

Microtubule organization in the green kingdom: chaos or self-order?

Geoffrey O. Wasteneys

Plant Cell Biology Group, Research School of Biological Sciences, The Australian National University, GPO Box 475, Canberra ACT 2601, Australia

(e-mail: geoffw@rsbs.anu.edu.au)

Journal of Cell Science 115, 1345-1354 (2002) © The Company of Biologists Ltd

Summary

Plant microtubule arrays differ fundamentally from their animal, fungal and protistan counterparts. These differences largely reflect the requirements of plant composite polymer cell walls and probably also relate to the acquisition of chloroplasts. Plant microtubules are usually dispersed and lack conspicuous organizing centres. The key to understanding this dispersed nature is the identification of proteins that interact with and regulate the spatial and dynamic properties of microtubules. Over the past decade, a number of these proteins have been uncovered, including numerous kinesin-related proteins and a 65 kDa class of structural microtubule-associated proteins that appear to be unique to plants. Mutational analysis has identified

MOR1, a probable stabilizer of microtubules that is a homologue of the TOGp-XMAP215 class of high-molecular-weight microtubule-associated proteins, and a katanin p60 subunit homologue implicated in the severing of microtubules. The identification of these two proteins provides new insights into the mechanisms controlling microtubule assembly and dynamics, particularly in the dispersed cortical array found in highly polarized plant cells.

Key words: Microtubule-associated protein, Microtubule-organizing centre, Katanin, MOR1, Centrosome, γ -Tubulin, Plant cell

Introduction

For over a billion years, plants, animals, fungi and protists have been acquiring unique characteristics. Microtubules are common to all eukaryotic kingdoms and are excellent markers of both conservation and specialization. On the one hand, microtubule fine structure and the tubulin subunits that make up microtubules are remarkably conserved between kingdoms, as are many microtubule-associated proteins (MAPs). On the other hand, microtubules are arranged and organized in highly diverse patterns, relying on a variety of mechanisms for assembly, orientation and function. We tend to think first about microtubules as the major structural components of mitotic spindles and flagellae. In these cases, microtubules emanate from centrosomes, microtubule-nucleating complexes that are focused around a microtubule-derived apparatus known as a centriole. Centrosomes can act interchangeably as spindle poles, anchors for the radial interphase array or basal bodies, in which case they are referred to as kinetosomes (Chapman et al., 2000). Textbooks tell us that minus ends of microtubules associate with centrosomes and that their growth by dynamic instability is coordinated by GTP caps at the fast-growing end. Without centrosomes, the concentration of tubulin subunits in cells would be far too low to allow microtubule nucleation.

It turns out, however, that centrosomes are a disadvantage for highly polarized cells. Many microtubules in neurons and epithelial cells are disconnected from the centrosome, metazoan oocytes have no centrosomes at all (Megraw and Kaufman, 2000) and, although yeast spindle pole bodies behave like centrosomes, they lack centrioles (Balczon, 1996). In fact, centrosomes are absent from up to half of known

eukaryotic species including most fungi, protists and vascular plants and from the spindles and interphase arrays of many algae. In vascular and many nonvascular plants, somatic cells have dispersed with centrosomes altogether (Vaughn and Harper, 1998). Among the 'higher' seed-producing plants, only two orders of gymnosperm, the cycads and ginkgoes, retain flagellated sperm (Southworth and Cresti, 1997). Dispersed plant microtubule arrays lack tightly focused organizing centres. In this context, the freedom from centrosomes may be a defining characteristic that has helped plants to evolve into organisms that are autotrophic and sessile but highly responsive to their environment.

Microtubule organization in plants is closely connected to the special features of cell walls

To understand microtubule organization in plant cells, it is necessary to take stock of the full range of arrays. Those arrays found in typical somatic cells are illustrated in Fig. 1. With the exception of mitotic spindles, whose function in separating chromosomes and chromatids is conserved, plant microtubule arrays do things that relate to the development of the plant cell wall and, hence, to plant cell shape and growth polarity. In the G2 phase of the cell cycle, the first sign of the approaching mitosis is the preprophase band*, comprising prominent bundles of microtubules that generally, but not always, encircle the midplane (Fig. 1A). Preprophase bands prepare the

*Preprophase bands appear to be closely appressed to the plasma membrane but in transverse sections examined by transmission electron microscopy, they form dense bundles that extend some distance into the cytoplasm.

eventual site of cell plate attachment, probably by locally altering the wall properties (Mineyuki, 1999), but disappear long before this event. The recent sighting of Golgi stacks at the division site during metaphase (Nebenfuhr et al., 2000) supports this idea. The signalling mechanisms that determine preprophase band location, particularly in asymmetrically dividing cells, which are critical for cell fate determination and tissue differentiation, remain a mystery. Once determined, however, a tight connection between the preprophase band and nucleus, via the microtubule- and actin filament-containing phragmosome*, seems essential for consolidating the preprophase band (Gunning and Wick, 1985). Using a microtubule-binding fluorescent reporter in living cells, Granger and Cyr recently confirmed that nuclear positioning is critical to downstream mitotic events (Granger and Cyr, 2001). They propose that the phragmosome-PPB complex plays a key role in the proper positioning of the preprophase nucleus, which in turn influences orientation of the mitotic and cytokinetic structures.

Dissolution of the preprophase band and nuclear envelope coincides with formation of the mitotic spindle (Fig. 1B). Spindle poles are typically broad, not tightly focused as in centrosome-containing cells. At the anaphase-telophase transition, the phragmoplast[†] forms (Fig. 1C,D). Like spindles, phragmoplasts are bipolar complexes with their plus ends meeting at the midplane. They direct the transport of Golgi-derived vesicles towards the centrifugally expanding cell plate, which matures to become the cross-wall separating daughter cells (Otegui and Staehelin, 2000a). Phragmoplast microtubules originate as a compact cylindrical bundle between the condensing chromatin of daughter nuclei (Fig. 1C), but gradually form a self-organizing double ring that increases in circumference in pace with the cell plate as it expands towards the parent wall (Fig. 1D). Phragmoplasts are also part of the cytokinetic apparatus during cellularization of syncytial cells, which include endosperm[‡], microspores[§] and the female gametophyte. In these cells, so-called adventitious phragmoplasts form at the nuclear-cytoplasmic domains, which are defined by the microtubules radiating from adjacent nuclei (Brown and Lemmon, 2001a; Otegui and Staehelin, 2000b). By observing the incorporation of fluorescent tubulin, it has been determined that phragmoplast microtubules continually add subunits at the plus ends, while units are lost at the minus ends (Asada and Shibaoka, 1991). This treadmill maintains the GTP cap, ensuring long-term microtubule survival.

As cells enter interphase or commit to terminal differentiation, microtubules are abundant at the periphery of the nucleus and appear to radiate towards the cell periphery (Fig. 1E). This perinuclear microtubule array is transient but real, as confirmed in living cells expressing a GFP-tubulin fusion protein (Hasezawa et al., 2000). Soon after this stage, microtubules are found throughout the cell periphery, often in

parallel order, in close association with the plasma membrane (Fig. 1F). These cortical microtubules play a critical role in controlling growth direction, both in cells that enlarge by diffuse growth* and in those that enlarge by tip growth[†] (Bibikova et al., 1999; Geitmann and Emons, 2000). They also play important roles in generating localized wall ingrowths after cells stop expanding in, for example, the formation of vascular tissues[‡] (Chaffey et al., 1997; Chaffey et al., 2000;) and transfer cells[§] (Bulbert et al., 1998; Singh et al., 1999). Microtubule involvement in wall formation and cell expansion is too complex a subject for this Commentary but is discussed in recent review articles (Baskin, 2001; Wasteneys, 2000).

The cortical array exemplifies the enigmatic nature of plant microtubules. Given no clear organizing centres, where do the cortical microtubules assemble and what determines their orientation? Despite nearly four decades of study, we know very little about microtubule assembly and orientation in the various arrays that coordinate the plant cell through division, polar expansion and terminal differentiation. In the next section, I speculate on how the acquisition and evolution of plant-specific features have, by necessity, influenced microtubule organization, and I outline two models for the self-organization of plant microtubule arrays.

Chloroplasts, cell walls and a different sort of motility

It can be argued that the combination of two defining features of plants, the cell wall and chloroplasts, was the impetus for the evolution of dispersed microtubule organization. For chloroplasts to be retained in cell lineages, primordial chloroplast division needed to be coordinated with that of the host cell. Many primitive plants including some algae, all bryophytes, certain vascular cryptogams and at least one group of ferns, have only one large chloroplast per cell. During division, the plastid surface serves as a microtubule-organizing centre for the formation of midzone and spindle microtubules (Brown and Lemmon, 2001b). The need to couple monoplastidic division with cytokinesis in ancestors of higher plants may have necessitated the abolition of the nucleus-associated centrosome. Plant cells in more advanced, multi-plastidic vascular plants lack plastid-associated microtubule-organizing centres but retain the legacy of microtubule-organizing centres that extend beyond the confines of the nucleus.

The other feature distinguishing plants is of course the cell wall. Coordinated synthesis and loosening of the largely polysaccharide wall material to turgor pressure drives plant cell expansion. But this type of growth requires the bulk of cell volume, which can be considerable, to be occupied by turgor-regulating vacuoles. As posited by Gunning, microtubule-based intracellular motility might be inadequate for the metabolic requirement for cytoplasmic mixing in highly

*Phragmosomes are bands of microtubules and actin filaments that span the distance between the nucleus and the preprophase band, often through transvacuolar strands.

[†]Phragmoplasts comprise centrifugally expanding bipolar arrays of microtubules that initiate as a concentrated bundle in the midzone between the daughter nuclei at telophase.

[‡]A triploid tissue derived from one sperm and two polar nuclei of the female gametophyte; nourishes the zygotic embryo.

[§]Microspores mature into pollen grains, the male gametophyte, by highly polarized cell divisions, giving rise in angiosperms to a diffuse vegetative nucleus and two sperm cells, carried within the pollen tube.

*Diffuse growth involves incorporation of new wall material and turgor-driven stretching of that material over the entire surface of the cell. Cells typically expand along one axis that is at right angles to the predominant microtubule orientation.

[†]In tip-growth, wall loosening and synthesis is localized to one part of the cell.

[‡]Vascular tissues provide conduits for water, nutrients and metabolites and provide mechanical support to enable land plants to become free-standing structures of impressive stature.

[§]Transfer cells have wall ingrowths to increase the surface area of plasma membrane for efficient exchange of nutrients.

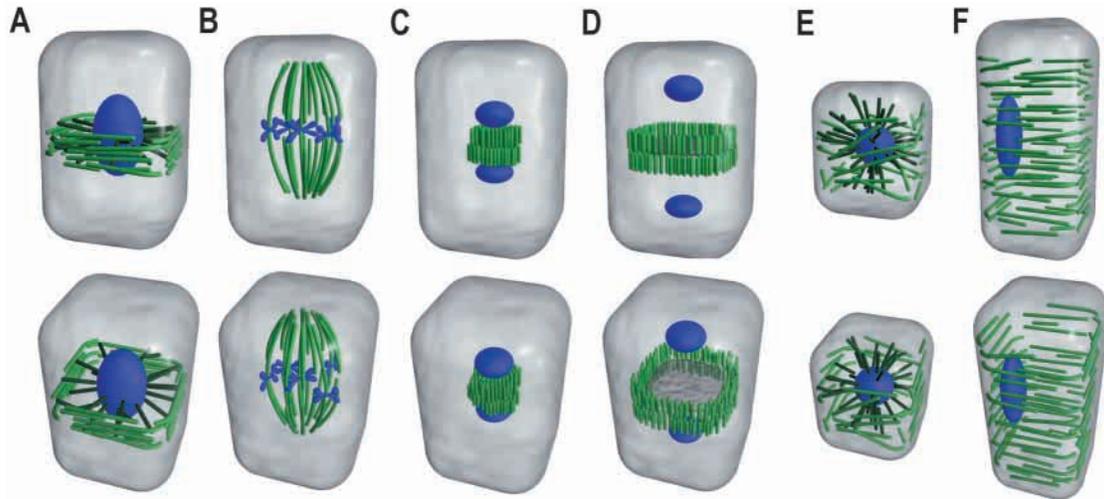


Fig. 1. These schematic illustrations, rendered in 3D at two aspects, show microtubule arrays through the plant cell cycle. (A) A preprophase band, linked to the nucleus by phragmosome microtubules, marks the future division site. (B) Metaphase spindle with a dispersed polar region. (C) In telophase, the phragmoplast forms as a concentrated cylinder of microtubules between daughter nuclei. (D) The cytokinetic phragmoplast expands centrifugally, leading the cell plate towards attachment sites previously established by the preprophase band. Microtubule plus ends meet at midplane. (E) Once cytokinesis is complete, microtubules extend from the nucleus toward the cell cortex and plasma membrane-associated microtubules appear. (F) Plant cells in interphase and those entering terminal differentiation often expand predominantly in one direction. During cell elongation, cortical microtubules are usually arranged in parallel arrays whose predominant orientation is at right angles to the axis of expansion.

vacuolated plant cells (Gunning, 1999). This, along with the need to position chloroplasts optimally in relation to light sources (Liebe and Menzel, 1995; Kandasamy and Meagher, 1999), is likely to have led to actomyosin becoming the dominant motile system in plant cells. Actin cables provide tracks for movement of myosin-coated vesicles, endoplasmic reticulum and other organelles, which in animal cells are largely moved about by microtubule-dependent motors (Boevink et al., 1998). At speeds up to 100 μm per second, myosin-driven movement in plant cells is in a class of its own. There is surprisingly little evidence that plant microtubules participate in active transport and cytoplasmic streaming but, unlike in animal cells, microtubules, not actin filaments, are the dominant element at the plasma membrane. The concept of a cytoskeleton–plasma-membrane–cell-wall continuum in plant cells (Wyatt and Carpita, 1993) necessarily emphasizes the microtubule component.

Motor proteins organize microtubule converging centres

If actomyosin-based activity drives much of the intracellular motility, what are all the kinesin-related proteins that have been identified doing? One emerging concept is that the ability to organize decentralized microtubule arrays depends to a large extent on microtubule self-organization. This may be coordinated largely by the activities of motor proteins, which in plants include a remarkable variety of minus- and plus-end-directed kinesins (Liu and Lee, 2001). In phragmoplasts, the relative activities of several kinesin-related proteins coordinate the extent of overlapping between antiparallel microtubules at the midplane (Liu and Lee, 2001; Lloyd and Hussey, 2001). Analysis of microtubule patterns in the multinucleate endosperm tissue led to the concept that microtubule-

converging centres link microtubule minus ends to control the predominant direction of elongation and shortening of microtubule arrays (Smirnova and Bajer, 1994; Smirnova and Bajer, 1998). Converging centres no doubt also operate at the minus ends of phragmoplast microtubules to maintain the integrity of the array as it expands centrifugally.

Do cortical microtubules grow on fractal trees?

A variation on the convergence mechanism seems to operate in the formation of plasma-membrane-associated arrays in walled cells during interphase and terminal differentiation. Analyzing cortical microtubule patterns after drug-induced disassembly (Fig. 2) led to the ‘branching cluster’ model for cortical microtubule assembly in plant cells (Wasteneys and Williamson, 1989a; Wasteneys, 1992). According to this model, microtubule-initiating factors move along existing microtubule ‘tracks’ and nucleate the assembly of new microtubules, which diverge from pre-existing microtubules at acute angles. The model predicts that plus-end-directed motors disperse initiating factors along microtubule tracks. The extent of motor activity is likely to be coupled to the rate of cell expansion; non-growing cells form tightly focused clusters, whereas rapidly expanding cells re-form cortical microtubules in more open, fractal tree patterns (Wasteneys and Williamson, 1989a). This form of self-organized assembly provides a plausible mechanism for generating even dispersal of microtubule-initiating factors in expanding cells.

Very recent discoveries of microtubule-associated proteins (MAPs) in plants provide the first opportunity to test these self-organization models. In the remainder of this article, the properties of two of these newly identified MAPs are detailed and I discuss their potential roles in microtubule organization

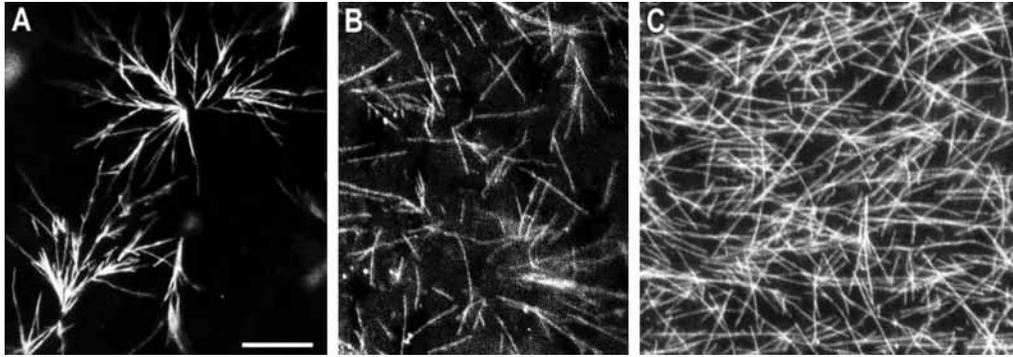


Fig. 2. Cortical microtubule recovery patterns after drug-induced microtubule disassembly. (A) Microtubules appear to diverge from the initial assembly site, forming fractal tree-shaped clusters, with microtubules diverging from each other at acute angles (figure adapted from Wasteneys and Williamson, 1989b). (B) Clusters eventually break up. (C) Later in recovery, parallel microtubule order begins to consolidate but some branching configurations and discordant microtubules persist. Bar, 10 μm .

and function, emphasizing the establishment of the cortical microtubule array at the onset of cell expansion.

Molecular approaches have identified several plant microtubule-organizing and accessory proteins

Microtubule-associated proteins (MAPs) that nucleate, stabilize and destabilize, and crosslink and anchor microtubules are all required to organize plant microtubules. The identity of these so-called structural MAPs remained elusive for some time in plant cells despite evidence for their presence in early electron micrographs (Hardham and Gunning, 1978). Extracting and identifying MAPs is hindered by the recalcitrance of plant material to biochemical approaches. Whereas MAPs are relatively easy to purify from brain tissue, which is teeming with microtubules, plant tissues have modest concentrations of microtubule proteins. Plant cells are usually cytoplasm-poor, most cell volume being occupied by vacuoles, whose rupture releases proteolytic enzymes that hamper purification strategies.

In contrast, genomic and proteomic approaches have proved to be very effective. Plant tubulins, the building blocks of microtubules, were quickly identified on the basis of their conserved amino acid sequences (Hussey et al., 1990; Kopczak et al., 1992), as were γ -tubulin (Liu et al., 1994) and numerous kinesin-like motor proteins (Asada and Collings, 1997). Homology searches for plant structural MAPs were at first largely unsuccessful, although some candidates, isolated by microtubule affinity, crossreact with antibodies to the animal MAPs *tau* (Vantard et al., 1991) and MAP4 (Maekawa et al., 1990; Higashiyama et al., 1996). As noted by Lloyd and Hussey, homology-based approaches are useful but do not uncover weakly related proteins, or novel proteins, which may not be recognized as having MAP function (Lloyd and Hussey, 2001).

A plant-specific MAP?

MAP65 was first isolated from cytoplasmic extracts of evacuated protoplasts (Chang-Jie and Sonobe, 1993). Patrick Hussey and colleagues recently isolated three clones from a tobacco BY-2 cDNA library using antibodies raised to

biochemically isolated protein (Smertenko et al., 2000). Detailed analysis of one clone, *NtMAP65-1*, suggests that its product may be involved in the overlapping of anti-parallel microtubules in the spindle and phragmoplast. This possibility suggests that the MAP65 protein works with several kinesin-related proteins to establish and regulate contact between antiparallel phragmoplast microtubules at the midplane (Lloyd and Hussey, 2001). *NtMAP65-1*-specific antibodies also label the preprophase band and a subset of cortical microtubules (Smertenko et al., 2000), and MAP65 isolated from carrot cultures forms regular crossbridges between adjacent microtubules in vitro (Chan et al., 1999). These results indicate that MAP65 also maintains inter-microtubule spacing in preprophase bands and interphase microtubule arrays. According to its sequence, the 65 kDa family of structural MAPs appears to be unique to plants.

Mutational analysis

In recent years, several groups have adopted mutational approaches to understand microtubule assembly and function. A collection of cytoskeleton-defective mutants, identified mainly in thale cress (*Arabidopsis thaliana*) and maize (*Zea mays*), is now available (Kost et al., 1999; Wasteneys, 2000; Azimzadeh et al., 2001). Two new mutant loci recently joined the collection. One locus encodes a homologue of the microtubule-severing protein, katanin p60 subunit (Bichet et al., 2001; Burk et al., 2001; McClinton et al., 2001). The other encodes a member of the TOGp-XMAP215-Dis1 family of high molecular weight MAPs (Whittington et al., 2001). The discovery of these mutants, whose phenotypes provide strong clues about the function of katanin p60 and MOR1 proteins, provides an opportunity to update our concepts of microtubule organization in plant cells.

Katanin p60 adds severing to the repertoire of mechanisms controlling cortical microtubule organization

The *Arabidopsis* homologue of katanin P60*, AtKSS

**Arabidopsis thaliana* katanin-like protein small subunit.

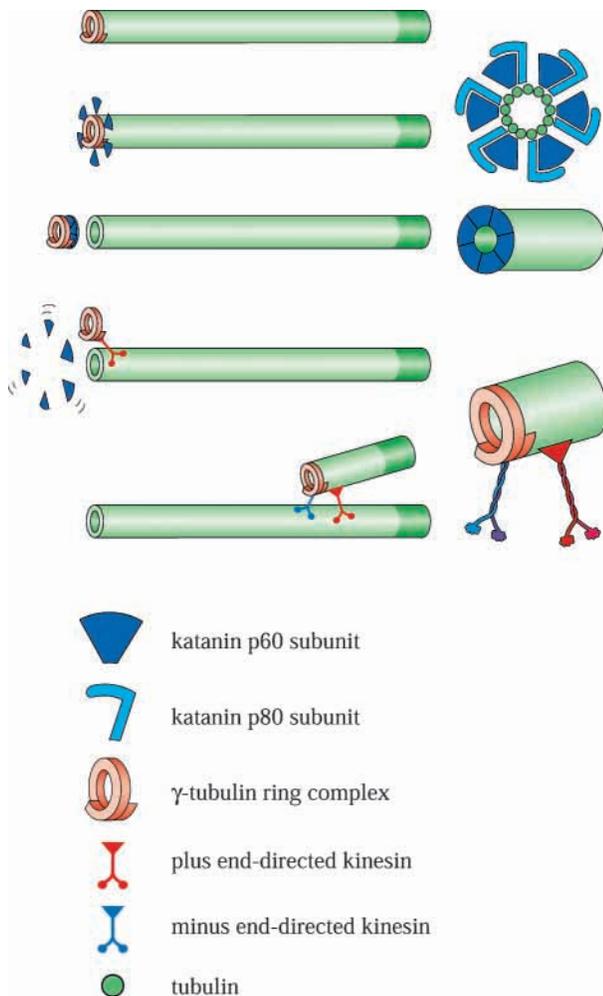


Fig. 3. Model for microtubule assembly by severing and transport of nucleating templates. In this model, a γ -tubulin ring complex associates with the minus end of a microtubule, while the microtubule extends by the addition of tubulin subunits at the fast-growing, GTP-tubulin-containing plus end (dark green). Severing of the minus end is achieved by the formation of a hexamer of katanin p60 subunits, whose association with the microtubule wall is coordinated by the larger p80 subunit, which may transiently dimerize with the p60 subunits. Microtubule-mediated ATPase activity results in inward movement of the p60 subunits, an action that cleaves the ring complex from the microtubule minus end. Katanin subunits dissociate but the lock-washer-shaped ring complex is transported along the microtubule by a plus-end-directed kinesin. The extent of transport along the microtubule may be regulated by the relative activities of plus- and minus-end-directed kinesins. The ring complex serves as a template for the assembly of additional microtubules. Repeated generation, severing and transport of nucleating templates at the minus end of the original microtubule may explain how the fractal tree complexes shown in Fig. 2A develop.

(McClinton et al., 2001), was independently identified on the basis of its sequence similarity to the catalytic subunit of a microtubule-severing protein complex (McNally, 2000; Quarmby, 2000). Mutant alleles including *botero1* (*bot1*) (Bichet et al., 2001) and *fragile fibre 2* (*fra2*) (Burk et al., 2001) were identified as radially swollen semi-dwarfs with brittle

stems. Cortical microtubule arrays were later found to be disordered, especially during the onset of post-cytokinetic cell expansion. The katanin P60 subunit is believed to sever microtubules near their minus ends in neurons (Ahmad et al., 1999), flagellae (Lohret et al., 1999) and mitotic cells (McNally and Thomas, 1998; McNally et al., 2000). At animal cell spindle poles, severing may increase the efficiency of the centrosomal release reaction to allow depolymerization of microtubule minus ends, a force-producing process that may drive anaphase chromosome movements (Desai et al., 1998). In plants, a similar role in spindle and phragmoplast microtubule treadmilling would be anticipated but mitosis and cytokinesis are apparently unaffected in the *bot1* and *fra2* mutants, which include null alleles (Bichet et al., 2001; Burk et al., 2001).

Severing microtubule minus ends to create microtubule-nucleating templates – why transplant an old tree when you can disperse a seed?

Studies of the function of the katanin P60 subunit in neurons suggest that it is required for severing microtubules from centrosomes (Ahmad et al., 1999) so that they can be translocated into the axons. There is some skepticism about this mechanism (Hollenback and Bamberg, 1999), a study by Chang et al. finding no evidence for microtubule translocation in axons (Chang et al., 1999). A similar debate continues in the plant microtubule community (Stoppin et al., 1994; Canaday et al., 2000). Clearly the nuclear surface is one of the preferred sites for microtubule assembly but cortical assembly has also been verified in stringent, semi-in-vitro polymerization assays (Wasteney et al., 1989; Kumagai et al., 1999) and in studies of microtubule recovery after disassembly (Cleary and Hardham, 1987; Falconer et al., 1988; Wacker et al., 1988; Galway and Hardham, 1989; Wasteney and Williamson, 1989a; Wasteney et al., 1993). If perinuclear microtubules were the exclusive source of cortical microtubules, translocation activity should be obvious in observations of fluorescent microtubules in living cells. In the absence of such evidence (Wasteney et al., 1993; Hush et al., 1994; Yuan et al., 1995; Kropf et al., 1997; Marc et al., 1998; Himmelspach et al., 1999; Ueda et al., 1999; Kumagai and Hasezawa, 2001), I propose that katanin p60 works directly within the cortical array (Fig. 3). Periodic severing of the minus ends of microtubules could generate microtubule-nucleating templates. Dispersing these templates by tracking along existing microtubules is consistent with the branching assembly model (Wasteney, 1992; Wasteney and Williamson, 1989a). Templates could be in the form of γ -tubulin lock-washer complexes (Moritz et al., 1995), and this prediction is consistent with the apparent dispersal of γ -tubulin along cortical microtubules in plant cells (Liu et al., 1993; McDonald et al., 1993; Liu et al., 1994; Stoppin-Mellet et al., 2000). Producing microtubule-nucleating templates by severing microtubule minus ends would generate a continual supply of microtubules with consistent protofilament number for the growing cell. Dispersing a seed is easier than moving a mature tree. Polarized animal cells could have adopted an analogous strategy for dispersing microtubules.

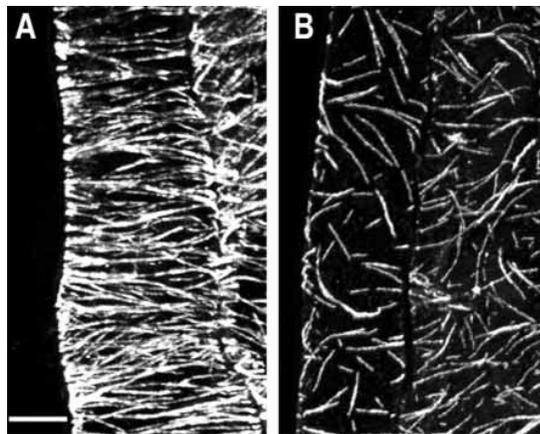


Fig. 4. Microtubule patterns in the epidermis of *Arabidopsis thaliana* cotyledons after 4 hours at 29°C. (A) Cortical microtubules are abundant and transversely oriented in wildtype. (B) In the *mor1* mutant, microtubules appear short and disoriented. Bar, 10 μ m.

MOR1 is a homologue of the TOGp-Dis1 class of high molecular weight MAPs, and is essential for cortical microtubule organization

Templated nucleation may explain microtubule assembly in remote parts of the cell but it does not explain how microtubules are stabilized at these sites. How, for example, are microtubules organized into the transversely oriented arrays that typify elongating cells? Reorientation of recently assembled microtubules by motor MAPs is one feasible mechanism but various observations of microtubules in living cells indicate that microtubules assemble at new orientations rather than being actively reoriented (Yuan et al., 1994; Yuan et al., 1995; Wymer et al., 1996; Himmelspach et al., 1999; Ueda and Matsuyama, 2000). The alternative selective stabilization (or biased turnover) model proposes that only those microtubules assembled in appropriate orientations are stabilized (Wasteneys and Williamson, 1989b).

The recent discovery that a high molecular weight MAP, called microtubule organization 1 (MOR1), has an essential function in organizing cortical microtubules provides a first glimpse at the mechanisms involved in stabilizing cortical microtubules (Whittington et al., 2001). MOR1 was identified by immunofluorescence microscopy screens for temperature-sensitive microtubule disruption in chemically mutagenized *Arabidopsis* seedlings. Two *mor1* alleles, whose phenotypes are similar, have normal cortical microtubule organization and growth at the permissive temperature of 21°C. At 29°C, microtubules rapidly shorten and lose their usual parallel alignment in expanding cells (Fig. 4). Consequently, cells lose control over their expansion direction. We isolated no null alleles or mutants with constitutive phenotypes, which suggested that the *MOR1* gene, which encodes a calculated 217 kDa protein that has significant sequence similarity to the XMAP215-TOGp-Dis1 class of structural MAPs (Whittington et al., 2001), is vital and non-redundant.

A *mor1* allele affects polarization and cell division in male and female gametophytes

Although *mor1* mutants have normal mitotic and cytokinetic

microtubule arrays, and cell division is unaffected at restrictive temperatures, this does not rule out a function for MOR1 in cell division. Twell and Park recently reported that the *gemini* pollen mutant phenotype (Park et al., 1998; Park and Twell, 2001b) is complemented by the wild-type *MOR1* gene (Eckardt et al., 2001; Park and Twell, 2001a). Homozygous *gem1* mutants are lethal, supporting a non-redundant function for MOR1 in *Arabidopsis thaliana*. In *gem1* mutant lines, aberrant cell division planes in haploid microspores result in a proportion of sperm-cell-free pollen. Polarization in the female gametophyte is also altered. These findings suggest that MOR1 organizes microtubules throughout the cell cycle, in common with all its known homologues (Wang and Huffaker, 1997; Chen et al., 1998; Matthews et al., 1998; Cullen et al., 1999; Charrasse et al., 2000; Dionne et al., 2000; Graf et al., 2000).

A temperature-sensitive HEAT repeat may be a key to MOR1 function in the cortical array

What is so peculiar about the proteins encoded by the two *mor1* mutant alleles that they cause temperature-dependent disruption of interphase microtubules but not preprophase bands, spindles or phragmoplasts? We determined that both mutant alleles introduce single amino acid substitutions in an N-terminal HEAT repeat, one of at least ten HEAT repeats found in MOR1 (Whittington et al., 2001). Several lines of evidence suggest that conformational changes at the higher temperature cause the altered microtubule behaviour (Whittington et al., 2001). Both disorganization and recovery of cortical microtubules in the *mor1* mutants occur within minutes of the temperature shift, a time scale too rapid for major transcriptional responses to take effect. In support of this, RT-PCR analysis demonstrates that mutant transcript is produced at the restrictive temperature. Finally, the specific amino acid substitutions found in the *mor1* mutants are the sort likely to compromise the function of HEAT repeats. In *mor1-1*, phenylalanine replaces leucine at position 174 and, in *mor1-2*, lysine replaces glutamic acid at position 195. Substituting a bulkier, aromatic residue or a charge change may well alter the structure of HEAT repeats, which are typified by a pattern of hydrophobic and hydrophilic residues, giving rise to two antiparallel α -helices connected by a short loop (Kobe et al., 1999). HEAT repeats have now been found in many different proteins, which have a variety of roles, including nuclear protein import/export, vesicle trafficking, regulation of phosphorylation and plasma membrane associations (Kunz et al., 2000). Thus, a conformational change in a HEAT repeat, which may be exacerbated at higher temperatures, is likely to reduce or eliminate a specific protein-protein interaction. Future studies will concentrate on characterizing the specific changes that occur at this functional motif at the restrictive temperature.

What is the function of this HEAT repeat in the MOR1 protein?

The N-terminal HEAT repeat targeted by the two *mor1* mutations is also found in homologues of MOR1 (Lemos et al., 2000; Cassimeris et al., 2001). This presents a conundrum. Does this motif mediate a function that is specific to cortical arrays, a specialization of plant cells, or does it have a more

general function? Cortical microtubules are exclusively affected by the *mor1* mutations; so the N-terminal HEAT repeat could connect microtubules to sites at the plasma membrane, as shown in Fig. 5A. This is consistent with evidence from *Xenopus* (Popov et al., 2001) and *Dictyostelium*

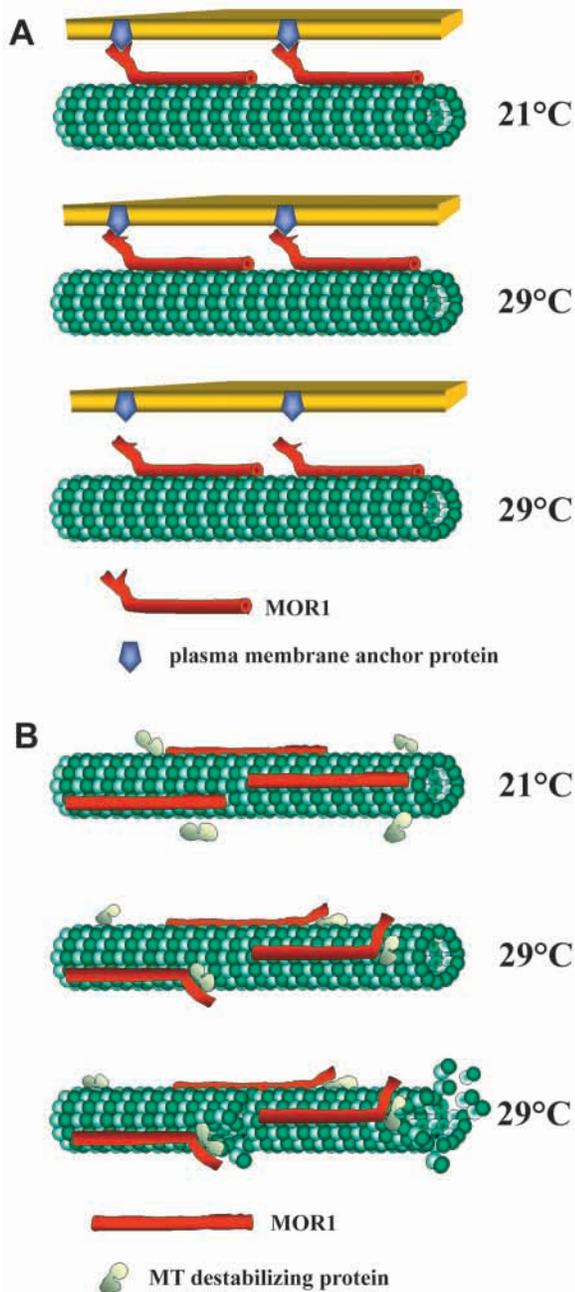


Fig. 5. Possible functions of the MOR1 HEAT repeat-1 (HR1) in microtubule stabilization. (A) HR1 links microtubules to the plasma membrane via a plasma-membrane-associated protein. At restrictive temperature, this loss of binding dissociates microtubules from the plasma membrane, promoting their destabilization. (B) HR1 competes with a destabilizing protein (probably a kin1-like kinesin) for binding. At permissive temperature, the high affinity of MOR1 for this site prevents destabilization. At 29°C, this affinity is lost, leading to kin1-dependent destabilization and microtubule shortening.

(Graf et al., 2000) homologues that the C-terminus binds the MAP to microtubules whereas the N-terminal domain has an alternative function. However, one difficulty with this idea is that the HEAT repeat is highly conserved in all homologues, many of which have no obvious association with the plasma membrane.

Alternatively, the N-terminal HEAT repeat could be a critical part of a general microtubule-stabilizing mechanism (Fig. 5B). The *Xenopus* homologue, XMAP215, has N-terminal-specific microtubule-stabilizing activity (Popov et al., 2001) and works in balance with XKCM1, a kin-1-class kinesin-related protein that opposes microtubule stabilization by XMAP215 (Tournebise et al., 2000). Could the N-terminal HEAT repeat identified in the *mor1* mutants mediate access of a kin-1 kinesin to microtubules? As outlined by Hussey and Hawkins, the N-terminal HEAT repeat of MOR1 could either interact directly with the destabilizing kinesin to modulate its microtubule binding or work less directly by competing for a common microtubule-binding site (Hussey and Hawkins, 2001).

Is there more than one MOR1?

How is MOR1 regulated during the cell cycle? Phosphorylation by increased cyclin-dependent kinase (CDK) activity is anticipated to reduce the affinity of MOR1 for microtubules, producing shorter, more dynamic microtubules (Vasquez et al., 1999). According to Hussey and Hawkins, candidate CDK targets are found in the MOR1 sequence but experimental confirmation awaits (Hussey and Hawkins, 2001). Another way to achieve differential regulation is through alternative splicing to generate a cell-cycle-specific isoform that is unaffected by the mutations. The *MOR1* gene has 52 introns; thus, it is a good candidate for transcriptional modification. At least two isoforms of XMAP215, expressed in different developmental stages, have been identified (Becker and Gard, 2000). Southern blotting confirms that the *Arabidopsis thaliana* genome has only one copy of *MOR1* (Whittington et al., 2001). However, a protein related to the XMAP215-TOGp family that conserves the HEAT repeat affected by the *mor1* mutations has been discovered in *Drosophila* (Lemos et al., 2000). A putative MAST homologue exists in *Arabidopsis thaliana*, and my group is currently investigating the possibility that it overlaps functionally with MOR1 during the cell cycle.

Determining expression, intracellular distribution and regulation of MOR1 through the cell cycle is an important part of its characterization and likely to provide significant new information on how microtubules are organized through this crucial morphogenetic process.

Conclusion/perspectives

Without centrosomes, plant microtubule arrays are largely self-organized by the relative activities of microtubule-associated proteins. The recent discoveries of katanin p60 and MOR1 provide important clues about how microtubule assembly and stabilization are achieved, particularly in the cortical arrays that regulate growth polarity and cell wall deposition in interphase and terminally differentiating cells. I have put forward the hypothesis that dispersed microtubule arrays are generated by

the severing of nucleating complexes from microtubule minus ends, and the transport of these complexes along existing microtubules. The N-terminal HEAT repeat altered in the two temperature-sensitive *mor1* mutants, and conserved in all eukaryotic homologues of MOR1, is likely to have a key role in the general mechanism of microtubule stabilization. One goal of future research will be to elucidate the exact function of this motif. The discovery of MAPs and microtubule regulatory proteins in plants is still at an early stage. An essential first step towards understanding how microtubule arrays are constructed is the isolation of mutants such as *bot1/fra2* (Bichet et al., 2001; Burk et al., 2001), *mor1/gem1* (Park and Twell, 2001b; Whittington et al., 2001), *zwichel* (Oppenheimer et al., 1997), *spiral 1* and *2* (Furutani et al., 2000), *ton* (Traas et al., 1995), *fass* (Torresruiz and Jurgens, 1994; McClinton and Sung, 1997) and *tan* (Smith et al., 2001). It is now possible to start finding out how plant MAPs interact and how kinases and phosphatases regulate their activity. This work will involve classical genetics and more contemporary functional genomics. Phenotyping double mutants will provide the first clues, and suspected interactions will be tested by double immunolocalizations, co-sedimentation assays, in vitro assays, or more elegantly by fluorescence resonance energy transfer (FRET) in living cells. After four decades of study, we may finally start to grapple with the real mysteries of plant microtubules. How are they assembled with such precision, what determines their orientation, and just how does this orientation control the direction of cell expansion?

I am especially grateful to Moira Galway (St Francis Xavier University) for many helpful discussions and critical reading throughout the preparation of this manuscript. I thank Brian Gunning (ANU), Ilse Foissner (University of Salzburg) and Dave Twell (University of Leicester) for their helpful insights and Angela Whittington, Ke Jun Wei and Madeleine Rashbrooke from my lab, for their analysis of MOR1's sequence. Finally, I acknowledge Thomas Magill and Catherine Eadie for expert production of Fig. 1 and Fig. 3, respectively.

References

- Ahmad, F. J., Yu, W. Q., McNally, F. J. and Baas, P. W. (1999). An essential role for katanin in severing microtubules in the neuron. *J. Cell Biol.* **145**, 305-315.
- Asada, T. and Collings, D. (1997). Molecular motors in higher plants. *Trends Plant Sci.* **2**, 29-37.
- Asada, T. and Shibaoka, H. (1991). Microtubule translocation in the cytokinetic apparatus of cultured tobacco cells. *Nature* **350**, 238-241.
- Azizzadeh, J., Traas, J. and Pastuglia, M. (2001). Molecular aspects of microtubule dynamics in plants. *Curr. Opin. Plant Biol.* **4**, 513-519.
- Balczon, R. (1996). The centrosome in animal cells and its functional homologs in plant and yeast cells. *Int. Rev. Cytol.* **169**, 25-82.
- Baskin, T. I. (2001). On the alignment of cellulose microfibrils by cortical microtubules: a review and a model. *Protoplasma* **215**, 150-171.
- Becker, B. E. and Gard, D. L. (2000). Multiple isoforms of the high molecular weight microtubule associated protein XMAP215 are expressed during development in *Xenopus*. *Cell Motil. Cytoskeleton* **47**, 282-295.
- Bibikova, T. N., Blancaflor, E. B. and Gilroy, S. (1999). Microtubules regulate tip growth and orientation in root hairs of *Arabidopsis thaliana*. *Plant J.* **17**, 657-665.
- Bichet, A., Desnos, T., Turner, S., Grandjean, O. and Höfte, H. (2001). BOTERO1 is required for normal orientation of cortical microtubules and anisotropic cell expansion in *Arabidopsis*. *Plant J.* **25**, 137-148.
- Boevink, P., Oparka, K., Santa Cruz, S., Martin, B., Betteridge, A. and Hawes, C. (1998). Stacks on tracks: the plant Golgi apparatus traffics on an actin/ER network. *Plant J.* **15**, 441-447.
- Brown, R. C. and Lemmon, B. E. (2001a). Phragmoplasts in the absence of nuclear division. *J. Plant Growth Regul.* **20**, 151-161.
- Brown, R. C. and Lemmon, B. E. (2001b). Sporogenesis in eusporangious ferns: I. Monoplastidic meiosis in Angiopteris (Marattiales). *J. Plant Res.* **114**, 223-235.
- Bulbert, M. W., Offler, C. E. and McCurdy, D. W. (1998). Polarized microtubule deposition coincides with wall ingrowth formation in transfer cells of *Vicia faba* l. cotyledons. *Protoplasma* **201**, 8-16.
- Burk, D. H., Liu, B., Zhong, R. Q., Morrison, W. H. and Ye, Z. H. (2001). A katanin-like protein regulates normal cell wall biosynthesis and cell elongation. *Plant Cell* **13**, 807-827.
- Canaday, J., Stoppin-Mellet, V., Mutterer, J., Lambert, A. M. and Schmit, A. C. (2000). Higher plant cells: Gamma-tubulin and microtubule nucleation in the absence of centrosomes. *Microsc. Res. Tech.* **49**, 487-495.
- Cassimeris, L., Gard, D., Tran, P. T. and Erickson, H. P. (2001). XMAP215 is a long thin molecule that does not increase microtubule stiffness. *J. Cell Sci.* **114**, 3025-3033.
- Chaffey, N. J., Barnett, J. R. and Barlow, P. W. (1997). Visualization of the cytoskeleton within the secondary vascular system of hardwood species. *J. Microsc.* **187**, 77-84.
- Chaffey, N., Barlow, P. and Barnett, J. (2000). Structure-function relationships during secondary phloem development in an angiosperm tree, *Aesculus hippocastanum*: microtubules and cell walls. *Tree Physiol.* **20**, 777-786.
- Chan, J., Jensen, C. G., Jensen, L. C., Bush, M. and Lloyd, C. W. (1999). The 65-kDa carrot microtubule-associated protein forms regularly arranged filamentous cross-bridges between microtubules. *Proc. Natl. Acad. Sci. USA* **96**, 14931-14936.
- Chang, S. H., Svitkina, T. M., Borisy, G. G. and Popov, S. V. (1999). Speckle microscopic evaluation of microtubule transport in growing nerve processes. *Nat. Cell Biol.* **1**, 399-403.
- Chang-Jie, J. and Sonobe, S. (1993). Identification and preliminary characterization of a 65 kDa higher-plant microtubule-associated protein. *J. Cell Sci.* **105**, 891-901.
- Chapman, M. J., Dolan, M. F. and Margulis, L. (2000). Centrioles and kinetosomes: Form, function, and evolution. *Q. Rev. Biol.* **75**, 409-429.
- Charrasse, S., Lorca, T., Doree, M. and Larroque, C. (2000). The *Xenopus* XMAP215 and its human homologue TOG proteins interact with cyclin B1 to target p34cdc2 to microtubules during mitosis. *Exp. Cell Res.* **254**, 249-256.
- Chen, X. P., Yin, H. and Huffaker, T. C. (1998). The yeast spindle pole body component Spc72p interacts with Stu2p and is required for proper microtubule assembly. *J. Cell Biol.* **141**, 1169-1179.
- Cleary, A. L. and Hardham, A. R. (1987). Depolymerization of microtubule arrays in root tip cells by oryzalin and their recovery with modified nucleation patterns. *Can. J. Bot.* **66**, 2353-2366.
- Cullen, C. F., Deak, P., Glover, D. M. and Ohkura, H. (1999). mini spindles: A gene encoding a conserved microtubule-associated protein required for the integrity of the mitotic spindle in *Drosophila*. *J. Cell Biol.* **146**, 1005-1018.
- Desai, A., Maddox, P. S., Mitchison, T. J. and Salmon, E. D. (1998). Anaphase a chromosome movement and poleward spindle microtubule flux occur at similar rates in *xenopus* extract spindles. *J. Cell Biol.* **141**, 703-713.
- Dionne, M. A., Sanchez, A. and Compton, D. A. (2000). ch-TOGp is required for microtubule aster formation in a mammalian mitotic extract. *J. Biol. Chem.* **275**, 12346-12352.
- Eckardt, N. A., Araki, T., Benning, C., Cubas, P., Goodrich, J., Jacobsen, S. E., Masson, P., Nambara, E., Simon, R., Sommerville, S. et al. (2001). Arabidopsis Research 2001. *Plant Cell* **13**, 1973-1982.
- Falconer, M. M., Donaldson, G. and Seagull, R. W. (1988). MTOCs in higher plant cells: an immunofluorescent study of microtubule assembly sites following depolymerization by APM. *Protoplasma* **144**, 46-55.
- Furutani, I., Watanabe, Y., Prieto, R., Masukawa, M., Suzuki, K., Naoi, K., Thitmadee, S., Shikanai, T. and Hashimoto, T. (2000). The SPIRAL genes are required for directional central of cell elongation in *Arabidopsis thaliana*. *Development* **127**, 4443-4453.
- Galway, M. E. and Hardham, A. R. (1989). Oryzalin-induced microtubule disassembly and recovery in regenerating protoplasts of the alga *Mougeotia*. *J. Plant Physiol.* **135**, 337-345.
- Geitmann, A. and Emons, A. M. C. (2000). The cytoskeleton in plant and fungal cell tip growth. *J. Microsc.* **198**, 218-245.
- Graf, R., Dauderer, C. and Schliwa, M. (2000). *Dictyostelium* DdCP224 is a microtubule-associated protein and a permanent centrosomal resident involved in centrosome duplication. *J. Cell Sci.* **113**, 1747-1758.

- Granger, C. L. and Cyr, R. J.** (2001). Use of abnormal preprophase bands to decipher division plane determination. *J. Cell Sci.* **114**, 599-607.
- Gunning, B. E. S.** (1999). Case Study 4.2. A perspective on plants: significance of cell walls. In *Plants in Action* (ed. B. J. Atwell, P. E. Kriedemann and C. G. N. Turnbull), pp. 137-142. South Yarra: Macmillan Education
- Gunning, B. E. and Wick, S. M.** (1985). Preprophase bands, phragmoplasts, and spatial control of cytokinesis. *J. Cell Sci. Suppl.* **2**, 157-179.
- Hardham, A. R. and Gunning, B. E.** (1978). Structure of cortical microtubule arrays in plant cells. *J. Cell Biol.* **77**, 14-34.
- Hasezawa, S., Ueda, K. and Kumagai, F.** (2000). Time-sequence observations of microtubule dynamics throughout mitosis in living cell suspensions of stable transgenic Arabidopsis – Direct evidence for the origin of cortical microtubules at M/G(1) interface. *Plant Cell Physiol.* **41**, 244-250.
- Higashiyama, T., Sonobe, S., Murofushi, H. and Hasezawa, S.** (1996). Identification of a novel 70 kDa protein in cultured tobacco cells that is immunologically related to MAP4. *Cytologia* **61**, 229-233.
- Himmelspach, R., Wymer, C. L., Lloyd, C. W. and Nick, P.** (1999). Gravity-induced reorientation of cortical microtubules observed in vivo. *Plant J.* **18**, 449-453.
- Hollenback, P. J. and Bamburg, J. R.** (1999). Axonal microtubules stay put. *Nat. Cell Biol.* **1**, E171-E173.
- Hush, J. M., Wadsworth, P., Callahan, D. A. and Hepler, P. K.** (1994). Quantification of microtubule dynamics in living plant cells using fluorescence redistribution after photobleaching. *J. Cell Sci.* **107**, 775-784.
- Hussey, P. J. and Hawkins, T. J.** (2001). Plant microtubule-associated proteins: the HEAT is off in temperature-sensitive mor1. *Trends Plant Science* **6**, 389-392.
- Hussey, P. J., Haas, N., Hunsperger, J., Larkin, J., Snustad, D. P. and Silflow, C. D.** (1990). The beta-tubulin gene family in *Zea mays*: two differentially expressed beta-tubulin genes. *Plant Mol. Biol.* **15**, 957-972.
- Kandasamy, M. K. and Meagher, R. B.** (1999). Actin-organelle interaction: association with chloroplast in Arabidopsis leaf mesophyll cells. *Cell Motil. Cytoskeleton* **44**, 110-118.
- Kobe, B., Gleichmann, T., Horne, J., Jennings, I. G., Scotney, P. D. and Teh, T.** (1999). Turn up the HEAT. *Structure* **7**, R91-R97.
- Kopczak, S. D., Haas, N. A., Hussey, P. J., Silflow, C. D. and Snustad, D. P.** (1992). The small genome of Arabidopsis contains at least six expressed alpha-tubulin genes. *Plant Cell* **4**, 539-547.
- Kost, B., Mathur, J. and Chua, N. H.** (1999). Cytoskeleton in plant development. *Curr. Opin. Plant Biol.* **2**, 462-470.
- Kropf, D. L., Williamson, R. E. and Wasteneys, G. O.** (1997). Microtubule orientation and dynamics in elongating characean internodal cells following cytosolic acidification, induction of pH bands, or premature growth arrest. *Protoplasma* **197**, 188-198.
- Kumagai, F. and Hasezawa, S.** (2001). Dynamic organization of microtubules and microfilaments during cell cycle progression in higher plant cells. *Plant Biol.* **3**, 4-16.
- Kumagai, F., Hasezawa, S. and Nagata, T.** (1999). Putative involvement of a 49 kDa protein in microtubule assembly in vitro. *Eur. J. Cell Biol.* **78**, 109-116.
- Kunz, J., Schneider, U., Howald, I., Schmidt, A. and Hall, M. N.** (2000). HEAT repeats mediate plasma membrane localization of Tor2p in yeast. *J. Biol. Chem.* **275**, 37011-37020.
- Lemos, C. L., Sampaio, P., Maiato, H., Costa, M., Omel'yanchuk, L. V., Liberal, V. and Sunkel, C. E.** (2000). Mast, a conserved microtubule-associated protein required for bipolar mitotic spindle organization. *EMBO J.* **19**, 3668-3682.
- Liebe, S. and Menzel, D.** (1995). Actomyosin-based motility of endoplasmic reticulum and chloroplasts in *Vallisneria* mesophyll cells. *Biol. Cell* **85**, 207-222.
- Liu, B. and Lee, Y. R. J.** (2001). Kinesin-related proteins in plant cytokinesis. *J. Plant Growth Regul.* **20**, 141-150.
- Liu, B., Marc, J., Joshi, H. C. and Palevitz, B. A.** (1993). A gamma-tubulin-related protein associated with the microtubule arrays of higher plants in a cell cycle-dependent manner. *J. Cell Sci.* **104**, 1217-1228.
- Liu, B., Joshi, H. C., Wilson, T. J., Silflow, C. D., Palevitz, B. A. and Snustad, D. P.** (1994). gamma-Tubulin in Arabidopsis: gene sequence, immunoblot, and immunofluorescence studies. *Plant Cell* **6**, 303-314.
- Lloyd, C. W. and Hussey, P. J.** (2001). Microtubule-associated proteins in plants – why we need a MAP. *Nat. Rev. Mol. Cell Biol.* **2**, 40-47.
- Lohret, T. A., Zhao, L. F. and Quarumby, L. M.** (1999). Cloning of *Chlamydomonas* p60 katanin and localization to the site of outer doublet severing during deflagellation. *Cell Motil. Cytoskeleton* **43**, 221-231.
- Maekawa, T., Ogihara, S., Murofushi, H. and Nagai, R.** (1990). Green algal microtubule-associated protein with a molecular weight of 90 kDa which bundles microtubules. *Protoplasma* **158**, 10-18.
- Marc, J., Granger, C. L., Brincat, J., Fisher, D. D., Kao, T. H., McCubbin, A. G. and Cyr, R. J.** (1998). A gfp-map4 reporter gene for visualizing cortical microtubule rearrangements in living epidermal cells. *Plant Cell* **10**, 1927-1939.
- Matthews, L. R., Carter, P., Thierrymieg, D. and Kempthues, K.** (1998). Zyg-9, a *Caenorhabditis elegans* protein required for microtubule organization and function, is a component of meiotic and mitotic spindle poles. *J. Cell Biol.* **141**, 1159-1168.
- McClinton, R. S. and Sung, Z. R.** (1997). Organization of cortical microtubules at the plasma membrane in Arabidopsis. *Planta* **201**, 252-260.
- McClinton, R. S., Chandler, J. S. and Callis, J.** (2001). cDNA isolation, characterization, and protein intracellular localization of a katanin-like p60 subunit from Arabidopsis thaliana. *Protoplasma* **216**, 181-190.
- McDonald, A. R., Liu, B., Joshi, H. C. and Palevitz, B. A.** (1993). Gamma-tubulin is associated with a cortical-microtubule-organizing zone in the developing guard cells of *Allium cepa* L. *Planta* **191**, 357-361.
- McNally, F.** (2000). Capturing a ring of samurai. *Nat. Cell Biol.* **2**, E4-E7.
- McNally, F. J. and Thomas, S.** (1998). Katanin is responsible for the m-phase microtubule-severing activity in xenopus eggs. *Mol. Biol. Cell* **9**, 1847-1861.
- McNally, K. P., Bazirgan, O. A. and McNally, F. J.** (2000). Two domains of p80 katanin regulate microtubule severing and spindle pole targeting by p60 katanin. *J. Cell Sci.* **113**, 1623-1633.
- Megraw, T. L. and Kaufman, T. C.** (2000). The centrosome in *Drosophila* oocyte development. *Curr. Top. Dev. Biol.* **49**, 385-407.
- Mineyuki, Y.** (1999). The preprophase band of microtubules: Its function as a cytokinetic apparatus in higher plants. *Int. Rev. Cytol. (A Survey of Cell Biology)* **187**, 1-49.
- Moritz, M., Braunfeld, M. B., Sedat, J. W., Alberts, B. and Agard, D. A.** (1995). Microtubule nucleation by gamma-tubulin-containing rings in the centrosome. *Nature* **378**, 638-640.
- Nebenfuhr, A., Frohlich, J. A. and Staehelin, L. A.** (2000). Redistribution of Golgi stacks and other organelles during mitosis and cytokinesis in plant cells. *Plant Physiol.* **124**, 135-151.
- Oppenheimer, D. G., Pollock, M. A., Vacik, J., Szymanski, D. B., Ericson, B., Feldmann, K. and Marks, M. D.** (1997). Essential role of a kinesin-like protein in Arabidopsis trichome morphogenesis. *Proc. Natl. Acad. Sci. USA* **94**, 6261-6266.
- Otegui, M. and Staehelin, L. A.** (2000a). Cytokinesis in flowering plants: more than one way to divide a cell. *Curr. Opin. Plant Biol.* **3**, 493-502.
- Otegui, M. and Staehelin, L. A.** (2000b). Syncytial-type cell plates: a novel kind of cell plate involved in endosperm cellularization of Arabidopsis. *Plant Cell* **12**, 933-947.
- Park, S. K. and Twell, D.** (2001a). Arabidopsis *GEMINI POLLEN1* is a putative microtubule-associated protein homologous to human ch-TOGp. *International Arabidopsis Meeting* <http://arabidopsis.org/news/Arab-Abstracts.pdf>.
- Park, S. K. and Twell, D.** (2001b). Novel patterns of ectopic cell plate growth and lipid body distribution in the *Arabidopsis gemini pollen1* mutant. *Plant Physiol.* **126**, 899-909.
- Park, S. K., Howden, R. and Twell, D.** (1998). The *Arabidopsis thaliana* gametophytic mutation *gemini pollen1* disrupts microspore polarity, division asymmetry and pollen cell fate. *Development* **125**, 3789-3799.
- Popov, A. V., Pozniakovskiy, A., Arnal, I., Antony, C., Ashford, A. J., Kinoshita, K., Tournebize, R., Hyman, A. A. and Karsenti, E.** (2001). XMAP215 regulates microtubule dynamics through two distinct domains. *EMBO J.* **20**, 397-410.
- Quarumby, L.** (2000). Cellular Samurai: katanin and the severing of microtubules. *J. Cell Sci.* **113**, 2821-2827.
- Singh, S., Lazzaro, M. D. and Walles, B.** (1999). Microtubule organization in the differentiating transfer cells of the placenta in *Lilium* spp. *Protoplasma* **207**, 75-83.
- Smertenko, A., Saleh, N., Igarashi, H., Mori, H., Hauser-Hahn, I., Jiang, C. J., Sonobe, S., Lloyd, C. W. and Hussey, P. J.** (2000). A new class of microtubule-associated proteins in plants. *Nat. Cell Biol.* **2**, 750-753.
- Smirnova, E. A. and Bajer, A. S.** (1994). Microtubule converging centers and reorganization of the interphase cytoskeleton and the mitotic spindle in higher plant *Haemanthus*. *Cell Motil. Cytoskeleton* **27**, 219-233.
- Smirnova, E. A. and Bajer, A. S.** (1998). Early stages of spindle formation and independence of chromosome and microtubule cycles in *Haemanthus* endosperm. *Cell Motil. Cytoskeleton* **40**, 22-37.
- Smith, L. G., Gerttula, S. M., Han, S. C. and Levy, J.** (2001). TANGLED1:

- a microtubule binding protein required for the spatial control of cytokinesis in maize. *J. Cell Biol.* **152**, 231-236.
- Southworth, D. and Cresti, M.** (1997). Comparison of flagellated and nonflagellated sperm in plants. *Am. J. Bot.* **84**, 1301-1311.
- Stoppin, V., Vantard, M., Schmit, A. C. and Lambert, A. M.** (1994). Isolated plant nuclei nucleate microtubule assembly – the nuclear surface in higher plants has centrosome-like activity. *Plant Cell* **6**, 1099-1106.
- Stoppin-Mellet, V., Peter, C. and Lambert, A. M.** (2000). Distribution of gamma-tubulin in higher plant cells: cytosolic gamma-tubulin is part of high molecular weight complexes. *Plant Biol.* **2**, 290-296.
- Torresruiz, R. A. and Jurgens, G.** (1994). Mutations in the *FASS* gene uncouple pattern formation and morphogenesis in *Arabidopsis* development. *Development* **120**, 2967-2978.
- Tournebize, R., Popov, A., Kinoshita, K., Ashford, A. J., Rybina, S., Pozniakovskiy, A., Mayer, T. U., Walczak, C. E., Karsenti, E. and Hyman, A. A.** (2000). Control of microtubule dynamics by the antagonistic activities of XMAP215 and XKCM1 in *Xenopus* egg extracts. *Nat. Cell Biol.* **2**, 13-19.
- Traas, J., Bellini, C., Nacry, P., Kronenberger, J., Bouchez, D. and Caboche, M.** (1995). Normal differentiation patterns in plants lacking microtubular preprophase bands. *Nature* **375**, 676-677.
- Ueda, K. and Matsuyama, T.** (2000). Rearrangement of cortical microtubules from transverse to oblique or longitudinal in living cells of transgenic *Arabidopsis thaliana*. *Protoplasma* **213**, 28-38.
- Ueda, K., Matsuyama, T. and Hashimoto, T.** (1999). Visualization of microtubules in living cells of transgenic *Arabidopsis thaliana*. *Protoplasma* **206**, 201-206.
- Vantard, M., Schellenbaum, P., Fellous, A. and Lambert, A. M.** (1991). Characterization of maize microtubule-associated proteins, one of which is immunologically related to tau. *Biochemistry* **30**, 9334-9340.
- Vasquez, R. J., Gard, D. L. and Cassimeris, L.** (1999). Phosphorylation by CDK1 regulates XMAP215 function in vitro. *Cell Motil. Cytoskeleton* **43**, 310-321.
- Vaughn, K. C. and Harper, J. D.** (1998). Microtubule-organizing centers and nucleating sites in land plants. *Int. Rev. Cytol.* **181**, 75-149.
- Wacker, I., Quader, H. and Schnepf, E.** (1988). Influence of the herbicide oryzalin on the cytoskeleton and growth of *Funaria hygrometrica* protonemata. *Protoplasma* **142**, 55-67.
- Wang, P. J. and Huffaker, T. C.** (1997). Stu2p: A microtubule-binding protein that is an essential component of the yeast spindle pole body. *J. Cell Biol.* **139**, 1271-1280.
- Wasteneys, G. O.** (1992). The characean cytoskeleton: spatial control in the cortical cytoplasm. In *The Cytoskeleton of the Algae* (ed. D. Menzel), pp. 273-295. Boca Raton, Ann Arbor, London, Tokyo: CRC Press.
- Wasteneys, G. O.** (2000). The cytoskeleton and growth polarity. *Curr. Opin. Plant Biol.* **3**, 503-511.
- Wasteneys, G. O. and Williamson, R. E.** (1989a). Reassembly of microtubules in *Nitella tasmanica*: assembly of cortical microtubules in branching clusters and its relevance to steady-state microtubule assembly. *J. Cell Sci.* **93**, 705-714.
- Wasteneys, G. O. and Williamson, R. E.** (1989b). Reassembly of microtubules in *Nitella tasmanica*: quantitative analysis of assembly and orientation. *Eur. J. Cell Biol.* **50**, 76-83.
- Wasteneys, G. O., Jablonsky, P. P. and Williamson, R. E.** (1989). Assembly of purified brain tubulin at cortical and endoplasmic sites in perfused internodal cells of the alga *Nitella tasmanica*. *Cell Biol. Int. Rep.* **13**, 513-528.
- Wasteneys, G. O., Gunning, B. E. S. and Hepler, P. K.** (1993). Microinjection of fluorescent brain tubulin reveals dynamic properties of cortical microtubules in living plant cells. *Cell Motil. Cytoskeleton* **24**, 205-213.
- Whittington, A. T., Vugrek, O., Wei, K. J., Hasenbein, N. G., Sugimoto, K., Rashbrooke, M. C. and Wasteneys, G. O.** (2001). MOR1 is essential for organizing cortical microtubules in plants. *Nature* **411**, 610-613.
- Wyatt, S. E. and Carpita, N.** (1993). The plant cytoskeleton-cell-wall continuum. *Trends Cell Biol.* **3**, 413-417.
- Wymer, C. L., Fisher, D. D., Moore, R. C. and Cyr, R. J.** (1996). Elucidating the mechanism of cortical microtubule reorientation in plant cells. *Cell Motil. Cytoskeleton* **35**, 162-173.
- Yuan, M., Shaw, P. J., Warn, R. M. and Lloyd, C. W.** (1994). Dynamic reorientation of cortical microtubules, from transverse to longitudinal, in living plant cells. *Proc. Natl. Acad. Sci. USA* **91**, 6050-6053.
- Yuan, M., Warn, R. M., Shaw, P. J. and Lloyd, C. W.** (1995). Dynamic microtubules under the radial and outer tangential walls of microinjected pea epidermal cells observed by computer reconstruction. *Plant J.* **7**, 17-23.