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Microtubules in Plants

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Microtubules (MTs) are highly conserved polar polymers that are key elements of the eukaryotic cytoskeleton and are essential for various cell functions. αβ-tubulin, a heterodimer containing one structural GTP and one hydrolysable and exchangeable GTP, is the building block of MTs and is formed by the sequential action of several molecular chaperones. GTP hydrolysis in the MT lattice is mechanistically coupled with MT growth, thus giving MTs a metastable and dynamic nature. MTs adopt several distinct higher-order organizations that function in cell division and cell morphogenesis. Small molecular weight compounds that bind tubulin are used as herbicides and as research tools to investigate MT functions in plant cells. The de novo formation of MTs in cells requires conserved γ-tubulin-containing complexes and targeting/activating regulatory proteins that contribute to the geometry of MT arrays. Various MT regulators and tubulin modifications control the dynamics and organization of MTs throughout the cell cycle and in response to developmental and environmental cues. Signaling pathways that converge on the regulation of versatile MT functions are being characterized.

INTRODUCTION

As early as the beginning of the 20th century, fibrous, filamentous, or tubular structures of similar diameter were described in various dividing eukaryotic cells. In the 1960s, high-resolution transmission electron microscopy images of these structures were obtained and it was determined with confidence that these filaments were actually hollow tubes, thus giving rise to the unified term "microtubules (MTs)" (Slautterback, 1963; Ledbetter and Porter, 1963). At around the same time, a group of MTs (currently known as cortical MTs) were identified just beneath the plasma membrane in the interphase cells of two angiosperms and one gymnosperm and were recognized as the same structural entities as those in mitotic spindles (Ledbetter and Porter, 1963). The building block of MTs, tubulin, was soon identified as a protein that binds to colchicine, a MT-destabilizing chemical (Weisenberg et al., 1968). Remarkably, shortly before MTs were identified, Green (1962) predicted that a cortical cytoskeleton that is sensitive to colchicine (as are mitotic spindles) guides the polar deposition of cellulose microfibrils in the plant cell wall. In the report by Ledbetter and Porter (1963), cortical MTs were found to align in parallel to cellulose microfibrils in the innermost (newest) layer of the cell wall, leading to the development of the MT-guided cellulose alignment hypothesis (Hepler and Palevitz, 1974; Baskin, 2001).

The static view of the cytoskeleton was transformed into a dynamic one with the publication of both in vitro (Horio and Hotani, 1986) and in vivo (Sammak and Borisy, 1988) time-lapse images of MTs. The dynamic instability model of MT dynamics was proposed in the 1980s by Mitchison and Kirschner (1984, 1986) and remains a useful representation of basic MT behavior. In living cells, various proteins modify the dynamic properties of MTs, associate multiple MTs into higher-order structures, use the MT lattice to transport cellular cargo, and use the force generated by disassembling MTs to move chromosomes during cell division. This review summarizes the structure and properties of this essential cytoskeleton component that is conserved in all eukaryotes, and focuses on plant (especially Arabidopsis) MTs and their cellular function.

MT STRUCTURE

MTs are hollow cylinders of approximately 24 nm in diameter that, in most eukaryotic cells, consist of 13 protofilaments (Ledbetter and Porter, 1964). Each protofilament is composed of longitudinally stacked heterodimers of α- and β-tubulins (Figure 1). Protofilaments are aligned in parallel and held together by lateral interactions, giving rise to the cylindrical polar structure of MTs. The end with β-tubulin exposed is the plus end, while the α-tubulin-exposed end is dubbed the minus end. When MTs disassemble from the plus end, stable rings and spirals of curled individual protofilaments are often observed (e.g., Mandelkow et al., 1991), indicating that longitudinal interactions between tubulin heterodimers within a protofilament are stronger than those between pro-

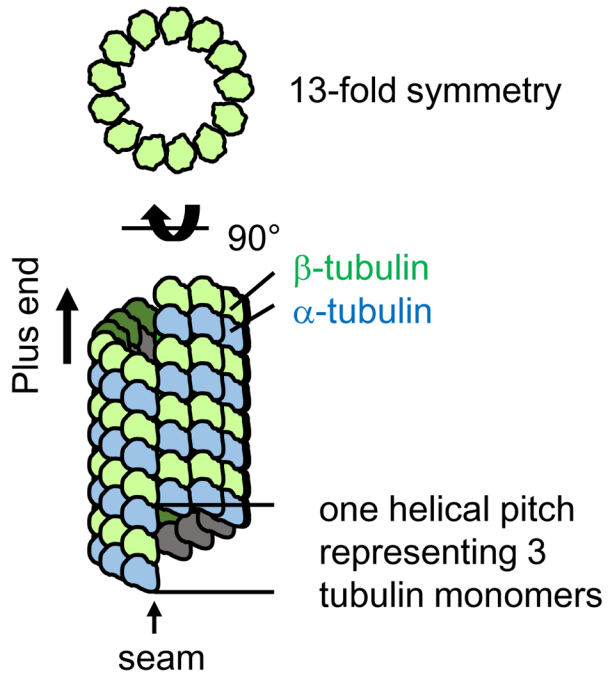

Figure 1. Microtubule (MT) structure.

Protofilaments are composed of multiple longitudinally stacked α-tubulin and β-tubulin heterodimers. When 13 protofilaments associate to form a hollow MT in vivo, their lateral association is staggered to generate a gap representing three tubulin monomers. In this B-type lattice structure, the gap represents a seam where the modes of lateral tubulin association between protofilaments are different from those in other regions of a MT.

tofilaments. Thus, protofilaments may be regarded as the basic units of MTs.

Protofilaments assemble into hollow cylinders in several configurations that vary based on the number of protofilaments and the number of tubulin monomers per helical pitch. MTs in eukaryotic cells commonly consist of 13 protofilaments arranged in a B-type lattice with a seam (Figure 1). In the B-type lattice, the α- and β-tubulin monomers of one protofilament associate with the α- and β-monomers, respectively, of the neighboring protofilament, except at the seam. Although a theoretical A-type lattice, in which the lateral interactions are between α-tubulin and β-tubulin, was originally postulated, ultrastructural analyses of MTs have established the true lattice structure as B-type (Desai and Mitchison, 1997). Theoretical predictions suggested the existence of MTs consisting of 10 to 17 protofilaments (Chrétien and Wade, 1991). Indeed, MTs with protofilament numbers ranging from 8 to 20 have been observed in in vitro-assembled MTs and also in vivo in some cell types and in particular organisms, such as *C. elegans* (see the references in Sui and Downing, 2010). Pseudoatomic models of MTs containing different numbers of protofilaments suggest that some degree of lateral deformation is tolerated in interprotofilament interactions (Sui and Downing, 2010). Because of this postulated capacity for high-curvature bending without breaking, MTs may bend sharply, as is frequently observed in vitro and in vivo, even though MTs are considerably more rigid than actin filaments (Brangwynne et al., 2006).

The tubulin heterodimer consists of globular α- and β-subunits of similar structure associated in a head-to-tail fashion (Figure 2), and the C-terminal residues of the α- and β-subunits are not visible by electron microscopy (Nogales et al., 1998). The C-terminal tubulin tail is highly acidic, is expected to be highly disordered, and undergoes various post-translational modifications in the case of animal tubulins (see below). Since each tubulin subunit binds one GTP molecule, an αβ-tubulin heterodimer possesses two GTP-binding sites. The N-site (non-exchangeable) in α-tubulin is buried within the tubulin dimer at the longitudinal interdimer interface. The GTP at the N-site is not hydrolyzed and is ascribed a structural role. The nucleotide at the exchangeable (E)-site in β-tubulin is exposed on the surface of an unpolymerized (free) dimer and at the terminal tubulin dimers of the MT plus end. Free tubulin dimers with GDP bound at the E-site readily exchange the nucleotide with GTP in the cytoplasm, thus generating a polymerization-competent pool of tubulins.

Tubulin heterodimers are preferentially added to the plus end. Upon addition of a tubulin dimer to a growing plus end, the α-tubulin subunit of the incoming dimer interacts with the E-site GTP of the terminal β-tubulin subunit, which triggers GTP hydrolysis (Figure 2). Thus, the α-tubulin subunit acts as a GTPase-activating protein (GAP) that potentiates GTPase activity at the E-site. Indeed, when the amino acid residue of the α-tubulin responsible for the GAP activity was mutated in Arabidopsis, the mutant tubulin was incorporated into MTs together with wild-type tubulins and generated stable MTs that labeled with End-Binding 1 (EB1; see

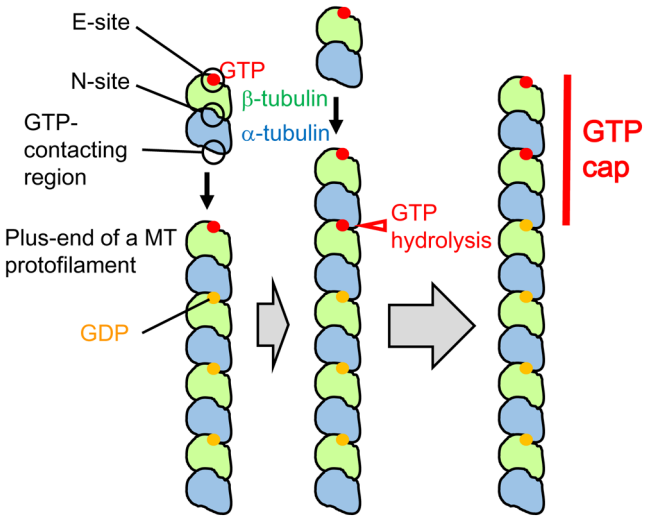

Figure 2. The addition of tubulin to the MT plus end promotes GTP hydrolysis.

GTP bound to β-tubulin at the exposed plus end of the MT (E-site GTP) is hydrolyzed to GDP when α-tubulin in the incoming tubulin heterodimer makes contact with the GTP molecule. This polymerization-triggered nucleotide hydrolysis creates a small GTP cap structure at the growing plus end of the MT. The non-exchangeable and stable GTP at the N site in α-tubulin is not shown. One protofilament is depicted for clarity.

below) along the lattice length, indicating that hydrolysis of the E-site GTP was inefficient and that GTP-tubulin persisted along the length of the lattice (Ishida et al., 2007). Protofilaments composed of GTP-tubulin are straight and form a stable MT lattice prone to polymerization, whereas protofilaments of GDP-tubulin are curved and form an unstable MT lattice that is held together by weaker inter-protofilament lateral interactions and readily depolymerizes. Thus, MT polymerization and GTP hydrolysis are mechanistically linked, resulting in the characteristic metastable behavior of MTs.

The GTP-cap model proposes that a MT continues to grow when GTP-tubulins occupy the plus end, but that loss of this cap due to outpaced hydrolysis and the stochastic dissociation of GTP-tubulin exposes the labile inner core of GDP-tubulin, leading to outward peeling of GDP-protofilaments and rapid depolymerization (Mitchison and Krischner, 1984). This model is well supported by numerous experimental results, but we still do not have a detailed understanding of the structure of the plus end during MT growth and catastrophe (i.e., the sudden conversion from polymerization to shrinkage). The plus end exists in three or more structural configurations in vitro and in vivo, as observed by electron tomography in Arabidopsis phragmoplast MTs (Austin et al., 2005). Growing MTs that undergo dynamic transitions between polymerization and shrinkage typically exhibit tapered ends, in which several slightly curved, laterally associated protofilaments extend from one side of the MT and exhibit a sheet-like organization, whereas depolymerizing MTs exhibit horn-shaped ends, in which individual protofilaments do not interact laterally with each other and are curved outward. MT plus ends can also be blunt when protofilaments are of almost equal length and remain parallel to each other. New MTs have been shown, both experimentally and in simulation, to initiate growth with a metastable blunt end that is recalcitrant to catastrophe and to gradually become more dynamic with frequent tapered ends (Gardner et al., 2012; Coombes et al., 2013). In MTs of the Arabidopsis phragmoplast, the blunt ends are often associated with cell plate membranes and thus appear to be static (Austin et al., 2005).

Rapidly depolymerizing plus ends suddenly and stochastically stop shrinking and switch to a polymerization state in vitro and in vivo (Figure 3). This intriguing event, called rescue, completes the dynamic stability cycle of MTs, and has been a subject of mechanistic investigations. A recombinant antibody that specifically recognizes GTP-tubulin in MTs was used to demonstrate that GTP-tubulin is present at the growing tip of MTs, as expected (Dimitrov et al., 2008). Surprisingly, the antibody also detected GTP-tubulin as discrete dots along the length of MTs both in vitro and in vivo. Thus, a small fraction of GTP-tubulin at the plus end cap appears to escape hydrolysis and remains in the inner body of MTs that mostly (but not completely) consist of GDP-tubulin. Moreover, rescue events frequently occurred at the locations of the GTP-tubulin remnants (Dimitrov et al., 2008). This is consistent with earlier observations that in vitro rescue events are not caused by the stochastic addition of GTP-tubulin to the depolymerizing MT end but rather are promoted by disrupted MT depolymerization (Walker et al., 1988).

The pause state, in which MT plus ends undergo neither growth nor shrinkage, are frequently observed in animal cells (e.g., Rusan et al., 2001), but are rare in interphase Arabidopsis cells (e.g., Shaw et al., 2003; Nakamura et al., 2004).

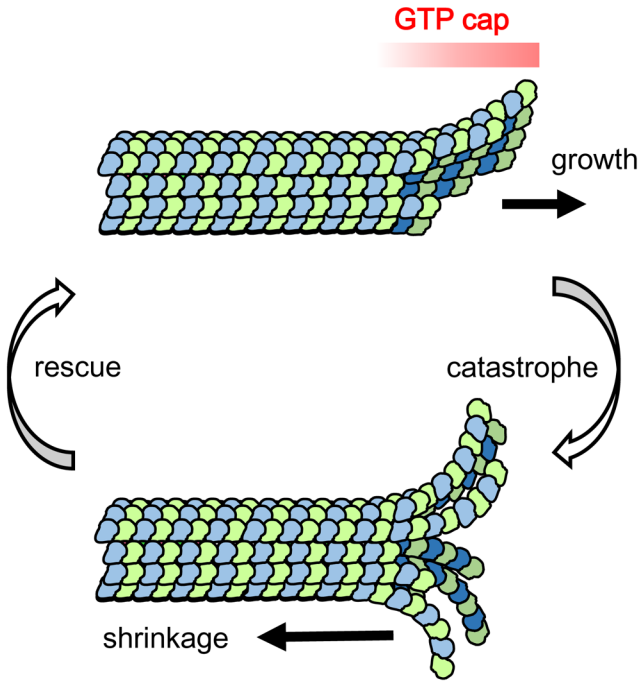

Figure 3. Dynamic instability.

The GTP cap at the plus end ensures steady MT growth, whereas its stochastic loss destabilizes the end structure and results in rapid shrinkage, in an event called catastrophe. When a GTP cap is restored, the MT starts to grow again. This transition is called rescue.

In animal and yeast cells, the minus ends of MTs are often associated with and stabilized by nucleation complexes at centrosomes and spindle pole bodies (Desai and Mitchison, 1997). Free minus ends that have been released from the nucleation complexes and are not stabilized at endomembranes or organelles are common in plant MTs and also in differentiated animal cells (Dammermann et al., 2003; Shaw et al., 2003). These free minus ends usually do not grow and exhibit slow and continuous depolymerization.

TUBULIN

Tubulin heterogeneity

The α- and β-tubulins share around 40% amino acid sequence identity, and their molecular structures are basically identical (Nogales et al., 1998). Each monomer is formed by a core of two β-sheets surrounded by α-helices, and by an acidic C-terminal tail that is not visible by electron crystallography and is probably unstructured. The genomes of multicellular eukaryotes encode multiple tubulin genes; *Arabidopsis thaliana*, for example, has six α-tubulin genes (Kopczak et al., 1992) and nine β-tubulin genes (Snustad et al., 1992). The majority of differences between Arabidopsis isotypes and between plant and animal tubulins are located on the extreme C-terminus. Some tubulin isotypes are expressed in specialized

cells or tissues during development (Hashimoto, 2013a; Breviario et al., 2013). In Arabidopsis, TUA1 α-tubulin (At1g64740) and TUB9 β-tubulin (At4g20890) genes are exclusively or highly preferentially expressed in pollen (Carpenter et al. 1992; Cheng et al., 2001). Whereas highly specialized MTs of some differentiated animal cells are composed of specific tubulin isotypes, the function of most MTs appears to be independent of their isotype composition (Janke, 2014). It is unknown whether and how tubulin heterogeneity controls MT functions in plant cells.

In addition to being composed of distinct tubulin isotypes, MT subtypes may be composed of tubulins that have undergone different post-translational modifications. Animal tubulins are subject to a variety of such modifications (Janke and Bulinski, 2011). Polyglycines and polyglutamines may be conjugated to the γ-carboxyl groups of glutamate residues at the acidic C-terminal tails of α- and β-tubulins, and are highly abundant in the axonemes of cilia and flagella. The widely conserved C-terminal tyrosine residue of animal α-tubulins is often enzymatically removed in stable and long-lived MTs. Besides these tail modifications, lysine 40 of animal α-tubulins, which faces the MT lumen, can be acetylated in stable and persistent MTs. These tubulin signatures may be recognized by molecular motors and MT regulators, and might specify the functions of local MT subpopulations, as proposed in the tubulin-code hypothesis (Sirajuddin et al., 2014). These post-translational modifications also appear to occur on plant tubulins, as antibodies raised against epitopes of animal tubulins detected positive signals in immunoblots and immunohistochemical preparations of plant cell samples (e.g., Smertenko et al., 1997). Future studies should address the occurrence, abundance, and physiological consequences of these modifications in plant tubulins.

β-tubulin can be phosphorylated by plant kinases in vitro (Ben-Nissan et al., 2008; Motose et al., 2011), but the in vivo functions of these putatively phosphorylated tubulins on MT organization in plant cells are largely unknown. Threonine 349 of α-tubulin in Arabidopsis plants is transiently phosphorylated upon acute hyperosmotic stress and then gradually de-phosphorylated (Ban et al., 2013; Fujita et al., 2013). This threonine residue is located at the longitudinal interdimer interface, and its phosphorylation effectively inhibits MT polymerization in vitro and induces rapid MT depolymerization in vivo (Fujita et al., 2013). Since the kinase responsible for this phosphorylation is only present in the plant lineage, phosphorylation-triggered MT depolymerization does not occur in animal and fungal cells.

Tubulin biogenesis

Tubulin is formed in eukaryotic cells as an obligate αβ-heterodimer with one N-site GTP buried at the interface of α- and β-tubulins; monomeric forms of α- and β-tubulins normally do not exist in cells. The biogenesis of tubulin heterodimers is highly complex and more than a dozen molecular chaperones facilitate the proper folding of nascent tubulin polypeptides and dimer assembly. Recombinant tubulins produced in *E. coli* are not properly folded and are non-functional, due to the lack of a proper protein folding machinery in prokaryotes (Lewis et al., 1997).

Tubulin biogenesis consists of an early pathway that involves prefoldin and the cytosolic chaperonin containing tailless complex polypeptide-1 (CCT), which assist in the folding of several cytosolic proteins, and the late tubulin folding pathway (Lundin et al., 2010). When nascent tubulin polypeptides are discharged from the ribosome upon completion of translation, the partially folded chains are captured by prefoldin, a jellyfish-shaped hexameric complex consisting of two α-type and four β-type subunits, and then delivered to CCT. Arabidopsis null mutants of prefoldin subunit genes were viable and able to complete their life cycle, but had reduced levels of tubulin proteins and errors in the organization of cortical MTs (Gu et al., 2008; Rodriguez-Milla and Salinas, 2009). It has been suggested that nascent tubulin polypeptides are delivered directly to CCT by means of a prefoldin-independent folding pathway (Lundin et al., 2010) and that prefoldin is required when tubulins are synthesized at high levels (Delgehyr et al., 2012). The prefoldin complex in the cytoplasm of Arabidopsis cells is localized through interaction with DELLA proteins to the nucleus, where prefoldin cannot mediate tubulin biogenesis (Locascio et al., 2013). Since gibberellin promotes cell elongation by degrading DELLA repressor proteins (Hauvermale et al., 2012), which is accompanied by the formation of transversely-oriented cortical MTs (see below), this growth-promoting phytohormone may modulate MT organization by interfering with the tubulin folding pathway.

CCT is a large cylindrical complex composed of two rings of eight related subunits, and is distantly related to the bacterial chaperonin GroEL (Valpuesta et al., 2002). CCT binds a partially folded tubulin polypeptide, undergoes ATP-dependent conformational changes, and releases a quasi-native folding intermediate. α- and β-tubulins are not the sole substrates of CCT; a limited subset of its target proteins include, among others, γ-tubulin and actin (Melki et al., 1993). The ATPase activity of substrate-bound CCT is modulated by its interaction with phosducin-like protein 3, a homolog of a retinal G-protein signaling regulator (Lundin et al., 2010). In Arabidopsis, this CCT-interacting protein (At3g50960 and At5g66410) is required for the proper functioning and organization of MTs during cell division and in interphase cells (Castellano and Sablowski, 2008).

The tubulin folding pathway is specific to tubulin heterodimer assembly. Five folding proteins (i.e., cofactors A, B, C, D, and E) and ARL2, an ARF family of regulatory GTPases, participate in the post-CCT pathway (Lewis et al., 1997; Szymanski 2002). The quasi-native α-tubulin folding intermediate interacts with cofactors B or E, whereas the β-tubulin intermediate forms complexes with cofactors A or D. The addition of cofactor C generates a super-complex consisting of cofactors C, D, and E, together with α- and β-tubulin, which releases a native heterodimer upon GTP hydrolysis. ARL2 interacts with cofactor D and regulates tubulin assembly, but the underlying molecular mechanism is not well understood. Arabidopsis knockout mutants of these tubulin-specific folding pathway genes are embryo lethal and show severe inhibition of tubulin biogenesis (McElver et al., 2000; Steinborn et al., 2002; Kirik et al., 2002).

SMALL MOLECULES THAT INTERACT WITH MTS

MTs are effective targets for anticancer therapy and for weed control. Most MT-interacting small molecules are derived from natural products, often of plant origin, and many of these are

used as anticancer drugs or herbicides. High resolution crystal structures of tubulins in complexes with MT-interacting anticancer drugs revealed that MT-stabilizing chemicals, such as taxol, promote tubulin assembly by enhancing lateral interactions between protofilaments or locking longitudinal tubulin interfaces in a GTP-tubulin-like conformation, whereas destabilizing compounds prevent MT polymerization by promoting a curved MT conformation or inhibiting a straightened conformation (Field et al., 2013; Alushin et al., 2014).

Several structurally distinct groups of herbicides destabilize plant MTs and have been used to control the emergence of annual grasses and certain broadleaf weeds (Morejohn, 1991; Figure 4). Molecular analyses of weed mutants that have acquired resistance to repeated herbicide usage revealed that dinitroaniline herbicides, such as oryzalin, bind α-tubulin at a region close to the interdimer interface (Anthony and Hussey, 1999). The benzamide class of herbicides, including propyzamide (also called pronamide), bind β-tubulin (Young and Lewandowski, 2000). These herbicides have low affinities for animal or fungal tubulins (e.g., Hugdahl and Morejohn, 1993), probably because their binding sites are not conserved in non-plant tubulins. Interestingly, protozoan parasites such as Trypanosoma are sensitive to dinitroaniline herbicides (Traub-Cseko et al., 2001), and their herbicide-resistant mutants have been used to provide additional support for the proposed dinitroaniline-binding site (Lyons-Abbott et al., 2010). Plant-derived allelochemicals may suppress the growth of neighboring plants by destabilizing their MTs (Chaimovitsh et al., 2010).

Oryzalin and propyzamide are commonly used to destabilize MTs or induce depolymerization of MTs, with oryzalin being a more potent destabilizer of MTs than propyzamide. Low concentrations of oryzalin (50-100 nM) or propyzamide (1-2 μM) suppress the dynamicity of cortical MTs, alter the MT array organization, and induce helical growth in elongating Arabidopsis epidermal cells (Nakamura et al., 2004). Such low doses of MT-destabilizing drugs have been used to destabilize MT arrays and to screen MT- and cellulose-related Arabidopsis mutants (Ishida et al., 2007; Naoi and Hashimoto, 2004; Paredez et al., 2008). Taxol, on the other hand, stabilizes and extensively induces bundling of MTs in plant cells. Disruption of MT dynamics and organization by either taxol or oryzalin perturb growth directionality in tip-growing root hairs (Bibikova et al., 1999).

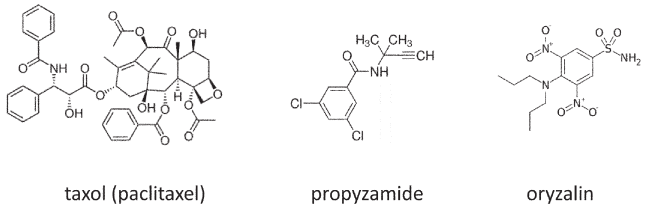

Figure 4. Structures of three chemicals that are frequently used in MT studies.

When taxol was commercially marketed by Bristol-Myers Squibb, its common name was changed to paclitaxel, but this compound is still described as taxol in the literature. While taxol stabilizes MTs of diverse origin, propyzamide and oryzalin effectively destabilize plant MTs and are used as herbicides.

PLANT MT ARRAYS

Interphase Arrays

Plant MTs adopt several distinct array organizations. In non-dividing interphase cells, MTs are commonly positioned beneath the plasma membrane. Electron micrographs show that these cortical MTs are not tightly associated with the plasma membrane, but are generally separated from it by a distance of several nanometers (e.g., Ledbetter and Porter, 1963). It is not yet clear how cortical MTs are anchored to the plasma membrane, although cross-bridge structures have occasionally been observed between these entities (e.g., Sonobe et al., 2001; Barton et al., 2008). A plasma membrane-bound phospholipase D has been proposed to function as the linker protein, since a *Nicotiana tabacum* (tobacco) phospholipase D associates with cortical MTs (Gardiner et al., 2001) and *n*-butanol, a potent activator of the enzyme, induces the release of cortical MTs from the plasma membrane and partially depolymerizes MTs (Dhonukshe et al., 2003). However, *n*-butanol was shown to depolymerize MTs directly in vitro (Hirase et al., 2006), and subsequent studies have yet to confirm MT detachment from the plasma membrane after *n*-butanol treatment (e.g., Zhang et al., 2012). Other candidate linker proteins that potentially join cortical MTs to the plasma membrane include a MT-binding protein CLASP (At2g20190; Ambrose and Wasteneys, 2008; also see below), a membrane-tethered formin AtFH4 (At1g24150) that interacts with both actin filaments and MTs (Deeks et al., 2010), MT-binding proteins (CSIs; At2g22125, At1g44120, and At1g77460) that associate with primary cellulose synthase complexes (Gu et al., 2010; Lei et al., 2013), and a phosphatidic acid-bound MT-bundling protein MAP65-1 (At5g55230), which mediates *n*-butanol- and phospholipase D-dependent MT depolymerization in vivo (Zhang et al., 2012). However, detachment of cortical MTs from the plasma membrane is only modestly enhanced or has not been observed in Arabidopsis mutants of these genes. Thus, redundant proteins with similar or distinct structures might also recruit interphase MTs to the cortical region of plant cells.

A major role of cortical MTs in interphase plant cells is to guide the movement of plasma membrane-embedded cellulose synthase complexes as they synthesize β-1,4-glucan chains (Figure 5; Paredez et al., 2006). Neighboring parallel glucan chains assemble into crystalline cellulose microfibrils when numerous hydrogen bonds form between them. These microfibrils provide the major mechanical resistance in the cell wall to external stresses and internal turgor pressure. According to the MT-microfibril alignment hypothesis for the directional (anisotropic) control of plant cell expansion, the orientation of microfibrils is determined by that of cortical MTs (Baskin, 2001). In growing plant tissues, bundles of cortical MTs are aligned perpendicular to the axis of cell expansion, and these MTs direct transverse assembly of cellulose microfibrils, which in turn promotes axial growth by constraining radial expansion. MT bundles form in the indentations of young leaf pavement cells to restrict local cell expansion. This MT-mediated constraint on growth is coordinated with local actin-mediated growth in the neck region of the neighboring cell, which results in the jigsaw puzzle shape of

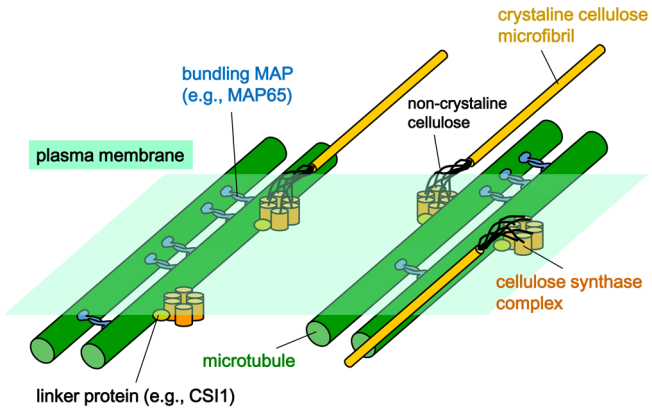

Figure 5. Cortical MTs guide cellulose deposition.

In this schematic model, a rosette-like structure containing multiple cellulose synthases co-aligns with cortical MTs and gives rise to over a dozen 1,3-β-glucan chains, which instantaneously form a crystalline cellulose microfibril in the cell wall. The cellulose synthase complex is recruited to the MT lattice by linker proteins, such as CSI1. Relatively stable MT bundles, which are formed by MAP65-family MT bundlers, are used as tracks for cellulose deposition.

mature pavement cells (Fu et al., 2005). When cortical MT arrays in longitudinally expanding cells are formed in an oblique orientation, due to MT-related mutations or treatment with MT-targeted drugs, the growth direction is tilted perpendicular to the MT alignment (Hashimoto, 2011). On the other hand, well-aligned transverse MT arrays appear to restrain auxin-mediated organ initiation at the shoot meristem in Arabidopsis (Sassi et al., 2014). In the trichoblast cells of *Lactuca sativa* (lettuce) roots, root hair initiation is suppressed by ordered arrays of cortical MTs (Takahashi et al., 2003). Thus, local growth isotropy caused by disorganized MT arrays may underlie developmentally or environmentally controlled initiation of organ formation and regional cell outgrowth.

Cellulose synthase complexes are primarily associated with MT bundles, which are more stable and persist in the cell cortex for a longer period than highly dynamic single MTs (Paredez et al., 2006). Cellulose synthase-interactive protein 1 (CSI1), a large protein containing Armadillo repeats and a C2 domain, binds to both cellulose synthase subunits and MTs, and facilitates interactions between them (Figure 5; Gu et al., 2010; Li et al., 2012; Bringmann et al., 2012). Cortical MTs also associate with small subcellular compartments containing cellulose synthases, and mediate the transfer of cellulose synthase complexes from Golgi bodies to the plasma membrane (Gutierrez et al., 2009; Crowell et al., 2009).

In contrast to typical animal cells, in which interphase MTs radiate from the centrosome near the nucleus toward the cell periphery and the growing MT plus ends interact with the plasma membrane, cortical MTs in plant interphase cells are spread in a quasi-2D sheet, in which interactions predominate between the growing plus end of one MT and the side wall of another MT that lies ahead of it (Hashimoto, 2003). After nucleation on a preexisting (mother) MT, the newly formed cortical MT is released from the mother MT, and the two resulting polymers exhibit distinct dynamic behaviors at their ends (Figure 6). The plus end shows typical dynamic in-

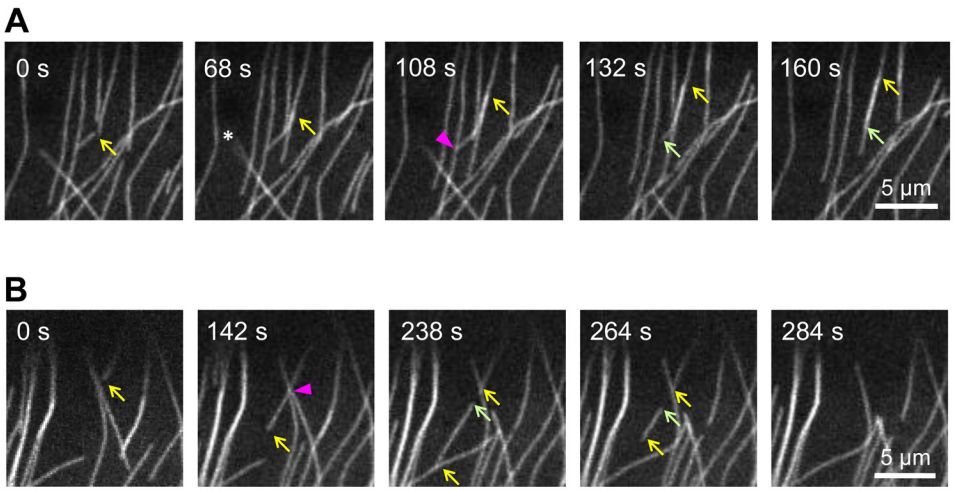

Figure 6. Cortical MT behavior.

A GFP-β-tubulin marker was expressed in onion epidermal cells. **(A)** A MT nucleated at a branched angle on an existing MT grows, while its minus end is still attached to the mother MT (its plus end is labeled with a yellow arrow in the 0-s frame). After 68 s, its plus end grows closely alongside a MT that lies ahead, and forms an anti-parallel bundle. Fluorescence in the bundled region is brighter than that of single MTs. In the 108-s frame, the bundled region is longer, and the minus end of the daughter MT is severed at the nucleation point (magenta arrowhead). The minus end (pale green arrow) of the daughter MT is released from the mother MT (132 s), and then is totally incorporated into the interacting MT (160 s). When the growing plus end is dissociated from the plasma membrane, it wobbles (asterisk in the 68-s frame) and subsequently depolymerizes. **(B)** A growing MT end (yellow arrow) is about to collide with the impeding MT (0 s), and crosses over it. The crossing MT is severed at the junction point (magenta arrowhead in the 142-s frame). After the front MT interacts with another MT (at 238 s), its plus end rapidly depolymerizes and its free minus end (pale green arrow) slowly retracts, leading to its extinction at 284 s. The exposed plus end of the rear MT after severing (yellow arrow at the crossover point in the 238-s frame) also starts to depolymerize after a pause.

stability, alternating between periods of growth and shrinkage, but is biased toward overall polymerization, whereas the free minus end undergoes slow depolymerization (Shaw et al., 2003). This net growth at one end and sustained shrinkage at the other, together with its lateral association with the plasma membrane, enables a cortical MT to migrate along the cell cortex. Migrating MTs frequently interact with other MTs that lie ahead of them. The outcome of MT-MT encounters depends on the angle at which they occur: steep-angle collisions are more likely to result in depolymerization of the incoming plus end or in crossing over of the impeding MT, whereas shallow-angle encounters tend to result in co-alignment of the two MTs and subsequent bundling (Dixit and Cyr, 2004; Figure 7). This angle-dependent modification of MT behavior can result in the cortical array being rearranged in the transverse orientation. Spontaneous ordering of MTs to the transverse array is typically seen in elongating single cells derived from isolated tobacco protoplasts (Hasezawa et al., 1988). Several computer simulations and mathematical models, which incorporate collision behaviors, MT nucleation, and cell geometry, have been proposed to explain the self-organization properties of the cortical array (Eren et al., 2012; Deinum and Mulder, 2013).

While MT-MT interactions can self-organize randomly oriented arrays into highly ordered configurations, what directional cues instruct the arrays to orient in a particular direction (e.g., transverse and longitudinal orientations) in distinct cell types? In the epidermal cells of the Arabidopsis shoot apex, cortical MTs are aligned in the direction of the predicted maximal principal stress. When a few epidermal cells of the shoot meristem were ablated by laser irradiation, MTs in the surrounding cells were rearranged along the circumference of the ablated cells, whereas a direct external force to the meristem caused MTs to align parallel to the maximal stress direction (Hamant et al., 2008). A feedback loop has been proposed in which the geometrical shape of a tissue generates stress patterns that align cortical MTs, which dictate the direction of cell growth and assist in shaping the meristem (Hamant et al., 2008). Computer simulations predict that if two opposing cell faces of newly formed cross walls are prone to induce more frequent MT depolymerization than do other cell faces, such face-dependent MT depolymerization is sufficient to form MT arrays with a net transverse orientation (Eren et al., 2012). The newly formed cross walls of cells that have just undergone cytokinesis have sharp transverse edges that induce MT depolymerization or buckling, thus acting as barriers to trespassing MTs, whereas the longitudinal edges of cross walls that formed earlier are more rounded and enriched with a MT stabilizing factor, thus allowing passage of in-coming MTs (Ambrose et al., 2011). Therefore, the intrinsic geometry of cells resulting from the history of developmentally programmed cell divisions provides an external cue that facilitates the ordering of cortical MTs.

In contrast to the vast majority of plant cells, which extend by wall loosening and deposition of new wall material along the entire length of expanding cell surfaces, some specialized cells, such as root hairs and pollen tubes, grow exclusively at the tip. Cortical MTs are found in all growth stages of root hairs and pollen tubes, but, in the subapical region of these tip-growing cells, MTs are often enriched in the core cytoplasm, forming endoplasmic MTs (Sieberer et al., 2005; Cheung et al., 2008). The nuclear surface of these cells actively initiates nascent MT formation, and is likely the origin of the many endoplasmic MTs (Ambrose and Wasteneys, 2014). The exact roles of endoplasmic MTs are unclear, but they may assist the actin cytoskeleton in delivering cell wall material to the growing tip.

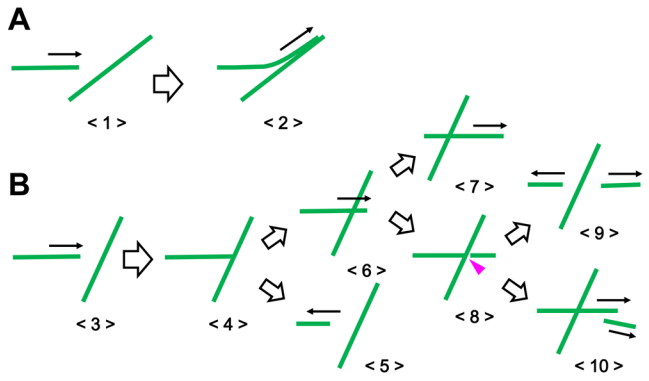

Figure 7. Schematic representation of MT-MT interactions and their outcomes.

(A) A growing plus end (arrow) approaches another MT that lies ahead of it at a relatively shallow angle **<1>**, and the end of the colliding MT changes its trajectory to align with the impeding MT, and forms a bundle **<2>**. **(B)** When a growing plus end (arrow) approaches another MT at a wide angle **<3>** and the two MTs meet **<4>**, the plus end of the colliding MT may start to depolymerize **<5>**. In other cases, the plus end crosses over the interacting MT lattice **<6>** and continues to grow **<7>**. The crossing MT may be severed at the junction point (magenta arrowhead) **<8>**. The exposed plus end of the rear MT frequently depolymerizes **<9>**, but sometimes starts to grow, generating two growing MT ends **<10>**.

Mitotic arrays

During progression of the cell cycle, MTs are transformed into several distinct arrays. At the late G2 phase, cortical MTs become enriched in a defined area of the cell, but extinct in another (Figure 8). The MT arrays composed of persistent MT bundles progressively narrow in width to form the mature preprophase band (PPB). During the formation of the PPB, MT dynamics are modulated and cortical MTs are selectively stabilized or destabilized. The PPB is initially slightly offset from the equator of the nucleus, but then the nucleus migrates just beneath the PPB. Cytoplasmic MTs that connect the nucleus and the PPB have been observed (e.g., Granger and Cyr, 2000). The PPB predicts the future site of cell plate insertion during somatic cell divisions. In symmetric cell divisions, the PPB is generally formed at the cell equator. However, when cells divide asymmetrically to generate new developmental patterns and cell types, the band is positioned at a tilted angle in a biased location to mirror the site of the future cell plate (Rasmussen et al., 2011). Although the PPB is common in vascular plants, endosperm cellularization and meiocyte development progress via orderly cell divisions that lack PPBs. Most nonvascular plants also lack PPBs, do not require a PPB for organized divisions, or have PPBs only in specialized cell types. The PPB structure may have evolved to control the precisely oriented

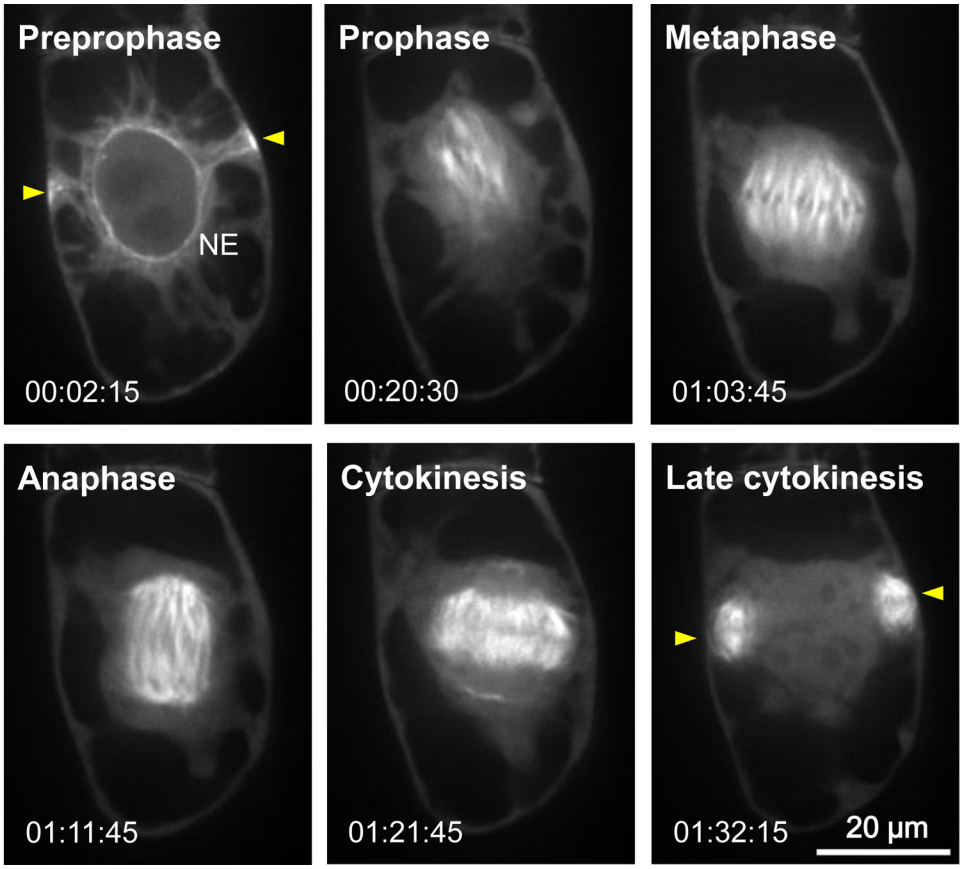

Figure 8. Mitotic MT arrays.

A tobacco BY-2 cultured cell expressing GFP-α-tubulin to visualize MTs. Times are indicated as hour: minute: second. In preprophase, MTs radiate from the surface of the nuclear envelope (NE) and form the preprophase band at the cell cortex (yellow arrowheads). The nuclear envelope disintegrates and prophase spindles begin to form during prophase. Complete spindles align the chromosomes along the equatorial plane during metaphase. In anaphase, the kinetochore MTs shorten to pull the newly formed daughter chromosomes to opposite sides of the cell. During cytokinesis, the phragmoplast expands from the center toward the cell cortex. The expanding phragmoplast may be tilted, but eventually is targeted to the exact cortical region where the preprophase band was once localized (yellow arrowheads).

cell divisions that occur during complex tissue formation in higher plants (Rasmussen et al., 2013).

The PPB begins to disassemble during prophase, but leaves spatial molecular markers at the position of the PPB, which remain present throughout cytokinesis. The deposited factors at the selected plasma membrane site then guide the centrifugal expansion of the cell plate to this site (van Damme, 2009; Rasmussen et al., 2013). Among several proteins that have been identified to function in PPB-dependent division plane determination, TONNEAU1 (At3g55000; TON1), its interacting partners, and the TON2/FASS (At5g18580)-containing protein phosphatase PP2A complex, are required for PPB formation and share unexpected sequence homology with animal proteins that localize to the centrosomes (Spinner et al., 2013). Since the site of MT nucleation is not centralized to a cellular structure in plant cells, such as the centrosome in animal cells, the functional significance of this sequence conservation in evolutionary distant organisms is unclear.

At prophase, the perinuclear spindle forms before the PPB disassembles and is organized into a bipolar spindle with its long axis perpendicular to the original plane of the PPB after nuclear envelope breakdown (van Damme, 2009). Small fractions of dividing cultured plant cells have double, offset, or abnormally shaped PPBs, or lack PPBs altogether (Granger and Cyr, 2000; Yoneda et al., 2005; Chan et al., 2005). Spindle MTs are frequently positioned obliquely in these cells with unusual PPBs, and the establishment of spindle bipolarity is delayed when no PPB is formed, indicating that the PPB promotes spindle formation and early positioning of the spindle poles.

Spindle MTs are polarized with their minus ends facing toward the two opposing poles. These minus ends are loosely focused, but do not converge on particular MT organizing centers, as found in the centrosome-based spindle organization of animal cells. Spindle MTs are disassembled at anaphase and a bipolar MT array is then formed in the spindle midzone at late anaphase

to telophase, which acts to push segregating daughter chromosomes apart from each other and toward the spindle poles.

At telophase, this bipolar MT array is transformed into the phragmoplast, the dynamic cytokinetic apparatus that is used to synthesize the expanding cell plate (Lee and Liu, 2013). The MT plus ends concentrated at or near the cell division site contain a subpopulation of interdigitating MTs with a narrow overlapping region that maintains the integrity of the array (Ho et al., 2011a). Golgi-derived vesicles are transported along the phragmoplast MTs toward the division site, where they deposit their enclosed cell wall materials. As the cell plate is being assembled, the phragmoplast expands centrifugally to the region of the plasma membrane of the mother cell where the PPB left spatial molecular markers. In the periphery of the expanding phragmoplast, newly polymerized MTs are bundled in an anti-parallel fashion, thus stabilizing the interdigitated core of the freshly assembled mini-phragmoplast module (Murata et al., 2013), whereas the older MTs in the center are disassembled.

Upon completion of cytokinesis and disassembly of the phragmoplast, the nuclear surface of the daughter cells initiates the assembly of endoplasmic MTs, which extend to the inner surface of the plasma membrane to create bipolar longitudinal cortical MT arrays (Ambrose and Wasteneys, 2014). In cultured tobacco cells, cortical MTs are initiated mainly at diagonal angles with respect to the cell axis, but gradually transition to the transverse orientation (Lindeboom et al., 2013a).

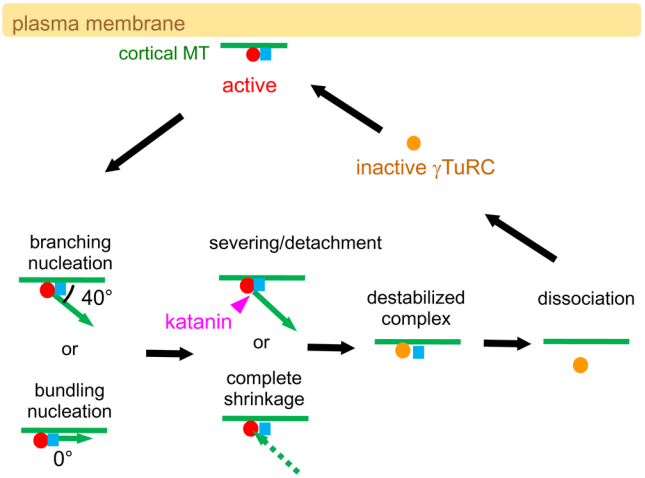

Figure 10. Model of MT-dependent MT nucleation in plant cortical arrays.

Inactive γTuRCs are present in the cytoplasm. When γTuRC is recruited to preexisting cortical MTs, it becomes competent for nucleation. Active γTuRC associates with augmin (blue square) and possibly with other regulatory factors. A daughter MT nucleates from the activated γTuRC either at an angle of approximately 40° or in parallel to the mother MT. When katanin severs the minus end of the daughter MT (magenta arrowhead) or the dynamic plus end of the daughter MT depolymerizes completely, the nucleation complex becomes destabilized, and then dissociates from the mother MT. The liberated γTuRC may be recycled for the next round of nucleation.

MT NUCLEATION

In cells, MT nucleation (i.e., the de novo initiation of nascent MT polymers) is highly regulated in space and time, and gives rise to the MTs needed to generate new MT configurations. In interphase plant cells, nascent MTs are predominantly formed on the lattice of preexisting cortical MTs (Murata et al., 2005) and are released from mother MTs to the cytoplasm just after daughter MTs are either severed at their minus ends or depolymerized completely from their dynamic plus ends (Nakamura et al., 2010) (Figures 9 and 10). MT-dependent MT nucleation in cortical arrays occurs either at branch angles of roughly 40° or along mother MTs, thus immediately promoting MT bundling (Chan et al., 2009). Phragmoplast MTs are nucleated mostly on MT bundles that are perpendicular to the cell plate (Murata et al., 2013). The nucleation angles of new MTs with respect to preexisting MT bundles peak at around 40°, with a significant proportion of nucleation events occurring at angles of less than 20°. During specific stages of interphase and in particular plant cell types, such as root hairs, MTs nucleate from the surface of the nucleus (Erhardt et al., 2002).

The γ-tubulin-containing ring complex (γTuRC) provides a template for MT nucleation in almost all multicellular eukaryotic cells. γTuRC is composed of γ-tubulin and five related subunits called γ-tubulin complex proteins (GCPs) (Kollman et al., 2011; Figure 11). One molecule each of GCP2 and GCP3 assembles with two molecules of γ-tubulin to form a Y-shaped subcomplex (γ-tubulin-containing small complex; γTuSC). In the most recent

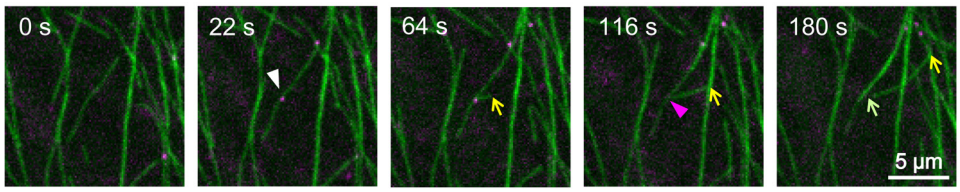

Figure 9. γTuRC nucleates a new MT on a preexisting cortical MT.

γTuRC and cortical MTs in a pavement cell of the Arabidopsis cotyledon were labeled with MOZART1a-GFP and mCherry-β-tubulin, respectively. After γTuRC appears on the lattice of a MT at 22 s, a new MT soon initiates in a branch-forming manner (see the growing plus end indicated by a yellow arrow at 64 s). The daughter MT is severed at the minus end (magenta arrowhead) at 116 s, and the liberated free minus end (pale green arrow) slowly depolymerizes at 180 s.

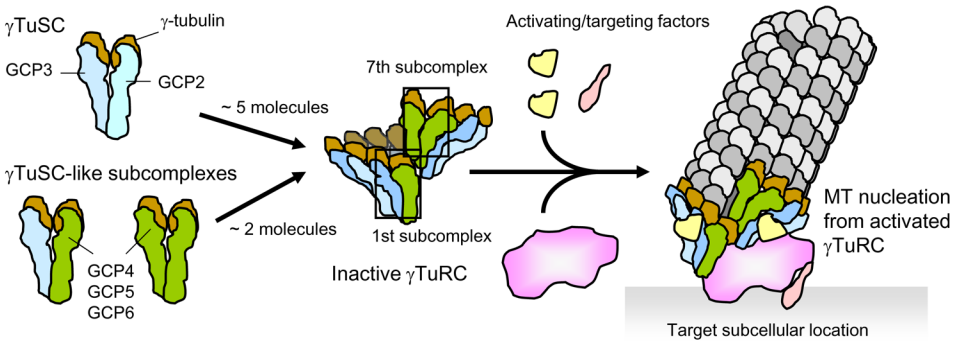

Figure 11. Schematic model of the assembly and targeting of γTuRC.

Two γ-tubulins, GCP2 and GCP3, combine to form γTuSC. GCP4, GCP5, and GCP6 are postulated to form γTuSC-like complexes. Approximately five γTuSCs and two γTuSC-like subcomplexes may assemble into inactive γTuRC. The γTuSC-like subcomplexes might be positioned at the gap in γTuRC, which corresponds to the seam of a nucleating MT. Targeting proteins, such as augmin, recruit γTuRC to a particular subcellular location, and activate the complex to initiate MT nucleation. This figure is adapted and modified from Figure 2 in Hashimoto (2013b).

model (Kollman et al., 2011), GCP2 and/or GCP3 in γTuSC may be replaced with GCP4, GCP5, or GCP6, to form γTuSC-like subcomplexes, and seven γTuSC or γTuSC-like subcomplexes then assemble helically into the lock washer-shaped γTuRC. In γTuRC, the first and seventh subcomplexes overlap by half their width, thus generating 13 γ-tubulins per helical turn. This geometry provides a suitable template for 13-protofilament MTs, which are the most frequently observed MTs in eukaryotic cells. The exact stoichiometry of γTuRC components has yet to be determined, but each human γTuRC is estimated to consist of 14 copies of γ-tubulin, 12 copies of GCP2 or GCP3, 2-3 copies of GCP4, a single copy of GCP5, and less than 1 copy of GCP6 (possibly due to proteolytic degradation or to sample heterogeneity) (Choi et al., 2010). Arabidopsis γTuRC is composed of the same subunits, with a stoichiometry similar to that reported for human γTuRC (Nakamura et al., 2010). Complete loss-of-function mutations of γTuRC genes are lethal in Arabidopsis, while partial knock-down of γTuRC components inhibits MT generation (Hashimoto 2013b).

γTuRC itself is considered to have only basal MT nucleation activity. To activate the complex, other cellular factors need to associate with it and recruit it to a particular cellular location. The activation and subcellular targeting of this complex may be coupled (Kollman et al., 2011; 2015). The most extensively studied regulator of γTuRC is augmin (from the Latin verb augmentare, “to increase”), an eight-subunit protein complex that increases the MT mass in a centrosome-independent manner (Goshima et al., 2008). Arabidopsis augmin is composed of six subunits with homologs in animals and two plant-specific subunits that represent functional homologs of the animal coiled-coil protein AUG7 (At5g17620) and the MT-binding subunit AUG8 (At4g30710) (Hotta et al., 2012). Although augmin was initially identified as a γTuRC regulator that functions within the spindle of animal cells (Goshima et al., 2008), it is required for the MT-dependent MT nucleation that occurs in the spindle (Ho et al., 2011b), the phragmoplast (Ho et al., 2011b), and the interphase cortical MT array (Liu et al., 2014) of Arabidopsis cells.

The WD-40 repeat protein NEDD1 (Neural precursor cell expressed, developmentally down-regulated protein 1) likely serves as an attachment factor between γTuRC and augmin (Goshima and Kimura, 2010). NEDD1 (At5g05970) co-purifies with γTuRC in Arabidopsis cell extracts (Nakamura et al., 2010), co-localizes with γTuRC in Arabidopsis cells (Zeng et al., 2009; Walia et al., 2014), and is required for MT nucleation in the spindle, phragmoplast, and cortical array (Zeng et al., 2009; Walia et al., 2014). In human cells, NEDD1 associates with the AUG6 subunit of augmin, and is thought to recruit γTuRC to augmin on the extant MT lattice (Zhu et al., 2008; Uehara et al., 2009). While the same γTuRC-NEDD1-augmin complex appears to generate nascent MTs on preexisting MTs in various plant MT arrays, it is not understood how the nucleation geometries of these daughter MTs are controlled (Sánchez-Huertas and Lüders, 2015). Additional regulatory factors and/or post-translational modification of the nucleation complex might be involved.

A conserved small polypeptide (approximately 8 kD), dubbed the Mitotic-spindle organizing protein associated with a ring of γ-tubulin 1 (MOZART1; At4g09550 and At1g73790), interacts with the γTuRC via GCP3 (At5g06680) and is required for MT nucleation in fission yeast (Masuda et al., 2013; Dhani et al., 2013), human (Hutchins et al., 2010), and Arabidopsis (Janski et al., 2012; Nakamura et al., 2012). In fission yeast, the core γTuRC still assembles in the absence of MOZART1, indicating that it is a peripheral regulatory component of γTuRC.

MT REGULATORS

MT dynamics, interactions with other cellular components, and higher-order organization are sophisticatedly regulated by various proteins that act on MT polymers or tubulin subunits. Historically, MT-binding proteins with sufficient affinity for MTs have been purified from cell extracts by co-purification with bundled MTs and biochemically identified as MT-associated proteins (MAPs) (see Hamada et al., 2014 for Arabidopsis MAPs). Novel MT regulators

have been identified in molecular genetics-based and other studies, and include proteins that bind to tubulin subunits or do not efficiently co-sediment with MTs after ultracentrifugation. Some plant MT regulators are evolutionarily conserved and have counterparts in animals and yeasts, while others appear to be specific to the plant lineage.

Many MAPs bind to the MT lattice. The MT-binding domains of some MAPs are enriched with basic amino acid residues that may interact with the highly acidic C-terminal tails of tubulins (Paschal et al., 1989), but a large variety of unrelated structural domains can interact with the MT surface in distinct binding modes. MAPs may stabilize MTs by reducing their dynamicity, and MAP-bound MTs often become bundled and exhibit increased tolerance to MT-destabilizing drugs. When a MAP is composed of a MT-binding domain and an antiparallel dimerization domain, the MAP homodimer may form a bridge between two adjacent MTs and induce MT bundling (Figure 5). MT bundling proteins of approximately 65 kD were first identified biochemically in plant extracts (Jiang and Sonobe, 1993), and homologous MAPs were later discovered in various eukaryotes. Members of the evolutionarily conserved MAP65 family contain an extended N-terminal dimerization domain and a C-terminal MT-binding domain with a spectrin fold, and crosslink two antiparallel MTs (Subramanian et al., 2010, 2013). Kinesin motors cooperate with MAP65s to control the dynamics and size of MT bundles (Walczak and Shaw, 2010). The Arabidopsis MAP65 family contains nine members, some of which display non-overlapping biochemical functions (Smertenko et al., 2008; Ho et al., 2012). Arabidopsis MAP65s localize to regions of MT overlap in kinetochore fibers (Fache et al., 2010) and the phragmoplast (Ho et al., 2011a), as well as to a subset of interphase cortical bundles (Lucas et al., 2011). The organization and functioning of the Arabidopsis phragmoplast depend on a cytokinesis-specific MAP65 protein (At5g51600; Műller et al., 2004; Ho et al., 2011).

Plus-end tracking proteins (+TIPs) are unique MAPs that specifically accumulate at growing MT plus ends. Some +TIPs possess intrinsic properties that cause them to accumulate at MT plus ends, as demonstrated in in vitro reconstitution experiments with dynamic MTs and recombinant +TIPs, whereas others do not possess true end-tracking activities but associate with bona fide +TIPs and are carried to the growing MT end in piggyback fashion (Akhmanova and Steinmetz, 2008). The evolutionarily conserved end-binding 1 (EB1) family is the canonical +TIP of the former class. The calponin-homology domain of EB1 binds to MTs at the vertex of four tubulin dimers and comes into contact with the regions of tubulin that coordinate GTP hydrolysis (Maurer et al., 2012; Alushin et al., 2014). Binding studies using GTP analogs suggest that EB1 recognizes the GTP cap. In animal cells, EB1 serves as a central adaptor that recruits various +TIPs of the latter class to the MT plus end through natively unstructured basic and serine-rich regions (Kumar and Wittmann, 2012; Jiang et al., 2012). Fluorescent protein fusions of EB1 appear as comets at the growing ends of MTs both in vitro and in vivo, and are currently widely used as markers to monitor MT dynamics in various eukaryotic cells (Figure 12). In non-plant eukaryotes, complete knockout of EB1 activity generally severely impairs MT functions or is lethal (Duellberg et al., 2013). Surprisingly, a triple knockout mutant of all three Arabidopsis EB1 genes (At3g47690, At5g62500, and At5g67270) grows normally and is almost indistinguishable from wild-type plants when grown under standard conditions (Komaki et al., 2009). In the mutant, the alignment of spindle and phragmoplast MTs is temporally compromised, but cell division and cytokinesis eventually proceed almost normally, and the nuclear-localized EB1 subtype is largely responsible for this mild phenotype. Apparently, EB1 does not function as a central hub that accumulates multiple physiologically important +TIPs in Arabidopsis cells.

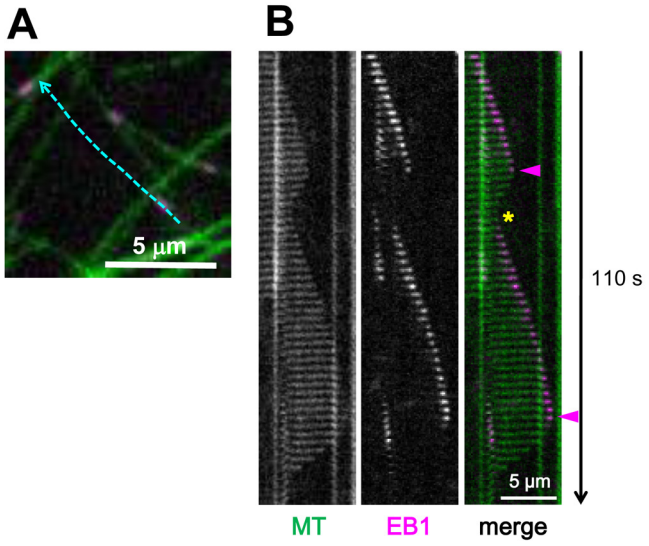

Figure 12. EB1 decorates the growing plus end of MTs. mCherry-β-tubulin and EB1b-GFP were co-expressed in a pavement cell of the Arabidopsis cotyledon.

(A) A cortical MT is marked by a dashed arrow. **(B)** Time series of a MT plus end. The mCherry-β-tubulin marker (green) monitors a full range of MT dynamics, whereas EB1 (magenta) marks only the growing tip and disappears from the shrinking end. The magenta arrowheads indicate two catastrophe events, while the asterisk shows a rescue event.

XMAP215 (Xenopus MAP215) family members are the evolutionarily conserved +TIPs that accelerate MT growth (Al-Bassam and Chang, 2011). XMAP215 tracks the growing MT plus end, and possesses five TOG domains (named after the human ortholog Tumor Overexpressed Gene; ch-TOG) and a basic MT lattice-binding region that together are important for MT polymerization (Brouhard et al., 2008; Widlund et al., 2011). The current model suggests that the second TOG domain and the internal lattice-binding region recruit the XMAP215-family +TIP to the growing plus end, where the most N-terminal TOG domain captures an unpolymerized curved tubulin dimer and then releases the straightened dimer upon its incorporation into the MT end, thereby concentrating the polymerization-competent tubulins at the growing plus end (Ayaz et al., 2012, 2014). The Arabidopsis homolog MOR1 (Microtubule Organization 1; At2g35630; Whittington et al., 2001) also contains five TOG domains at the N-terminus and a C-terminal MT-binding region (Twell et al., 2002), as found in fly and mammal XMAP215 members (Al-Bassam and Chang, 2011). The temperature-sensitive *mor1-1* allele possesses an amino acid substitution in the first TOG domain (Whit-

tington et al., 2001) and, at the restrictive temperature, disrupts cortical MT arrays (Whittington et al., 2001) as well as mitotic and cytokinetic arrays (Kawamura et al., 2006). Cortical MTs in the *mor1-1* cells become less dynamic upon the temperature shift (Kawamura and Wasteneys, 2008).

Cytoplasmic linker-associated proteins (CLASPs) contain TOG-like domains that show sequence homology with the XMAP215 family TOG domains and bind to tubulin dimers in a similar manner (Al-Bassam et al, 2010). Animal and yeast CLASPs bind to MT lattices directly and are also targeted to MT plus ends by their interactions with EB1 and other +TIPs (Al-Bassam and Chang, 2011). The Arabidopsis CLASP is enriched at the growing plus ends of MTs (Ambrose et al., 2007; Kirik et al., 2007), but it is unknown whether it tracks plus ends directly or requires EB1 for its tip localization. Animal and yeast CLASP proteins promote MT rescue, suppress MT catastrophe, and selectively stabilize interphase MTs at particular cellular sites (Al-Bassam and Chang, 2011). In Arabidopsis leaf epidermal cells, CLASP partly contributes to the attachment of interphase MTs to the cortex (Ambrose and Wasteneys, 2008). In addition, Arabidopsis CLASP suppresses catastrophe when MT plus ends encounter sharp cell edges head-on, and guides MTs around sharp edges (Ambrose et al., 2011). Arabidopsis CLASP interacts with the retromer component sorting nexin 1 to facilitate the association between endosomes and MTs, and to regulate auxin transport by trafficking the auxin efflux carrier Pin-Formed 2 (Ambrose et al., 2013).

SPIRAL1 (SPR1; At2g03680) is a plant-specific protein of approximately 11 kD, belonging to a six member family with overlapping functions in Arabidopsis (Nakajima et al. 2004, 2006; Sedbrook et al. 2004). In Arabidopsis cells, SPR1 is localized to the MT lattice and partially accumulates at the growing plus ends of MTs, forming an extended comet that is much longer than the EB1 comet (Sedbrook et al. 2004). This in vivo association of SPR1 with cortical MTs does not require EB1 (Galva et al. 2014). It is not clear how SPR1 associates with MTs and is preferentially recruited to the plus end, since SPR1 is not recovered in MAP preparations from Arabidopsis cell extracts (Hamada et al. 2013) and a recombinant SPR1 protein with no additional purification tags does not bind to MTs in vitro (unpublished results of S. Komaki, D. Coleman, H. Takahashi, and TH). A recombinant SPR1 protein fused to a glutathione *S*-transferase tag, which would form an artificial homodimer (Waugh, 2005), however, showed affinities for both MTs and unpolymerized tubulin in vitro (Galva et al. 2014). The Arabidopsis *spr1* mutant shows right-handed helical growth of elongating axial organs (Furutani et al. 2000), and this twisting phenotype is exaggerated in multiple mutants of the *SPR1* gene family (Nakajima et al. 2006).

Katanin (derived from the Japanese word for sword) and structurally related proteins (i.e., spastin and fidgetin) internally break MTs in an ATP-dependent manner. The 60-kD catalytic subunit of katanin belongs to the ATPase associated with diverse cellular activities (AAA) protein superfamily that uses the energy of ATP hydrolysis to remodel large molecular complexes within the cell (Roll-Mecak and NcNally, 2010). The canonical 80-kD regulatory subunit of katanin contains an N-terminal WD40 repeat domain and a C-terminal conserved domain, and targets the katanin severing activity to defined sites of cellular MTs. The Arabidopsis genome contains four genes that encode proteins homologous to the katanin regulatory subunits and two genes that encode spastin- and fidgetin-like proteins (Roll-Mecak and NcNally, 2010), but their functions have not been characterized. Katanin is monomeric when bound to ADP, but assembles into a hexamer on the MT lattice in the presence of ATP (Hartman and Vale, 1999). MT severing is a remarkable biological activity of katanin, as the enzyme must break both longitudinal and lateral interactions in the rigid MT lattice. A current model proposes that the disordered and negatively charged C-terminal tail of tubulin is tugged into the central pore of the katanin hexamer, thereby partially unfolding tubulin or locally destabilizing tubulin-tubulin interactions within the MT lattice (Roll-Mecak and Vale, 2008). ATP hydrolysis may trigger conformational changes in katanin that generate a mechanical force to pry apart the tubulin subunits.

Arabidopsis mutants of the katanin catalytic subunit gene (At1g80350) are defective in anisotropic cell expansion, show delays in the disappearance of perinuclear MT arrays at the onset of interphase, and have highly randomized cortical MT arrays in elongating root cells (Burk et al., 2001; Bichet et al., 2001). In the cortical MT arrays of *katanin* mutant cells, the severing events at the minus ends of daughter MTs (Nakamura et al., 2010) and at the crossover sites of two intersecting MTs (Zhang et al., 2013; Lindeboom et al., 2013b) are abolished. Indeed, GFP-tagged katanin catalytic subunit transiently associates with these severing sites. At MT crossover sites, the overlying MTs are preferentially severed, possibly reflecting that metazoan katanin localizes at defective sites (e.g., kinks and discontinuities) in the MT lattice (Zhang et al., 2013). It is not known what mechanism targets katanin to the basal region of newly nucleated daughter MTs.

Arabidopsis SPIRAL2 (SPR2; At4g27060) and its closest homolog SPIRAL2-Like (SP2L; At1g50890) are composed of nine repeat regions that share weak sequence similarity to the TOG domains, and directly bind to MTs in vitro through these repeat regions (Yao et al., 2008). In vitro, SPR2 and SP2L proteins promote dynamicity of the MT plus end by suppressing the static pause state, whereas plus end growth is occasionally stalled in cells lacking functional SPR2 and SP2L. The MT arrays in the epidermal cells of hypocotyls of the *spr2* and *spr2 sp2l* mutants are arranged in left-handed helices, and the cells show right-handed helical growth, whereas the MT arrays of wild-type elongating cells are transversely arranged (Shoji et al., 2004; Buschmann et al., 2004; Yao et al., 2008). GFP fusions of SPR2 and SP2L, expressed under the control of their native regulatory elements in Arabidopsis cells, are localized along the MT lattice in particles of various sizes. The SPR2-GFP particles are motile in the epidermal cells of hypocotyls and cotyledons, but accumulate at the MT crossover sites in cotyledon pavement cells (Wightman et al., 2013). Intersecting MTs are more frequently severed in the pavement cells of the *spr2* mutant than in those of the wild type, suggesting that SPR2 prevents katanin-based MT severing at MT crossover sites.

Kinesins are MT-based motor proteins containing a catalytic motor core with an ATP-binding site and a MT-binding site, frequently juxtaposed with a neck region. The position of the motor domain in a kinesin polypeptide is often used to classify the kinesin as an N-terminal, C-terminal, or internal motor kinesin. The Arabidopsis genome has at least 61 kinesin genes, which can be grouped into about a dozen subfamilies based on the phylogenetic analysis of their motor domains (Lee and Liu, 2004). Many kinesins are MT plus end-directed motors, but some C-terminal

motor kinesins move toward the minus ends of MTs. Several Arabidopsis kinesins organize and facilitate the function of distinct MT arrays during the cell cycle (Lee and Liu, 2004; Zhu and Dixit 2012). A unique MT-dependent signaling role is found for the plant-specific AtNACK1 kinesin (At1g18370; also called HINKEL), which is required for cell-plate expansion (Krysan et al. 2002). The tobacco NACK1 physically interacts with a mitogen-activated protein kinase (MPK) kinase kinase, and recruits it to the equatorial midzone of the phragmoplast, where the kinase is activated (Nishihama et al., 2002). The NACK1 phosphorylation cascade in Arabidopsis constitutes the MPK kinase MKK6 (At5g56580) and its downstream MPK4 (At4g01370) (Sasabe and Machida 2012). Activated tobacco MPK4 phosphorylates MAP65 and decreases its MT-bundling activity, thereby contributing to controlled expansion of the phragmoplast (Sasabe et al. 2006). Animal kinesins of the Kinesin-13 family, which often contain motor domains in the central region of the protein, are not motor proteins but are MT depolymerases that couple ATP hydrolysis to the removal of GTP-tubulin dimers from both ends of MTs (Howard and Hyman 2007). Arabidopsis Kinesin-13A (At3g16630) depolymerizes MTs in an ATP-dependent manner in vitro, but the depolymerization of cortical MTs in vivo requires its association with the MIDD1 MAP (At3g53350), an effector of the activated Rho of Plant (ROP) GTPase (Oda and Fukuda 2013).

Cortical actin filaments occasionally and transiently co-align with cortical MTs. When actin filaments were first depolymerized by a drug and were then reassembled following washout of the drug, new actin polymers recovered along cortical MTs (Sampathkumar et al. 2011). A plant-specific actin-nucleating formin, AtFH4, is a membrane-integrated protein that can interact with both actin and MTs (Deeks et al. 2010), and a 190-kD protein in tobacco interacts with both actin and MT bundles in vitro (Igarashi et al. 2000). Protein complexes consisting of the actin-binding protein NET3C (At2g47920) and the membrane-anchored protein VAP27 (At3g60600), which has an affinity for MTs, are proposed to specify the contact sites between the cortical endoplasmic reticulum network and the plasma membrane (Wang et al. 2014).

The Arabidopsis plasma membrane-associated Ca^{2+}-binding proteins PCaP1 (At4g20260) and PCaP2 (At5g44610) bind to the Ca^{2+}-calmodulin complex and phosphatidylinositol phosphates (Nagasaki et al. 2008; Kato et al., 2010). Interestingly, recombinant PCaP proteins depolymerize, in a Ca^{2+}-dependent manner, both MTs (thus called MAP18 and MT-Destabilizing Protein 25; Wang et al. 2007; Li et al. 2011) and actin filaments (Zhu et al. 2013; Qin et al. 2014). Knock-down or knock-out of PCaP genes in Arabidopsis impairs polarized tip growth in root hairs (Kato et al. 2013) and in pollen tubes, and is associated with disorganized apical actin filaments in the latter (Zhu et al. 2013; Qin et al. 2014). It remains to be established whether these plasma membrane-localized Ca^{2+}-regulated proteins directly disassemble cortical MT arrays in vivo or influence MT organization indirectly by way of the disrupted actin cytoskeleton.

An increasing number of MAPs and MT regulators are being discovered in plant cells, as described in several recent review articles (Gardiner 2013; Hamada 2014).

SIGNALING PATHWAYS

Plant MT arrays are reorganized in response to endogenous cues and environmental stimuli. Plant phytohormones have long been known to cause cortical MTs to reorient to transverse or longitudinal arrays in elongating epidermal cells of various plant tissues (Shibaoka 1994). Gibberellins and brassinosteroids generally promote the transverse organization of the arrays, whereas ethylene and abscisic acid commonly induce longitudinally oriented arrays. The effect of auxin on cortical MT arrays depends on the concentration of auxin and the tissue used. In light-grown Arabidopsis hypocotyl cells, synchronous treatment with auxin and gibberellin induces the formation of transverse MT arrays (Vineyard et al. 2013). In the roots and etiolated hypocotyls of Arabidopsis, the exogenous application of elongation-inhibitory concentrations of auxin induces rapid MT remodeling from transverse to longitudinal arrays. This rapid response to auxin mostly depends on auxin binding protein 1 (ABP1; At4g02980) and its downstream signaling components, such as ROP6 GTPase (At4g35020) and ROP-interacting CRIB motif-containing protein RIC1 (At2g33460) (Chen et al. 2014). In interdigitating pavement cells in Arabidopsis, the same ABP1-ROP6-RIC1 pathway promotes ordering of cortical MTs in the indentation region (Xu et al. 2010), where MT severing by katanin is modulated by RIC1 (Lin et al. 2013). It should be noted that Arabidopsis *abp1* null mutants were recently shown to exhibit no detectable defects in auxin signaling and in plant development (Gao et al., 2015), challenging the validity of previous studies based on other *abp1* alleles and *ABP1*-related transgenic lines. Several MAP genes are up-regulated by phytohormones, and may contribute to the longer-term re-organization of cortical MT arrays (Wang et al. 2012).

Light strongly affects the organization of cortical MT arrays, especially on the Arabidopsis hypocotyls. Blue light rapidly triggers the reorientation of cortical arrays to longitudinal by stimulating katanin-mediated MT severing at MT intersections (Lindeboom et al. 2013b). Light may modulate hypocotyl elongation by modulating the abundance of MAPs via proteasome-dependent degradation (Liu et al. 2013).

Environmental stresses affect the organization and stability of plant MTs. Dehydration, high salinity, low temperature, and aluminum are reported to destabilize cortical MTs in plants (Schwarzerová et al. 2002; Sivaguru et al. 2003; Abdrakhamanova et al. 2003; Shoji et al. 2006; Wang et al. 2007; Krtková et al., 2012; Ban et al. 2013). Postmeiotic radial MT arrays in Arabidopsis male gametes are depolymerized in response to cold stress (De Storme et al. 2012). Proteasome-dependent degradation of SPR1 protein is stimulated in response to high salinity (Wang et al. 2011). The best-characterized response of MTs to environmental stress involves Propyzamide-hypersensitive 1 (PHS1; At5g23720). An Arabidopsis *phs1-1d* allele was isolated as a gain-of-function mutant that displayed partial destabilization of cortical MTs and possessed a missense mutation in the putative kinase-interacting motif of a MPK phosphatase (Naoi and Hashimoto, 2004). Null alleles of PHS1 are phenotypically indistinguishable from the wild type under standard growth conditions (Pytela et al. 2010), but do not induce MT depolymerization upon hyperosmotic stress (Fujita et al. 2013). In addition to the MPK phosphatase domain, PHS1 contains an atypical kinase domain

that phosphorylates Thr349 of α-tubulin (Fujita et al. 2013). This threonine residue is well conserved among eukaryotic α-tubulins and is located at the longitudinal interdimer interface of the tubulin heterodimer. Tubulins phosphorylated at this residue are not incorporated into MT polymers in vitro and in vivo. Thus, the tubulin kinase activity of PHS1 is normally suppressed by its phosphatase activity, but is unmasked immediately after exposure to osmotic stress, inducing rapid depolymerization of cortical MTs.

Cyclin-dependent kinases (CDKs) and aurora kinases, together with other kinases and phosphatases, regulate the progression of the cell cycle and organization of mitotic MT arrays, but how the phosphorylation cascades affect MT functions is not well understood. A protein complex consisting of TONNEAU1 (TON1) and protein phosphatase 2A is recruited to the preprophase MTs via the redundant TON1-recruiting motif family of proteins (Spinner et al. 2013). Although the TON1 complex is essential for the formation of the preprophase band, little is known about the mechanism underlying the generation of the phosphatase-regulated preprophase band. A MPK kinase kinase NPK1 and the NPK1-activating kinesin-like protein NACK1 are phosphorylated by CDKs during the early mitotic phases in cultured tobacco cells, which ensures that the active NPK1-NACK1 complex is not formed before cytokinesis when the complex promotes the regulated progression of the phragmoplast MT array (Sasabe et al. 2011).

Differentiation of special cell types may be accompanied by rearrangements of cortical MTs. During xylem vessel differentiation, cortical MTs are remodeled into distinct configurations that direct the formation of secondary cell walls with annular, spiral, reticulate, pitted, or other thickenings. In developing xylem cells, ROP11 GTPase (At5g62880) is locally activated by the membrane-anchored guanine nucleotide exchange factor ROP-GEF4 (At2g45890) and inactivated by the cytoplasmic GTPase-activating protein ROP-GAP3 (At2g46710) ; these three ROP-related components are sufficient to reconstitute evenly spaced patches of active ROP11 domains in non-xylem cells (Oda and Fukuda 2012). Locally activated ROP11 recruits a ROP11 effector MIDD1 to the plasma membrane domains, where cortical MTs are disassembled by the MIDD1-activated MT depolymerizer Kinesin-13A (Oda and Fukuda 2013).

CONCLUDING REMARKS AND FUTURE PROSPECTS

Although the structure and the intrinsic dynamic properties of MTs are highly conserved in eukaryotes, the cytoskeleton in plant cells adopts higher order array assemblies than that in animal and fungal cells, and displays distinct means of regulating polymer stability. The underlying molecular mechanisms involve a variety of MT regulators that are evolutionarily conserved, have diverged in their regulatory modes, or appear to have evolved specifically in the plant kingdom. Developmental, environmental, and physiological cues are transduced to remodel the spatio-temporal organization of the MT arrays, but these signaling pathways are just beginning to be unraveled. Genetic approaches and molecular manipulations have highlighted the importance of MT regulators in the cellular organization of MT arrays and in the proper growth and development of Arabidopsis plants. The mechanisms by which the MT regulators modulate basic polymer functions and higher-order assemblies, however, have not been sufficiently explored, and require in vitro characterizations of the regulators and the functional complexes. Once the major regulatory factors have been identified and fully characterized, the nucleation and release of nascent MTs, MT-MT interactions, and transformation of a MT array into a new configuration may be reconstituted in vitro. Considerable effort has been devoted to modeling cortical MT organization. Our ability to model MT organization accurately would be proof of our understanding of this versatile cytoskeleton component.

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