# Generation of noncentrosomal microtubule arrays

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*Accepted 23 August 2006*
*Journal of Cell Science 119, 4155-4163 Published by The Company of Biologists 2006*
*doi:10.1242/jcs.03227*

## Summary

**In most proliferating and migrating animal cells, the centrosome is the main site for microtubule (MT) nucleation and anchoring, leading to the formation of radial MT arrays in which MT minus ends are anchored at the centrosomes and plus ends extend to the cell periphery. By contrast, in most differentiated animal cell types, including muscle, epithelial and neuronal cells, as well as most fungi and vascular plant cells, MTs are arranged in noncentrosomal arrays that are non-radial. Recent studies suggest that these noncentrosomal MT arrays are generated by a three step process. The initial step involves formation of noncentrosomal MTs by distinct mechanisms depending on cell type: release from the centrosome, catalyzed nucleation at noncentrosomal sites or breakage of pre-existing MTs. The second step involves transport by MT motor proteins or treadmilling to sites of assembly. In the final step, the noncentrosomal MTs are rearranged into cell-type-specific arrays by bundling and/or capture at cortical sites, during which MTs acquire stability. Despite their relative stability, the final noncentrosomal MT arrays may still exhibit dynamic properties and in many cases can be remodeled.**

Key words: Microtubules, Centrosome, *S. pombe*, Myotubes, Neurons, Epithelia, Plants

## Introduction

Microtubules (MTs) are prominent elements of the cytoskeleton that contribute to cell division, migration and polarity. They do so by moving chromosomes (in division) and by serving as tracks for transport of vesicles, organelles, other cytoskeletal elements, protein assemblies and mRNA. In many cells, MTs also contribute to polarity by maintaining the steady-state positions of cellular organelles, including the nucleus, Golgi apparatus, endoplasmic reticulum, mitochondria and lysosomes.

MTs in cultured animal cells are strongly influenced by the presence of centrosomes. The centrosome consists of a pair of centrioles and pericentriolar material that contains the γ-tubulin ring complexes (γ-TuRCs) that are responsible for nucleating MTs. γ-TuRCs are highly conserved structures composed of γ-tubulin and associated proteins, and in fission yeast all γ-tubulin complex (γ-TuC) components are needed for cytoplasmic MT organization (see Weise and Zheng, 2006, in this issue). Other proteins, such as pericentrin, NEDD1 and ninein contribute to centrosome function by recruiting and tethering γ-TuRCs to the centrosome and anchoring newly formed MTs (Dictenberg et al., 1998; Luders et al., 2006; Mogensen et al., 2000). Typically, interphase cells have a single centrosomal array, whereas mitotic cells have two centrosomal arrays. In each case, the arrays are radial; the fast-growing plus end of the MT is distal to the centrosome and the slow-growing minus end is located at or near the centrosome. In mitosis, the two radial arrays give rise to the bipolar mitotic spindle. In migrating cells, whether they retain proliferative capacity or are end-stage cells, such as neutrophils, MTs are also generally radially arrayed.

The processes that contribute to the generation of these radial arrays include MT nucleation and anchoring by the centrosome (Andersen, 1999; Hyman and Karsenti, 1998), self-assembly of MTs from tubulin subunits, dynamic instability of MTs (Cassimeris and Spittle, 2001), and capture of MTs by chromosomes and cortical sites (Gadde and Heald, 2004; Gundersen, 2002; Gundersen et al., 2004; Schuyler and Pellman, 2001). These properties allow the overall radial array of MTs to be maintained while individual MTs turn over. Key players in these processes are members of the family of +TIPs (such as CLIP-170 and EB1) that localize preferentially at the plus end of growing MTs (Akhmanova and Hoogenraad, 2005; Howard and Hyman, 2003). These +TIPs can also mediate interactions with cortical or intracellular structures and have been implicated in signal transduction pathways that regulate MT organization and stability (Gundersen, 2002; Gundersen et al., 2004).

The non-radial MT arrays in differentiated animal and plant cells are less well understood. Many of the same processes (assembly of tubulin subunits and dynamic instability) contribute to the formation of MTs in differentiated cells; yet there are major differences. Whereas the radial MT arrays in proliferating and migrating cells are centrosome-based, those in differentiated cells are noncentrosomal and usually linear. Also, whereas most MTs in proliferating and migrating cells are very dynamic, in differentiated cells many MTs become stabilized (Bulinski and Gundersen, 1991; Gundersen and Bulinski, 1986).

Here, we consider recent data from the study of noncentrosomal arrays and use this information to propose a three-step process as a conceptual framework for understanding how these arrays are formed. We do not discuss centrosome-independent mechanisms for generating radial MT arrays in cells, such as those regulating the formation of acentrosomal spindles in female animal meiotic cells and plant

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**Table 1. Cell types with predominantly centrosomal or noncentrosomal microtubule arrays**

| Mammalian | Other |
|---|---|
| Centrosomal | |
| Fibroblasts | *D. discoideum* |
| Lymphocytes | *S. cerevisiae* |
| Macrophages | |
| Neutrophiles | |
| Melanocytes | |
| Myoblasts | |
| Osteoblasts | |
| Non-centrosomal | |
| Neurons | *S. pombe* |
| Epithelia | Non-mammalian erythrocytes |
| Myotubes | Somatic plant cells |
| | Epithelia in *D. melanogaster* |

mitotic cells or experimental situations where the centrosome has been removed. The reader is referred to excellent reviews on this topic (Gadde and Heald, 2004; Keating and Borisy, 1999).

## Linear, noncentrosomal MT arrays are typical of polarized, nonmigratory cells

Cells that exhibit linear, noncentrosomal arrays range from single-celled organisms such as *Schizosaccharomyces pombe* (*S. pombe*) to differentiated cells in multicellular organisms from both the plant and animal kingdoms (Table 1). The great diversity of cell types in which noncentrosomal arrays are found seems to indicate that there is no unifying feature that distinguishes cells with linear, noncentrosomal arrays. However, these cells are all axially polarized and they are all non-migratory. Below, we describe five systems in which recent studies have begun to shed light on how linear, noncentrosomal arrays of MTs are generated and how they function.

### *S. pombe*

The interphase MT array of the fission yeast *S. pombe* is a relatively simple noncentrosomal array. It is required for delivery of polarity factors, actin-regulatory factors and membrane components to sites of growth, which normally occurs only at the cell ends (Chang, 2001; Chang et al., 2005; Chang and Peter, 2003; Hayles and Nurse, 2001). It also positions the nucleus at the cell center by exerting pushing forces through MT polymerization (Tran et al., 2001). Interphase MTs are arranged into three to five bundles of MTs that parallel the long axis of the cell, their plus ends directed towards the cell ends and their minus ends overlapping near the nuclear surface (Fig. 1). One or two of the interphase bundles are attached to the spindle pole body (SPB), which is the yeast equivalent of the centrosome and is tightly apposed to the nuclear membrane. Other MT bundles are attached to interphase MT-organizing centers (iMTOCs). Thus, the

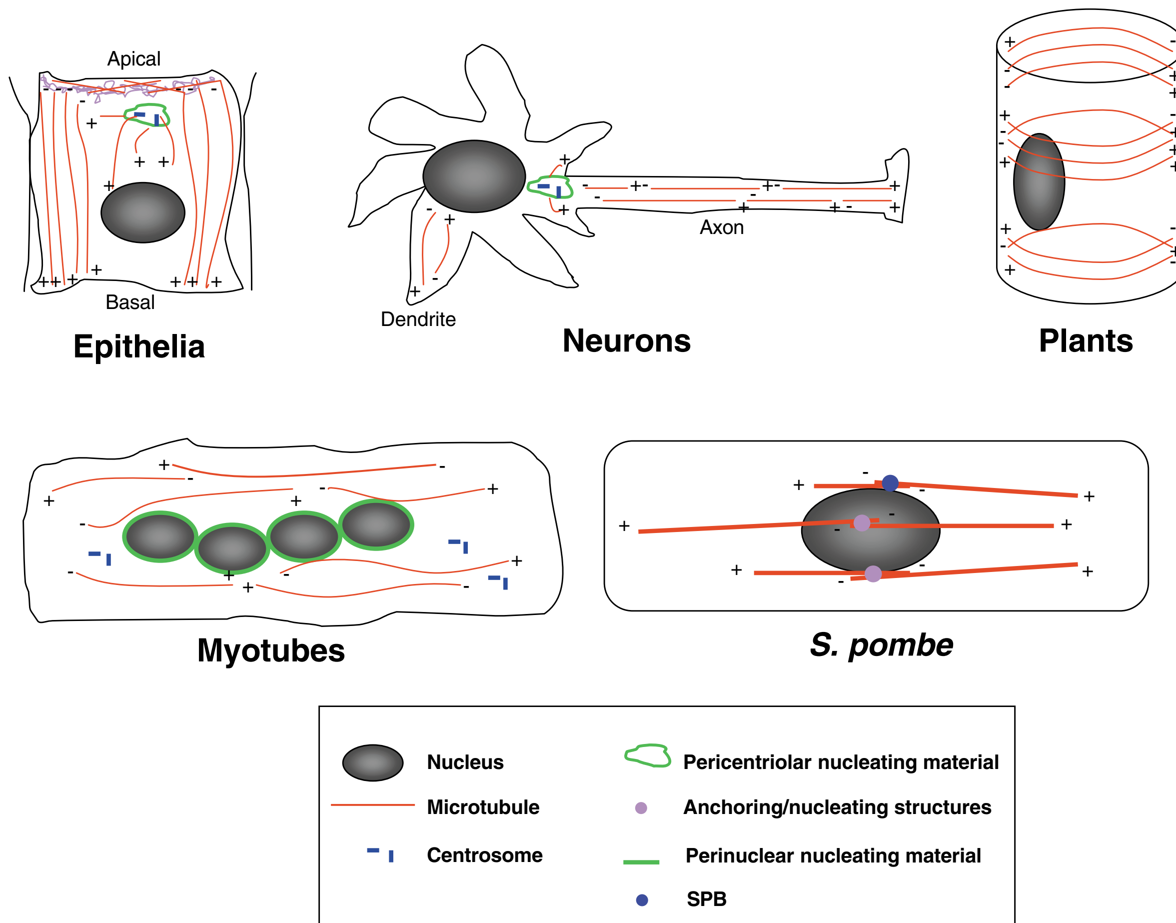


**Fig. 1.** Cells with noncentrosomal MT arrays.

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interphase MT array in *S. pombe* is actually a mixture of centrosomal and noncentrosomal MTs. We consider the *S. pombe* MT array among the linear, noncentrosomal arrays, because most of the MTs are not contributed by the SPBs and the overall array is linear.

### Muscle cells

Skeletal muscle fibers (myotubes) are large multinucleated cells derived from the fusion of mononucleated muscle precursor cells (myoblasts). In developing myotubes before the formation of actin-myosin sarcomeres, there is an abundant array of MTs and these are distributed along the long axis of the cells, their plus ends being distal and their minus ends localized at dispersed sites on or around the nuclei, which cluster toward the center of the myotube (Fig. 1) (Tassin et al., 1985). Upon cell fusion, the radial, centrosomal MT array characteristic of myoblasts is replaced by noncentrosomal MTs arranged parallel to the long axis of the multinucleated cell (Tassin et al., 1985). During this process, MT-nucleating material clusters around the nuclei shortly after fusion and centrioles completely disappear in older myotubes (Connolly et al., 1986; Tassin et al., 1985). Notably, many MTs in the array become stabilized as the cells differentiate (Gundersen et al., 1989).

The linear, noncentrosomal array of MTs in myotubes has been implicated in myofibrillogenesis as well as in establishing the elongated shape of the cell (Antin et al., 1981; Hill et al., 1986; Pizon et al., 2005; Tassin et al., 1985). Experiments using inhibitors of MTs have also implicated MTs in the mobility of acetylcholine receptors in primary cultures of chick embryonic muscle cells, suggesting a function for these arrays in the development of neuromuscular junctions (Connolly, 1984; Connolly and Oldfin, 1985). MTs may also be required for the clustering of muscle nuclei at neuromuscular junctions (Englander and Rubin, 1987).

### Neuronal cells

Neurons have a combination of centrosomal and noncentrosomal MTs, some in the cell body being attached to the centrosomes. In terminally differentiated neuronal cells, MTs are assembled into linear, noncentrosomal arrays in both axons and dendrites. These highly polarized structures emerge from the cell during differentiation and are sites of connection with other neurons via synapses. The MT arrays within these processes are necessary to maintain the processes and may indirectly contribute to synaptic activity. They are believed to provide structural support for each process as well as tracks for the delivery of membrane vesicles (Horton and Ehlers, 2003; Overly et al., 1996; Signor and Scholey, 2000).

Ultrastructural studies show that axons and dendrites have distinct arrays of MTs. In axons, MTs are generally long and uniformly oriented, their plus ends distal to the cell body, whereas in dendrites MTs are much shorter and exhibit mixed polarity (Fig. 1) (Baas et al., 1989; Baas et al., 1988; Baas et al., 1991; Burton and Paige, 1981; Heidemann et al., 1981). In neither case are MTs attached to the centrosome or any known nucleating structure. In addition, cross sections through neuronal processes have shown that the MTs are not randomly distributed but are regularly spaced by cross-bridges ranging in length from ~65 nm in dendrites to ~25 nm in axons (Chen et al., 1992).

### Epithelial cells

MTs organization in epithelial cells depends on the type of epithelium and whether the cells are in culture or in a tissue. One characteristic array of MTs that appears in most polarized epithelial cells is a prominent linear, noncentrosomal array in which the MTs are aligned along the apical-basal axis of the cell (Fig. 1). In these arrays, the plus ends of MTs are located basally, whereas the minus ends are apical (Bacallao et al., 1989). Like in muscle cells and neurons, these MTs tend to be more stable than those in radial interphase arrays (Bre et al., 1987; Pepperkok et al., 1990). In some epithelia, in addition to the apical-basal array, there is a separate apical array of shorter noncentrosomal MTs organized into a meshwork. These MTs display mixed polarity so that MT plus and minus ends are near the membrane. A basal meshwork of noncentrosomal MTs has also been described in cultured epithelia (Reilein and Nelson, 2005; Reilein et al., 2005), although it is not yet clear whether these MTs are derived from the apical-basal linear array or are formed independently. Some epithelia also possess a small number of centrosomal MTs.

The linear noncentrosomal arrays in epithelial cells contribute to the structural and functional polarity of epithelia. Apical and basolateral membrane domains are separated by tight junctions in epithelia and there is evidence that MTs contribute directly to the delivery and maintenance of proteins in the apical domain (reviewed by Musch, 2004; Rodriguez-Boulan et al., 2005). Surprisingly, there is less evidence that the prominent linear apical-basal MT array contributes to the delivery or maintenance of the basolateral membrane domain. Nevertheless, that basolaterally targeted proteins may depend on MTs is reinforced by studies in which inhibition of kinesin motor proteins interferes with basolateral protein targeting (Kreitzer et al., 2000).

### Plants

As opposed to animal and yeast cells, somatic plant cells lack a discrete MT-organizing center (MTOC), such as the centrosome or the SPB, and do not have cytoplasmic dynein to help localize MT minus ends at the MTOC. Instead they are characterized by ordered cortical MT arrays containing bundles of MTs that have non-uniform polarity (Fig. 1). In rapidly growing interphase cells, these bundles of MTs are perpendicular to the main growth axis of the cell. The ability of plant cells to dynamically modify their shape relies on the regulated deposition of several layers of cellulose microfibrils, which are also typically organized transversely around the cell. The orientation of each layer depends on the distribution of a parallel set of cortical MTs that are assembled beneath the plasma membrane when cells enter interphase or commit to differentiation (Lloyd and Chan, 2004; Smith and Oppenheimer, 2005). Like cellulose microfibrils, these MTs can be arranged in spiral patterns maintained by MT-MT cross-bridges and by tight association with the plasma membrane through protein linkers.

## A three-step model for generating linear, noncentrosomal MT arrays

On the basis of evidence from the five systems described above, we propose a general model for the formation of linear, noncentrosomal arrays of MTs. The key feature of this model is that linear, noncentrosomal arrays of MTs are formed in

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three sequential steps: (1) generation of noncentrosomal MTs; (2) movement of noncentrosomal MTs to sites of assembly and; (3) assembly of noncentrosomal MTs into higher-order arrays (Fig. 2).

## Generation of noncentrosomal MTs

Noncentrosomal MTs are the key building blocks for the formation of noncentrosomal arrays. They are generated by three mechanisms: release from centrosomes, nucleation from noncentrosomal sites and breakage distal to the centrosome. In general, noncentrosomal MTs form at sites distant from where they will be assembled into the final array, perhaps reflecting the tendency for nucleating factors such as γ-tubulin to be localized centrally in the cell.

### Release from the centrosome

Release from the centrosome appears to be the major mechanism for generating noncentrosomal MTs in epithelial and neuronal cells. The rate of release in epithelial cells has been determined by direct imaging of fluorescently tagged MTs and is an order of magnitude higher than in fibroblasts, in which the MTs are predominantly radial and remain attached to the centrosome (Keating et al., 1997; Vorobjev et al., 1997; Waterman-Storer and Salmon, 1997). The rate of MT release has only been studied in sparse epithelial cells; so it is not yet clear whether similar enhanced centrosomal release accounts for the noncentrosomal arrays found in polarized epithelial cells. Release of MTs from the centrosome in neuronal cells has not been imaged directly but has been inferred from fixed-cell experiments in which MTs are allowed to repolymerize transiently after being exposed to high concentrations of nocodazole (Ahmad and Baas, 1995; Baas, 1996). MTs initially regrow as an aster at the centrosome and are later observed at peripheral locations, few MTs remaining attached to the centrosome. MTs appear to be frequently released from neuronal centrosomes, but direct imaging studies are needed to rule out alternative interpretations and to establish that this occurs under steady-state conditions.

In neuronal cells, the MT-severing protein katanin appears to be involved in centrosomal release, which is consistent with its centrosomal localization (Hartman et al., 1998; McNally et al., 1996). Microinjection of a neutralizing anti-katanin antibody strongly interferes with the release of MTs from the centrosome and the generation of neuronal extensions (Ahmad et al., 1999). Whether katanin or the related severing protein spastin (Evans et al., 2005) is involved in release of MTs in epithelia or other animal cells has not been explored.

In plants, severing of MTs by katanins may also contribute to the generation of cortical MT arrays. Several *Arabidopsis* mutations (*fra2*, *bot1*, *ehr3* and *lue1*) map to a genomic region that encodes a katanin p60-like protein. Interestingly, all these mutants exhibit disorganized cortical MT arrays, which lead to defects in isotropic cell growth, inflorescence stem fragility and ectopic root hairs (Bichet et al., 2001; Bouquin et al., 2003; Burk et al., 2001; Webb et al., 2002).

An alternative to severing is release of MTs from nucleating sites at the centrosome. A key issue is whether MTs released from centrosomes are released with their γ-TuRCs. If so, this would solve the question of what prevents MT minus ends from depolymerizing once they are released from centrosomes. Recent studies have identified at least one protein (GCP-WD,

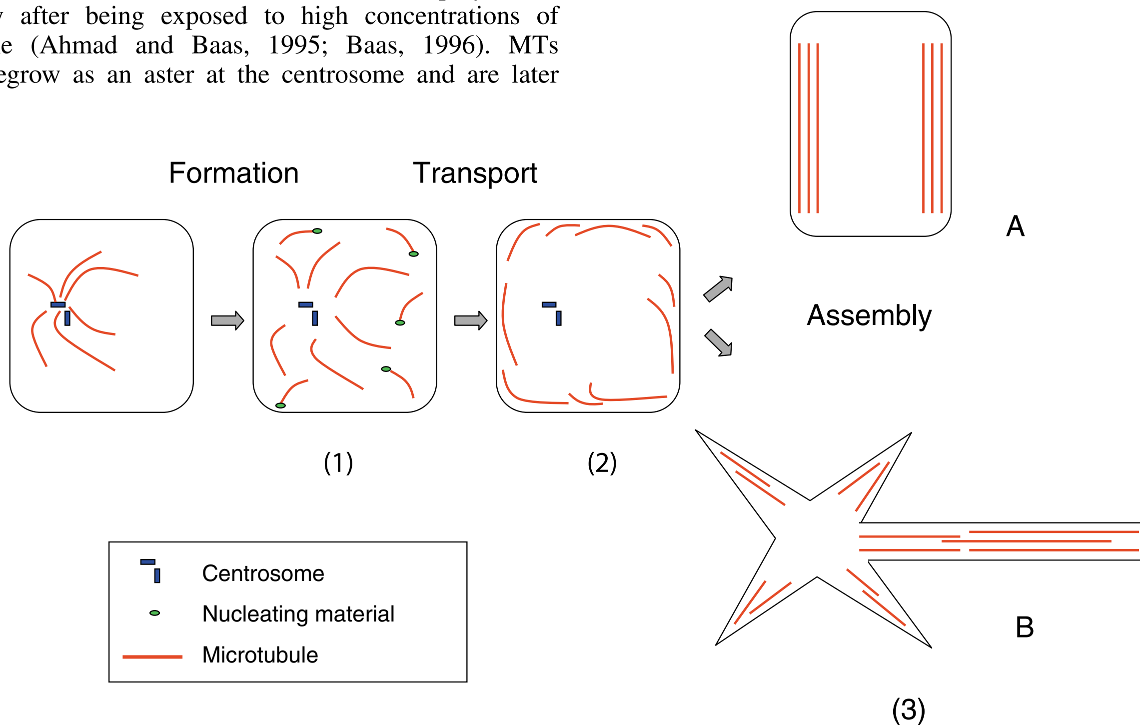


**Fig. 2.** The three-step hypothesis. Schematic diagram of the three steps in the formation of noncentrosomal MT arrays. (1) Upon receiving an initiating signal, MTs can be generated de novo at noncentrosomal sites or be released from the centrosome where they were nucleated. (2) Once formed, noncentrosomal MTs are translocated to sites of assembly, typically by MT-dependent motors. (3) Noncentrosomal MTs are stabilized and assembled into ordered arrays by MT capture and bundling. Depending on the cell type, noncentrosomal MTs can be arranged either (A) along the major axis of the cell as is the case in epithelia, myotubes, plant cells or *S. pombe* or (B) parallel to cellular processes as in neurons.

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also known as NEDD1) that appears to tether γ-TuRCs at the centrosome without being necessary for the integrity of the γ-TuRC, and this would be an obvious candidate for sites of regulation and release of MTs with bound γ-TuRCs (Luders et al., 2006). Despite the attractiveness of this hypothesis, there is little indication that γ-tubulin is present on minus ends of stable noncentrosomal MTs, and in neurons there is evidence that is not (Baas and Joshi, 1992).

Perhaps other, unidentified proteins stabilize these ends. One possible candidate is ninein, a protein originally described as a centrosomal MT-anchoring factor (Bouckson-Castaing et al., 1996; Mogensen et al., 2000). In polarized cells, ninein is also found at noncentrosomal sites, and in neurons it has been implicated in the recapture and stabilization of free MT minus ends as the MTs translocate into developing neurites (Baird et al., 2004). There is evidence that ninein also has a role in MT nucleation and anchoring by providing a direct link between centrioles and γ-TuRCs (Delgehyr et al., 2005).

Free minus ends might not need stabilizing factors. Although free MT minus ends are unstable in certain cells, such as fibroblasts, they are quite stable in neurons and epithelial cells. The latter might lack active minus-end depolymerases present in fibroblasts or have minus-end stabilizers missing in fibroblasts. In fact, many studies show that newly formed minus ends are relatively stable. For example, generation of free minus ends by UV microbeam severing of MTs does not rapidly lead to depolymerization in vitro or in vivo (Tao et al., 1988; Walker et al., 1989). The minus ends might therefore persist for some time and only need additional factors for long-term stabilization.

## Nucleation at noncentrosomal sites

Nucleation of MTs from noncentrosomal sites has been extensively studied in plant cells, which are acentriolar. At late G2 phase and just after cytokinesis, MTs originate from the nuclear periphery and grow towards the cell cortex (Granger and Cyr, 2001; Yuan et al., 1994). The idea that nucleation occurs from sites on the nucleus is supported by the finding that MTs assemble on isolated nuclei (Hasezawa et al., 2000; Stoppin et al., 1994). However, during interphase, cortical MTs appear to be nucleated at scattered sites near the cell cortex as shown by imaging of GFP-tubulin in epidermal cells of *Arapidopsis* (Shaw et al., 2003). New MTs appear both in association with existing MTs and in areas devoid of MTs. Evidence for MT nucleation at cortical sites also comes from the observation that SPC98 (a component of the γ-TuRC) and EB1 associate with newly formed MTs in the periphery of plant cells (Chan et al., 2003; Erhardt et al., 2002). MT polymerization sites marked by GFP-tagged EB1 associate with and dissociate from the cortex, which suggests that nucleating material constantly redistributes.

By contrast, another study has found that MT-dependent MT nucleation is the predominant pathway for generating the cortical array in plants (Murata et al., 2005). This study implicated γ-tubulin as the nucleating material at the cell cortex and suggested a model in which cytosolic γ-TuRCs are actively recruited to pre-existing cortical MTs for the nucleation of new MTs. Some of these new MTs are incorporated into bundles of MTs whereas others undergo catastrophe.

Interestingly, a similar process occurs in *S. pombe*. The generation of the interphase MT array depends on the translocation of MT-nucleating material from the equatorial MTOC (eMTOC) formed in late anaphase to the iMTOCs and to the SPB at the periphery of the nucleus (Zimmerman et al., 2004). A key player in this process is rsp1p, a protein containing a DNAj/hsp40 domain that is involved in the dispersion of γ-TuCs, presumably by directing the recruitment of Hsp70 to the eMTOC (Zimmerman et al., 2004). rsp1p and γ-tubulin both reside at iMTOCs near the nucleus and in small particles that move along MTs in a motor-dependent way. Translocation of γ-TuCs on MTs is also observed in cells lacking mto1p, a centrosomin-related protein involved in the recruitment of γ-TuCs to iMTOCs (Sawin et al., 2004; Zimmerman and Chang, 2005).

Studies of mto2p, to which mto1p binds directly (Samejima et al., 2005), provide further evidence that nucleating components are transported along pre-existing MTs in *S. pombe* (Janson et al., 2005). Analyses of the *mto2Δ* phenotype showed that mto2p is involved in MT nucleation from noncentrosomal γ-TuCs and that MT nucleation occurs from cytoplasmic γ-TuCs bound to existing MTs. Nucleation material at the minus end of a new MT occasionally moves along a pre-formed MT bundle until it encounters an iMTOC proximal to the nuclear envelope. This regulated arrangement of nucleating material onto pre-existing MTs could generate and maintain the bipolar overlap of the interphase bundles.

In MT-regrowth experiments in vertebrate cells, MTs frequently form at noncentrosomal sites. However, few studies have shown that these MTs are nucleated by specific factors and their formation during regrowth may simply reflect spontaneous nucleation due to the high tubulin concentration. Nonetheless, MTs regrow from specific sites at the periphery of the nucleus in early syncytial myotubes and in cardiac myocytes (Bugnard et al., 2005; Kronebusch and Singer, 1987; Tassin et al., 1985). Nuclear surfaces of mammalian osteoclasts have also been reported to nucleate MTs at sites where pericentrin localizes (Mulari et al., 2003). Indeed, both pericentrin, ninein and γ-tubulin localize to the outer nuclear envelope in skeletal myotubes, and MT regrowth from nuclear sites is blocked by injection of inhibitory anti-γ-tubulin antibodies (Bugnard et al., 2005).

## Breakage of pre-existing MTs

Breakage at sites distant from the centrosome may also contribute to the formation of noncentrosomal MTs. Breakage by forces generated by actin retrograde flow generates noncentrosomal MTs in migrating epithelial cells and neuronal growth cones (Rodriguez et al., 2003; Waterman-Storer and Salmon, 1997). As yet, there is no evidence that noncentrosomal MTs formed by this mechanism contribute to noncentrosomal arrays, and the MT fragments generated by this process appear to breakdown.

MT-severing enzymes may also contribute to breakage of MTs distal to the centrosome. Defective katanin function results in an increase in the overall length of MTs extended from the cell body (Ahmad et al., 1999), which indicates a role for MT severing at sites other than the centrosome. The localization of spastin to the Golgi (Evans et al., 2005) suggests that severing contributes to the formation of noncentrosomal MTs at these sites. Severing enzymes may generate shorter polymers that are easier to transport during neurite outgrowth

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and to regulate MTs during collateral branching (Baas et al., 2005; Yu et al., 1994). Consistent with this idea is the observation that the p60 catalytic subunit of katanin is highly expressed at the tips of growing neuronal processes, peak expression levels occurring at dendritogenesis (Yu et al., 2005).

## Transport of noncentrosomal MTs to sites of assembly

Once noncentrosomal MTs are generated, they must be brought to the sites where they are incorporated into linear arrays. Increasing evidence indicates that in animal cells this is accomplished by MT motor proteins. Treadmilling is an alternative mechanism for moving MTs into noncentrosomal arrays and appears to play a role in plant cells.

### Motor proteins

In neurons, the observation that tubulin is a component of slow axonal transport led to the early hypothesis that this provides a source of tubulin to assemble MTs in axons and dendrites (Tytell et al., 1984). What was unclear from these early studies was whether tubulin was transported as subunits or as assembled MTs. Recent studies have now shown that assembled MTs are moved into neuronal processes by MT motors. A combination of photobleaching and difference imaging in cultured nerve cells showed that a subset of MTs in axons move fast and asynchronously (Wang and Brown, 2002). Injection of recombinant dynamitin arrests the translocation of MTs from the cell body into the axon, implicating cytoplasmic dynein in the process (Ahmad et al., 1998). Depletion of cytoplasmic dynein by RNAi in cultured rat sympathetic neurons has confirmed that cytoplasmic dynein is a key player in the anterograde transport of MTs (He et al., 2005).

For cytoplasmic dynein to move MTs into the axon, it has to be anchored (through its cargo domain) to a structure with higher resistance to translocation than the MTs it is moving. One possible candidate is the actin cytoskeleton. Cytoplasmic dynein itself moves with slow axonal transport at a rate similar to that of actin (Dillman et al., 1996). Also, drug-induced depolymerization of actin filaments in neurons modifies the organization of MTs in growth cones and specifically reduces the rate of anterograde MT transport (Hasaka et al., 2004). Understanding how dynein interacts with actin would provide further support for this model.

Kinesin motor proteins may also contribute to MT transport in neuronal processes by generating forces against other MTs (Myers et al., 2006). One candidate is the minus-end-directed kinesin CHO2/HSET, which is involved in the anterograde transport of MTs in developing neurites (Sharp et al., 1997). Other kinesins have been implicated in MT transport. For example, the plus-end-directed motor CHO1/MKLP1 is required for translocation of MTs into dendrites (Sharp et al., 1997; Yu et al., 2000).

In *S. pombe*, motor-dependent MT-sliding along pre-existing MTs contributes to the establishment of uniformly polarized antiparallel bundles. The minus-end-directed kinesin-like protein Klp2 of the Kar3/Ncd family has been implicated in this process (Carazo-Salas et al., 2005). To date, very little is known about MT translocation in epithelia or muscle although it is likely that motor-mediated movement rather than modified treadmilling, which has only been observed in plants, translocates MTs in these systems.

### MT treadmilling

MT treadmilling occurs when there is balanced addition of tubulin subunits at the plus end and loss at the minus end. In plants, treadmilling rather than MT translocation by motors appears to be the prominent mechanism for moving noncentrosomal MTs to their sites of assembly in the cortex. Evidence for this comes from photobleaching analyses of individual GFP-labeled MTs in *Arabidopsis* epidermal cells (Shaw et al., 2003). The MTs exhibit dynamic instability at both ends but have a bias toward depolymerization at the minus ends and a bias toward polymerization at the plus ends, which results in treadmilling of the MT. All of the MTs examined in these experiments translocated by treadmilling, which suggests this is the major mechanism to reposition cortical MTs in plants.

## Assembly of noncentrosomal MT arrays

Once noncentrosomal MTs are formed and moved, they need to be assembled into their final arrays. Capture of MTs at cytoplasmic or cortical sites and bundling seem to be two general mechanisms that contribute to the assembly process. A common feature of the MTs in noncentrosomal arrays, particularly in metazoan cells, is that they become stabilized (Bulinski and Gundersen, 1991; Gundersen and Bulinski, 1986). This probably reflects the need to sustain long-lived arrays during differentiation and morphogenesis. An obvious implication is that capture and bundling not only contribute to the assembly of the 3D array but also help stabilize and thus maintain it.

### MT capture

MT capture is typically mediated by +TIPs and their cortical receptors (Gundersen, 2002; Gundersen et al., 2004; Schuyler and Pellman, 2001). In many cases, this is facilitated by bridging proteins that guide MTs to these receptors by interacting with actin filaments (Gundersen et al., 2004; Rodriguez et al., 2003).

Molecules that participate in MT capture in cells that have radial centrosomal arrays have also been implicated in regulation of noncentrosomal MT arrays. For example, EB1, APC and MACF/ACF7 mediate MT capture in fibroblasts and endodermal cells (Kodama et al., 2003; Wen et al., 2004). EB1, APC and Shortstop (*Drosophila* MACF/ACF7) are found at the myotendenous junction in *Drosophila* and are necessary for the arrangement of the noncentrosomal MTs found there (Subramanian et al., 2003). EB1, APC, shortstop and members of the orbit/mast/CLASP family of +TIPs have also been implicated in regulation of MTs in axonal growth cones and epithelial cells in both *Drosophila* and mammalian systems (Lee and Kolodziej, 2002; Reilein and Nelson, 2005; Reilein et al., 2005; Zhou et al., 2004; Lee et al., 2004). Cortical capture of MTs has been extensively described in *S. pombe* and most of the +TIPs (tip1p, the CLIP-170 orthologue, and mal3p, the EB1 orthologue) are required to regulate MT dynamics that allow MTs to find the ends of the cell (Chang, 2001; Chang and Peter, 2003; Hayles and Nurse, 2001).

It is not known whether MT capture in cells that have noncentrosomal arrays resembles capture of plus ends of MTs in cells with radial MT arrays. In *S. pombe*, MTs grow from organizing sites on the nucleus until they reach the cell ends, where they pause for 90-100 seconds before undergoing catastrophe (Tran et al., 2001). This pausing helps maintain the axial array of MTs. Several +TIPs prevent MTs undergoing

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catastrophe before they reach the cell ends (Chang et al., 2005; Hayles and Nurse, 2001).

In MDCK epithelial cells, a basal array of MTs is arranged in a meshwork (Reilein and Nelson, 2005; Reilein et al., 2005). MTs in this meshwork appear to be anchored along their length to cortical sites and in some cases to each other through their ends. APC and EB1 appear to be involved in both processes. MTs within the meshwork are dynamic, which allows the meshwork to be remodeled while maintaining its overall arrangement.

MT plus ends are also important for cortical attachment of MT arrays in plants. For example, the plus ends of most somatic phragmoplast MTs are stabilized by association with the cortex (Austin et al., 2005). EB1 and other +TIPs may regulate the association of plus ends with cortical sites in plants as in yeast and animal cells. SPR1, a plant-specific microtubule-associated protein (MAP) (Nakajima et al., 2004; Sedbrook et al., 2004), and the *Arabidopsis* kinesin ATK5, a member of the kinesin-14 subfamily (Ambrose et al., 2005), preferentially localize to MT plus ends and could participate in cortical interactions. It would also be interesting to test whether the Ras interacting with calmodulin (RIC) proteins, which regulate cortical MTs and the actin cytoskeleton downstream of the plant-specific ROP-family of small G proteins, also target MT plus ends (Fu et al., 2005).

Lateral capture of cortical MTs has also been observed in plant cells, in which cross-bridges between cortical MTs and the plasma membrane arrange them in cortical arrays. Specific inhibition or activation of p90/PLD, an enzyme that associates with MTs and membranes (Gardiner et al., 2001; Marc et al., 1996), affects the organization of interphase cortical MTs, leading to either the disruption of cortical MTs or their uncoupling from the plasma membrane (Dhonukshe et al., 2003; Gardiner et al., 2003).

### MT bundling

MT bundling, particularly in neurons, *S. pombe* and plant cells, is a major contributor to the assembly of noncentrosomal, linear MT arrays. MAPs, such as tau, MAP1, MAP2 and MAP4, and motors shape the array and stabilize the MTs within it. Although MTs are clearly bundled in many noncentrosomal arrays, the existence of bona fide MT-bundling proteins in animal cells has been difficult to establish, except for the kinesins that bundle spindle MTs (Chui et al., 2000; Kapitein et al., 2005). A confounding factor is that many proteins that interact with MTs can bundle MTs when overexpressed but clearly are not normally involved in bundling (e.g. +TIPs).

Bundling of MTs is thought to be important for the consolidation of MTs in growing neuronal processes and provide structural support for mature axons and dendrites. Tau, which is preferentially found in axons (Binder et al., 1985), and MAP2, which is preferentially found in dendrites (Bernhardt and Matus, 1984), might bundle MTs because overexpression of these proteins in insect cells generates processes that have MT-MT spacings that reflect the size of the MAP (Chen et al., 1992). Nonetheless, mice lacking either tau or MAP2 have relatively normal neurons, which suggests that other factors are involved (Harada et al., 1994; Teng et al., 2001). Motor proteins, which help bundle MTs in *S. pombe* and plant cells, are other likely candidates. They might also ensure that the bundles in dendrites are antiparallel (Sharp et al., 1997; Yu et al., 2000).

In plants, growing MTs collide with and join smaller bundles. Cortical MT stability is regulated by protein phosphorylation (Naoi and Hashimoto, 2004), and the organization of the cortical array involves MT-dependent nucleation followed by selective stabilization of MTs formed in the appropriate orientation (Murata et al., 2005). Several MAP-like proteins are involved (for a review, see Hussey et al., 2002). At least one member of the MAP65 family seems to align the cortical MTs possibly by cross-bridging the 25-30 nm gap between them (Chan et al., 1999). MOR1/TMBP200 is the tobacco orthologue of the *Xenopus* MAP215 and can regulate MT dynamics in vitro (Hussey et al., 2002). Mutations in *mor1* cause temperature-dependent loss of organization of parallel MT arrays in vivo (Whittington et al., 2001).

A MAP65 family protein known as ase1p is another MAP that has a dual role organizing MTs in mitosis and interphase in *S. pombe* (Loiodice et al., 2005). In *ase1*-deleted cells, the nucleus is misplaced at interphase as a consequence of a failure to stabilize antiparallel MT bundles where they overlap. Ase1 dynamically localizes to iMTOC and may engage a MT motor.

MTs are stabilized in both muscle and epithelial cells (Gundersen et al., 1989; Pepperkok et al., 1990), but whether a capture mechanism or bundling is involved is unclear. The muscle-specific RING finger proteins MURF-2 and MURF-3 both regulate muscle differentiation by modulating MT stability and so probably play a part (McElhinny et al., 2004; Spencer et al., 2000).

## Concluding remarks

Most of the differentiated cells in animals and plants contain noncentrosomal MT arrays. We have used evidence from five different systems to propose a general model for how these noncentrosomal MTs arrays are generated. Although the model focuses on the events that contribute to the formation of noncentrosomal MT arrays, which usually display enhanced stability compared with radial MT arrays, it is important to bear in mind that the final noncentrosomal MT arrays are generally far from static structures. In plants, for example, cortical bundles contain MTs of mixed polarity, and these alternate between phases of depolymerization and polymerization; some MTs within the array depolymerize within minutes (Tian et al., 2004). Similarly, in epithelial cells the basal meshwork of noncentrosomal MTs can incorporate new tubulin subunits, while at the same time maintaining its overall organization (Reilein and Nelson, 2005). Whether the dynamics of these relatively stable arrays reflects homeostasis of the array or stimulus-dependent regulation of its activity needs further attention. Thus, in addition to understanding how noncentrosomal arrays are formed, we will also need to consider how their dynamics are regulated if we are to understand their function.

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