the basic structure of plant CMT arrays from high-resolution

electron microscopy of glutaraldehyde-fixed root tip sections of *Azolla pinnata*, *Impatiens balsamina*, and *Zea mays*. They were the first to report that the CMT array is made up of relatively short overlapping microtubules, with an average length considerably shorter than the cell circumference, rather than forming continuous hoops of individual microtubules that could encircle the entire cell. These early investigators of CMT structure observed microtubules in CMT arrays that were randomly aligned and lying deeper in the cytoplasm than the more organized microtubules adjacent to the plasma membrane (Ledbetter and Porter, 1963; Hardham and Gunning, 1978). However, they could not make more detailed studies of these microtubules, as their positions were difficult to track through serial ultrathin sections. Hardham and Gunning (1978) also noted the presence of cross-bridges between individual microtubules, which had been observed in several previous studies (reviewed in Hepler and Palevitz, 1974).

In recent years, genetic studies, combined with fluorescent labeling of proteins and high-resolution microscopy, have led to the identification of a number of microtubule-associated proteins (MAPs) with key functions in CMT array organization and dynamics. Some of the main players are γ -tubulin, katanin, MAP65 proteins, and END BINDING1 (EB1) proteins. γ -Tubulin acts to nucleate microtubule formation at sites dispersed along the length of existing microtubules (Shaw et al., 2003; Murata et al., 2005), and katanin severs new microtubules from their nucleation sites (Stoppin-Mellet et al., 2002, 2006). EB1 is localized to microtubule plus ends and has a function in promoting end growth (Bisgrove et al., 2004) and also plays a role in root responses to touch and gravity signals (Bisgrove et al., 2008). MAP65 proteins form cross-bridges between microtubules, which likely function to maintain and stabilize microtubule bundles within the CMT array (Van Damme et al., 2004).

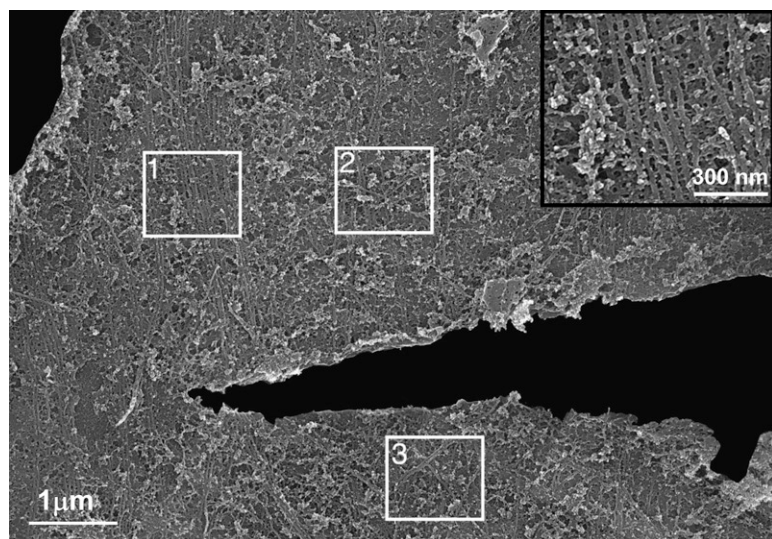
In this issue of *The Plant Cell*, **Barton et al. (pages 982–994)** analyzed CMT ar-

rays of *Tradescantia virginiana* using high-resolution scanning electron microscopy (HRSEM). The authors conducted HRSEM on segments of epidermal peels from the outer surface of young leaves and used immunogold labeling with antibodies against α -tubulin, EB1, and katanin to determine protein localization. They also compared HRSEM results to those obtained from confocal light microscopy, allowing for a clear illustration of the resolution limitations of light microscopy.

In HRSEM images, they identified two spatially discrete subpopulations of microtubules, one consisting predominantly of highly ordered bundles positioned directly adjacent to the plasma membrane and another of more randomly aligned discordant microtubules, showing less stability and lying deeper in the cytoplasm (see figure), confirming the similar observations by earlier workers (Ledbetter and Porter, 1963; Hardham and Gunning, 1978). Barton et al. propose that the discordant layer represents the most newly formed microtubules and suggest that nucleation on the cytoplasmic side of the ordered reticulum adjacent to the membrane would allow new microtubules to grow unimpeded by other microtubules. They further speculate that the discordant layer might play a role in sensing environmental signals, as it could respond (in terms of influencing cell growth and morphology) more quickly than the stable layer of the CMT array on the plasma membrane. This prompts intriguing questions, not least of which is, how might experiments be designed to assess whether or not the discordant layer has a specific function? First, more convincing evidence is needed that the discordant layer represents the most newly formed microtubules. Other questions include, how do CMTs in the discordant layer interact with those of the ordered array adjacent to the plasma membrane? Are there differences in the presence or activities of MAPs associated with the two layers?

Barton et al. observed microtubule interactions either as steep angle encounters

IN THIS ISSUE



Fine Structure of a Cortical Microtubule Array.

The image shows a portion of a montage composed of 104 HRSEM images revealing the intricate construction of a CMT array of a *T. virginiana* leaf epidermal cell. The complete montage is available as supplemental data online, wherein it is possible to zoom in to see individual microtubules. The inset within the black box (top right) shows a magnification of Box 1. Numbered boxes correspond to individual HRSEM images presented in Figures 1 and 3 in Barton et al. (2008).

resulting in crossovers or shallow angle encounters resulting in bundling, consistent with observations of microtubule encounters in live cells. Dixit and Cyr (2004) showed that microtubules in cultured tobacco cells tend to depolymerize at acute angles of collision and co-align at shallow angles of collision, where the critical angle of transition was $\sim 40^\circ$. It has also been shown that new microtubules arise from existing microtubules at a characteristic angle of 40° (Murata et al., 2005) and therefore appear to be optimally positioned to adopt one of these two fates. The definitive angle measured by Barton et al. was $\sim 20^\circ$ and also related to the planes in which microtubules interacted. Shallow angle encounters were observed more often on the plasma membrane, whereas steep angle encounters occurred more often in the discordant layer where microtubules crossed other microtubules. This is one of the observations leading them to conclude that the discordant layer contains newly formed microtubules. They rarely observed bundling at angles over 30° , possibly because the growing microtubule ends had initiated depolymerization.

The authors also found that their data support the estimate of Hardham and Gunning (1978) that the average microtubule length in a cell is equal to about one-eighth of the cell circumference and suggest that innate cell dimensions might influence microtubule length. Considering that CMT arrays disassemble before mitosis and reassemble during cytokinesis, it is also conceivable that microtubule length might influence cell size and shape. It has been shown that cellulose synthase complexes follow precisely in tracks created by microtubules in the CMT array (Paredes et al., 2006). Wasteneys and Fujita (2006) have shown that microtubule organization in the CMT array can influence the length of cellulose microfibrils that are produced, which in turn has a major effect on cell size and shape, but whether or how microtubule length itself might influence cellulose biosynthesis has not been examined.

Barton et al. examined localization of the MAPs EB1 and katanin using immunogold labeling coupled with HSREM. EB1 was found dispersed along the lengths of cortical microtubules and decorated junctions

where adjacent microtubules aligned into bundles. EB1 proteins were also observed at microtubule ends and on the plasma membrane directly past microtubule ends. Chan et al. (2003) also reported localization of an EB1-GFP fusion at discrete foci on the plasma membrane. Barton et al. suggest that EB1 may form part of a complex that links microtubules to the plasma membrane and plays a direct role in influencing cell growth and morphology. Katanin was also found to be localized along cortical microtubules. Katanin labeling was occasionally observed on microtubule ends in close proximity and in line with neighboring microtubule ends, suggesting that the two ends might have formed a single microtubule that was severed by katanin activity.

The work of Barton et al. provides detailed high-resolution images of the CMT array in plant cells that makes an important contribution to our understanding of microtubule dynamics and CMT array organization and function. The work is also an important reminder of the limitations of light microscopy. Finally, the observations raise interesting questions about the nature and function of the discordant layer of microtubules.

Nancy A. Eckardt
News and Reviews Editor
neckardt@aspb.org

REFERENCES

- Barton, D.A., Vantard, M., and Overall, R.L. (2008). Analysis of cortical arrays from *Tradescantia virginiana* at high resolution reveals discrete microtubule subpopulations and demonstrates that confocal images of arrays can be misleading. *Plant Cell* **20**: 982–994.
- Bisgrove, S.R., Hable, W.E., and Kropf, D.L. (2004). +TIPs and microtubule regulation. The beginning of the plus end in plants. *Plant Physiol.* **136**: 3855–3863.
- Bisgrove, S.R., Lee, Y.R., Liu, B., Peters, N.T., and Kropf, D.L. (2008). The microtubule plus-end binding protein EB1 functions in root responses to touch and gravity signals in *Arabidopsis*. *Plant Cell* **20**: 396–410.
- Chan, J., Calder, G.M., Doonan, J.H., and Lloyd, C.W. (2003). EB1 reveals mobile microtubule nucleation sites in *Arabidopsis*. *Nat. Cell Biol.* **5**: 967–971.

IN THIS ISSUE

- Dixit, R., and Cyr, R.** (2004). Encounters between dynamic cortical microtubules promote ordering of the cortical array through angle-dependent modifications of microtubule behavior. *Plant Cell* **16**: 3274–3284.
- Hardham, A.R., and Gunning, B.E.S.** (1978). Structure of cortical microtubule arrays in plant cells. *J. Cell Biol.* **77**: 14–34.
- Hepler, P.K., and Newcomb, E.H.** (1964). Microtubules and fibrils in the cytoplasm of *Coleus* cell undergoing secondary wall deposition. *J. Cell Biol.* **20**: 529–533.
- Hepler, P.K., and Palevitz, B.A.** (1974). Microtubules and microfilaments. *Annu. Rev. Plant Physiol.* **25**: 309–362.
- Ledbetter, M.C., and Porter, K.R.** (1963). A “microtubule” in plant cell fine structure. *J. Cell Biol.* **19**: 239–250.
- Lucas, J., and Shaw, S.L.** (2008). Cortical microtubule arrays in the *Arabidopsis* seedling. *Curr. Opin. Plant Biol.* **11**: 94–98.
- Murata, T., Sonobe, S., Baskin, T.I., Hyodo, S., Hasezawa, S., Nagata, T., Horio, T., and Hasebe, M.** (2005). Microtubule-dependent microtubule nucleation based on recruitment of γ -tubulin in higher plants. *Nat. Cell Biol.* **7**: 961–968.
- Paredes, A.R., Somerville, C.R., and Ehrhardt, D.W.** (2006). Visualization of cellulose synthase demonstrates functional association with microtubules. *Science* **312**: 1491–1495.
- Shaw, S.L., Kamyar, R., and Ehrhardt, D.W.** (2003). Sustained microtubule treadmilling in *Arabidopsis* cortical arrays. *Science* **300**: 1715–1718.
- Stoppin-Mellet, V., Gaillard, J., and Vantard, M.** (2002). Functional evidence for *in vitro* microtubule severing by the plant katanin homologue. *Biochem. J.* **365**: 337–342.
- Stoppin-Mellet, V., Gaillard, J., and Vantard, M.** (2006). Katanin's severing activity favors bundling of cortical microtubules in plants. *Plant J.* **46**: 1009–1017.
- Van Damme, D., Van Poucke, K., Boutant, E., Ritzenthaler, C., Inze, D., and Geelen, D.** (2004). In vivo dynamics and differential microtubule-binding activities of MAP65 proteins. *Plant Physiol.* **136**: 3956–3967.
- Wasteneys, G.O., and Fujita, M.** (2006). Establishing and maintaining axial growth: wall mechanical properties and the cytoskeleton. *J. Plant Res.* **119**: 5–10.

High-Resolution Imaging of Cortical Microtubule Arrays

Nancy A. Eckardt

Plant Cell 2008;20;817-819; originally published online April 25, 2008;

DOI 10.1105/tpc.108.060228

This information is current as of July 19, 2018

References	This article cites 17 articles, 10 of which can be accessed free at: /content/20/4/817.full.html#ref-list-1
Permissions	https://www.copyright.com/ccc/openurl.do?sid=pd_hw1532298X&issn=1532298X&WT.mc_id=pd_hw1532298X
eTOCs	Sign up for eTOCs at: http://www.plantcell.org/cgi/alerts/ctmain
CiteTrack Alerts	Sign up for CiteTrack Alerts at: http://www.plantcell.org/cgi/alerts/ctmain
Subscription Information	Subscription Information for <i>The Plant Cell</i> and <i>Plant Physiology</i> is available at: http://www.aspb.org/publications/subscriptions.cfm