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JB Review

Regulatory mechanisms and cellular functions of non-centrosomal microtubules

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Proper organization of microtubule (MT) arrays is essential for numerous cellular functions, including intracellular transport and cell migration. Although the centrosome generally serves as the primary MT-organizing centre in proliferating animal cells, MTs are also organized at the Golgi apparatus in a wide range of cell types to regulate Golgi ribbon formation that is required for polarized cell migration. Furthermore, differentiated epithelial cells and neurons possess organized non-centrosomal MTs predominantly at the apical cortical regions and the axonal and dendritic neurites, respectively, to establish and maintain their highly polarized morphology. Unlike radial arrays of centrosomal MTs, non-centrosomal MTs are organized into non-radial asymmetric network, which facilitates polarized transport and cell polarization. In this review, we will focus on recent advances in our understanding of the regulatory mechanisms and cellular functions of non-centrosomal MTs.

Keywords: cell polarity; Golgi apparatus; microtubule minus end; microtubule organizing centre (MTOC); non-centrosomal microtubule.

Abbreviations: γ-TuRC, γ-tubulin-ring complex; AKAP450, A-kinase anchor protein 450; CAMSAP, calmodulin-regulated spectrin-associated protein; CC, coiled-coil; CLASP, CLIP-associated protein; CLIP, cytoplasmic linker protein; IFT20, intraflagellar transport 20; MMG, myomegalin; MT, microtubule; MTOC, MT-organizing centre; PCM, pericentriolar matrix.

Microtubules (MTs) are cytoskeletal filaments assembled from heterodimers of two closely related globular proteins, α- and β-tubulin. They are present in all eukaryotic cells and play fundamental roles in diverse cellular processes, such as cell morphology and polarity, formation of the cilia and flagella, and intracellular transport of secretory vesicles, organelles and other cellular components. They also form mitotic spindles to ensure proper segregation of chromosomes. An individual MT is a hollow, 25-nm (in diameter) cylindrical tube organized mostly by 13 longitudinal protofilaments (*1*). Each protofilament is composed of head-to-tail arrays of the tubulin dimers, consequently giving rise to polarity with two distinct ends, plus end (at which β-tubulin is exposed) and minus end (at which α-tubulin is exposed) (*2*).

Although pure α/β-tubulin heterodimers polymerize spontaneously *in vitro* in a temperature-dependent manner, when their concentrations exceed the critical one, cells require nucleating factors to initiate their polymerization, which occurs at the distinct subcellular sites called MT-organizing centres (MTOCs) in a cell-type specific manner (*3*). The centrosome, a non-membrane bound organelle composed of a pair of centrioles surrounded by pericentriolar matrix (PCM), is the best-studied MTOC and traditionally considered as the major site of MT nucleation and anchoring (*4*). The centrosomal MTs are typically organized into a radial array with plus ends extending towards the periphery of the cell and minus ends attached to the nucleating factor, γ-tubulin-ring complex (γ-TuRC), localized at the centrosome. A number of proteins are known to regulate localization of γ-TuRC to the centrosome, including ninein, CEP192, AKAP450, pericentrin and CDK5RAP2 (*4*).

In addition to the centrosome, other subcellular sites are known to act to organize non-centrosomal MTs, which are observed predominantly in some differentiated cell types. Polarized epithelial cells generally have MT arrays along the apical–basal polarity with their minus ends accumulated around the apical cortex (*5*). In mature neurons, non-centrosomal MTs are arranged in the axons and dendrites (*6*). In muscle cells, non-centrosomal MTs are organized around the nuclear envelope and at the Golgi apparatus (*7*, *8*). MT organization at the Golgi apparatus is also observed in other cell types, including epithelial cells, neurons and cancer cells (*9*–*11*). Although the arrangement and localization of non-centrosomal MTs appear to vary

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among different cell types, important advances in our understanding of the proteins that regulate the minus ends of non-centrosomal MTs have been made in recent years. In this review, we overview current understanding of the regulatory mechanisms and cellular functions of non-centrosomal MTs.

Dynamic Organization of MTs

MTs are highly dynamic structures that undergo rapid cycles of assembly and disassembly at the ends. This behaviour known as 'dynamic instability' is driven by hydrolysis of the GTP bound to β-tubulin (*12*). Although both α- and β-tubulin within the dimers are in the GTP-bound state when they are polymerized, only the GTP bound to β-tubulin can be hydrolyzed shortly after polymerization, which gives rise to a cap of GTP-bound β-tubulin at the newly polymerized MT tips, whereas MT shaft contains GDP-bound β-tubulin (*13*) (Fig. 1). As GTP-bound MT cap is highly stable, MTs continue growing in the presence of the GTP-cap. In contrast, GTP hydrolysis triggers the conformation changes in tubulin dimers, which make MTs less stable (*14*). Therefore, loss of the GTP-cap causes catastrophe, the transition of MTs from growth to shortening, whereas regaining the cap leads to rescue, the transition of MTs from shortening to growth (Fig. 1). Thus, growth or shortening of MTs is determined by the rate of tubulin incorporation relative to the rate of GTP hydrolysis.

In vitro studies using purified tubulin have shown that MTs can grow and shrink at both plus and minus ends, but the plus end grows faster and undergoes catastrophe more frequently (*13*, *15*). Furthermore, *in vitro* severing of an MT gives rise to the new plus end that undergoes rapid depolymerization, whereas the new minus end is relatively stable and can resume growth (*16*, *17*). Thus, the two ends have intrinsically different dynamic properties. In cells, the plus ends repeat rapid growth and shortening to search

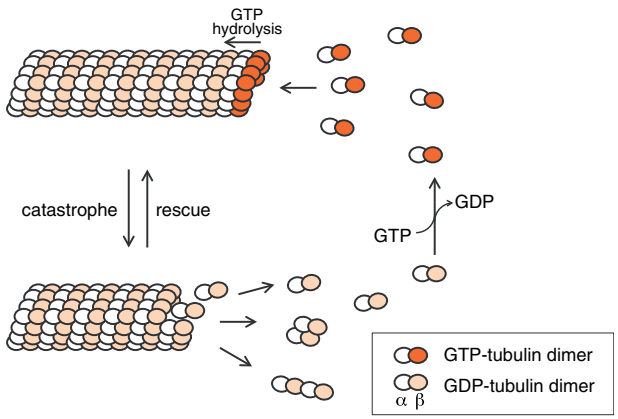

Fig. 1 Assembly and disassembly of MTs. The GTP-tubulin dimers assemble on the MT plus ends, forming the GTP cap. The GTP bound to β-tubulin can be hydrolyzed shortly after incorporation. Loss of the GTP-cap causes catastrophe, whereas regaining the cap leads to rescue. The released tubulin dimers will be available again for another MT assembly/disassembly cycle following the nucleotide exchange.

the 3D space of the cell (*14*). Once the plus ends encounter the cellular structures, such as the kinetochores or cell cortex, they are captured and stabilized by cellular factors, including the plus-end-tracking proteins (+TIPs), a structurally and functionally diverse group of proteins that can accumulate at growing plus ends (*18*). For example, cytoplasmic linker protein (CLIP)-170 and CLIP-associated proteins (CLASPs) are +TIPs that coordinate MT attachment to both the kinetochores and cell cortex (*18*). CLASPs are also known to suppress catastrophes and promote rescues by binding to MT lattices and recruiting soluble tubulin dimers (*19*).

Regulation of the MT-Minus Ends

Despite their ability to grow *in vitro*, the MT-minus ends generally fail to grow in cells, possibly due to their capping by specific cellular factors (*20*). Once the free minus ends are generated by release from the sites of nucleation or breakage/severing of existing MTs, they are either stabilized or depolymerized depending on their context (*20*). By acting as an MT nucleator at the centrosome, γ-TuRC caps the MT-minus ends and thereby protects their growth and depolymerization (*21*). Accumulating evidence demonstrates that γ-TuRC is also involved in the nucleation of non-centrosomal MTs and/or anchoring their minus ends in various types of cells, including neurons, epithelial cells and muscle cells (*3*, *20*). Furthermore, γ-TuRC can cap the minus ends of preexisting MTs (*21*). However, not all the MT-minus ends can associate with γ-TuRC (*22*).

Various γ-TuRC binding proteins have been shown to regulate organization of non-centrosomal MTs. A-kinase anchor protein 450 (AKAP450, also known as AKAP350, AKAP9 or CG-NAP), a scaffolding protein localized at the centrosome and Golgi apparatus (*23*), has been shown to recruit γ-TuRC to the Golgi apparatus either directly or indirectly through CDK5Rap2 and/or myomegalin (MMG), and thereby plays an essential role in the nucleation of Golgi-derived MTs (*24–27*) (Fig. 2). AKAP450 is recruited to the *cis*-Golgi by a *cis*-Golgi matrix protein, GM130 (*28*). Intraflagellar transport 20 (IFT20), a component of IFT-B complex normally required for the assembly and maintenance of the primary cilia, has also been shown to localize at the *cis*-Golgi and interact with both AKAP450 and GM130 (*11*, *29*). IFT20 promotes the nucleation of Golgi-MTs probably through regulating the proper interaction between AKAP450 and GM130 at the *cis*-Golgi (*11*). Interestingly, Greer *et al.* (*30*) have reported that displacement of AKAP450 from the Golgi disrupts localization of IFT20 at the *cis*-Golgi, suggesting that IFT20 and AKAP450 are mutually required for their localization at the *cis*-Golgi and both are necessary for the efficient recruitment of γ-TuRC. MTs nucleated at the *cis*-Golgi are stabilized by CLASPs localized at the *trans*-Golgi network (*31*). Furthermore, the MT-cross linking protein, MTCL1, interacts with both AKAP450 and CLASPs and thereby stabilizes Golgi-MTs (*32*) (Fig. 2).

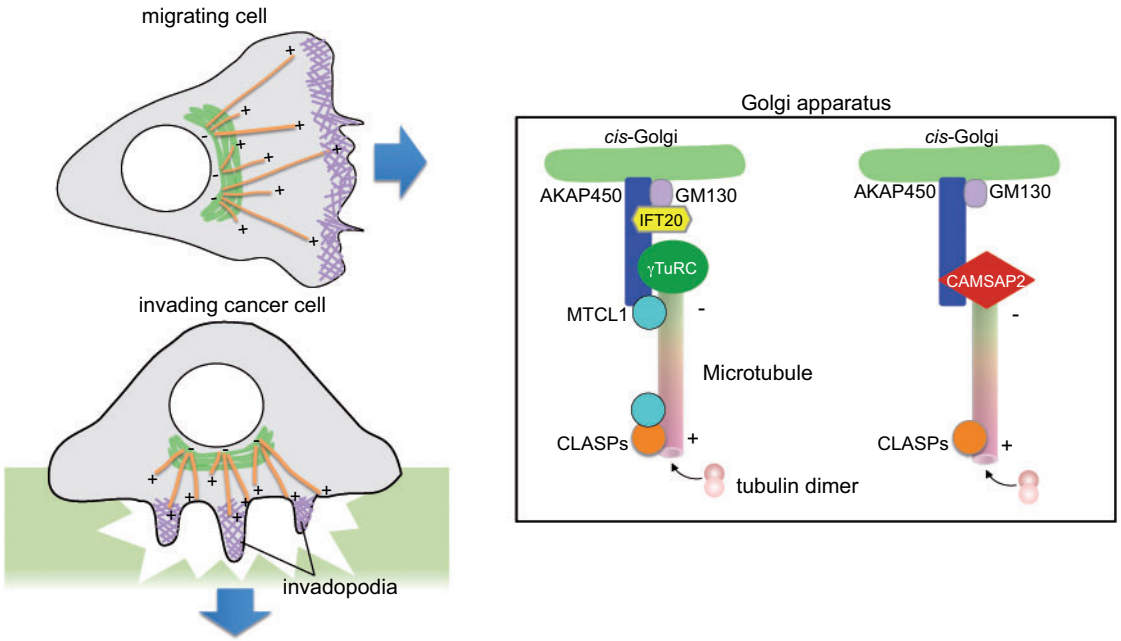

Fig. 2 Organization of Golgi-MTs during polarized cell migration and cancer cell invasion. Vertebrate cells possess the polarized Golgi ribbon, which organizes asymmetric MT arrays with their plus ends oriented towards the direction of cell migration or invasion. At the *cis*-Golgi, GM130 and AKAP450 form a complex, with the help of IFT20, to recruit γ-TuRC that nucleates MTs. Nucleated Golgi-MTs are stabilized by CLASPs. MTCL1 interacts both AKAP450 and CLASPs and crosslinks Golgi-MTs. CAMSAP2 binds to and stabilizes free MT-minus ends and tethers them to the *cis*-Golgi through interacting with AKAP450.

Other proteins interacting with γ-TuRC include ninein, which is originally identified as an essential centrosomal protein in anchoring the MT-minus ends to the mother centriole (*33*, *34*). Interestingly, during epithelial cell differentiation, centrosomal MTs are released together with ninein, which relocates and reanchors their minus ends to the apical sites, resulting in reorganization of radial MTs into their apical–basal arrays (*35*) (Fig. 3). It has been shown that a putative *Caenorhabditis elegans* homologue of ninein, NOCA-1, functions with γ-tubulin to organize non-centrosomal MTs (*36*). During skeletal muscle differentiation, ninein also relocates from the centrosome to the nuclear envelope together with γ-tubulin and pericentrin (*37*).

The multi-subunit protein complex augmin recruits γ-TuRC to preexisting MTs and thereby nucleates MT branches at their lateral surfaces independently of the centrosome (*38*, *39*). Such a mechanism appears to provide an effective means of amplifying the number of MTs with the same polarity within the spindle (*39*). In post-mitotic neurons, the augmin–γ-TuRC module is also crucial for organizing highly bundled and uniformly polarized non-centrosomal MTs to ensure proper neuronal morphogenesis and intracellular transport (*40*) (Fig. 4).

Recently, CAMSAP (calmodulin-regulated spectrin-associated protein)/Patronin family of proteins has emerged as specific regulators of free MT-minus ends. These family members include CAMSAP1, CAMSAP2 and CAMSAP3 (also called Nezha or Marshalin) in mammals, Patronin in *Drosophila* and PTRN-1 in *C. elegans*. They associate with the MT-minus ends directly and protect them from depolymerization (*41–44*). The CAMSAP/Patronin family of proteins shares a signature domain at their C-terminal regions called the CKK (C-terminal domain common to CAMSAP1, KIAA1078 and KIAA1543), which serves as an MT-binding domain. In addition, the family members have one calponin homology and three coiled-coil (CC) domains (CC1–3) at their N-terminal and middle regions, respectively. Through interacting with the free MT-minus ends, CAMSAP1 dynamically tracks the growing minus ends, whereas CAMSAP2 and CAMSAP3 stably decorate stretches of the MT-minus ends, forming stabilized MT lattices that are resistant to depolymerization (*41*, *42*). The function of CAMSAP2 and CAMSAP3 appears to be independent of γ-TuRC, suggesting that they recognize the MT-minus ends that are released from γ-TuRC or newly generated by MT breakage/severing (Figs 2–4). It has been shown that CAMSAP2-decorated MT stretches are tethered to the Golgi membrane through a complex of AKAP450 and MMG (*45*) (Fig. 2). Although AKAP450 and MMG are essential for γ-TuRC-dependent MT nucleation at the Golgi as described above, CAMSAP2 is dispensable for this process (*45*). CAMSAP2-bound Golgi-MTs are further stabilized by CLASPs, whereas CLASPs are not involved in Golgi tethering of MTs (*45*) (Fig. 2). Thus, γ-TuRC and CAMSAP2 appear to act sequentially, together with AKAP450, to organize Golgi-MTs. Consistent with this view, in *C. elegans*, PTRN-1 functions in parallel to NOCA-1, which acts with γ-tubulin, to assemble circumferential MT arrays in the larval epidermis (*36*).

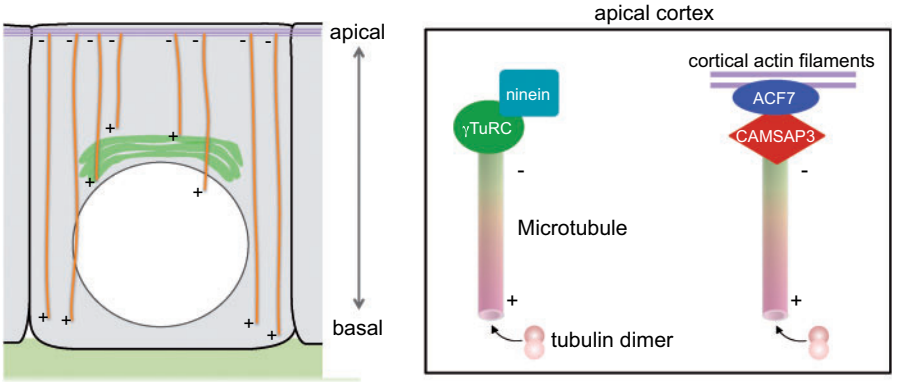

Fig. 3 Organization of non-centrosomal MTs in epithelial cells. Polarized epithelial cells possess a characteristic MT array aligned along the apical–basal axis with their minus ends accumulated around the apical cortex of the cell. During epithelial cell differentiation, centrosomal MTs are released together with ninein, which relocates and re-anchors their minus ends to the apical sites. A putative *C. elegans* homologue of ninein functions with γ-tubulin to organize non-centrosomal MTs. CAMSAP3 stabilizes free MT-minus ends and tethers them to the apical cortical F-actin through interacting with ACF7.

Cellular Function of Non-Centrosomal MTs

Roles of Golgi-derived MTs

The Golgi apparatus varies in shape greatly from one organism to another. For example, many *protozoa* have a single Golgi stack, the basic structural and functional unit of the Golgi apparatus, whereas in *Drosophila*, multiple Golgi stacks are dispersed throughout the cytoplasm. In contrast, in vertebrate cells, individual Golgi stacks are laterally linked to form a continuous membrane system called the Golgi ribbon, which also varies in shape and geometry depending on the cell type. The Golgi apparatus has been shown to be a site that nucleates and stabilizes non-centrosomal MTs in many cell types, including neurons, muscle cells and cancer cells. In vertebrate cells, Golgi-MTs are proposed to tether the Golgi stacks to form the Golgi ribbon (*28*, *31*, *46*). Accordingly, inhibition of Golgi-MT formation results in fragmentation of the Golgi ribbon into the individual stacks (Golgi mini-stacks), which are clustered around the centrosome, forming the circular Golgi apparatus, or dispersed around the nucleus depending on the cell type (*11*, *28*, *32*, *46*). The cell type-dependent distribution of the fragmented Golgi mini-stacks may reflect their intrinsic proportions of MT subsets that affect the shape and geometry of the Golgi apparatus. It has been known that centrosomal MTs are responsible for pericentrosomal localization of the Golgi, although it cannot support Golgi ribbon formation (*46*). In contrast, Golgi-MTs allow Golgi mini-stacks to move toward each other, linking the min-stacks laterally for properly oriented Golgi ribbon formation (*46*, *47*).

Unlike the centrosome, which organizes radial symmetric MT array, the Golgi ribbon generally organizes asymmetric MT network, which can serve as tracks for post-Golgi transport, thereby facilitating polarized transport to establish and/or maintain cell polarity (*46–48*). This property of the Golgi ribbon is essential for polarized cell migration, which relies on polarized transport through the Golgi (Fig. 2). In fact, RPE1 cells lacking Golgi-MTs are incapable of polarized migration in spite of the fact that the circular Golgi apparatus can reorient together with the centrosome towards the leading edge (*28*, *49*). Thus, Golgi-MT-dependent Golgi ribbon formation is critically required for maintaining polarized cell migration.

Similar to directionally migrating cells, invasive cancer cells possess the Golgi apparatus, polarized towards the actin-based membrane protrusions, invadopodia or pseudopodia, where matrix metalloproteinases are targeted and secreted to mediate focal degradation of extracellular matrices (*11*, *45*, *50*) (Fig. 2). In fact, inhibition of Golgi-MT formation is associated with impaired cell polarization, invadopodia/pseudopodia formation and invasion of highly invasive cancer cells (*11*, *45*). Interestingly, signalling emanated from Ror2 receptor tyrosine kinase induces expression of IFT20, which in turn promotes the nucleation of Golgi-MTs through the mechanisms involving AKAP450–GM130 complex in non-ciliated cancer cells, as described above (*11*) (Fig. 2). In ciliated cells, IFT20 acts as a component of the IFT-B complex, serving as a cargo adaptor for ciliary transport. Interestingly, among the ~20 IFT proteins, only IFT20 has been shown to localize at the Golgi apparatus, in addition to the basal body and cilia (*29*). Although IFT20 is believed to mediate protein transport from the Golgi apparatus to the primary cilia (*29*, *51*), it is currently unknown whether IFT20 regulates Golgi-MT organization also in ciliated cells. With this regard, it is noteworthy that physiological function of AKAP450 at the Golgi is also essential for ciliogenesis (*30*, *49*), suggesting that IFT20 might also act with GM130–AKAP450 complex in nucleating Golgi-MTs for ciliogenesis in normal ciliated cells. Besides its key role in regulating Golgi-MT organization, IFT20 also plays a role in efficient anterograde transport within the Golgi (*11*), although it remains unknown how the organization of Golgi-MTs links to the processes of Golgi transport, such as cisternal maturation and tubular transport.

Roles of non-centrosomal MTs in epithelial cells

Epithelial cells show apical–basal polarity that is linked to their functions, such as directional transport and secretion. Fully differentiated epithelial cells in

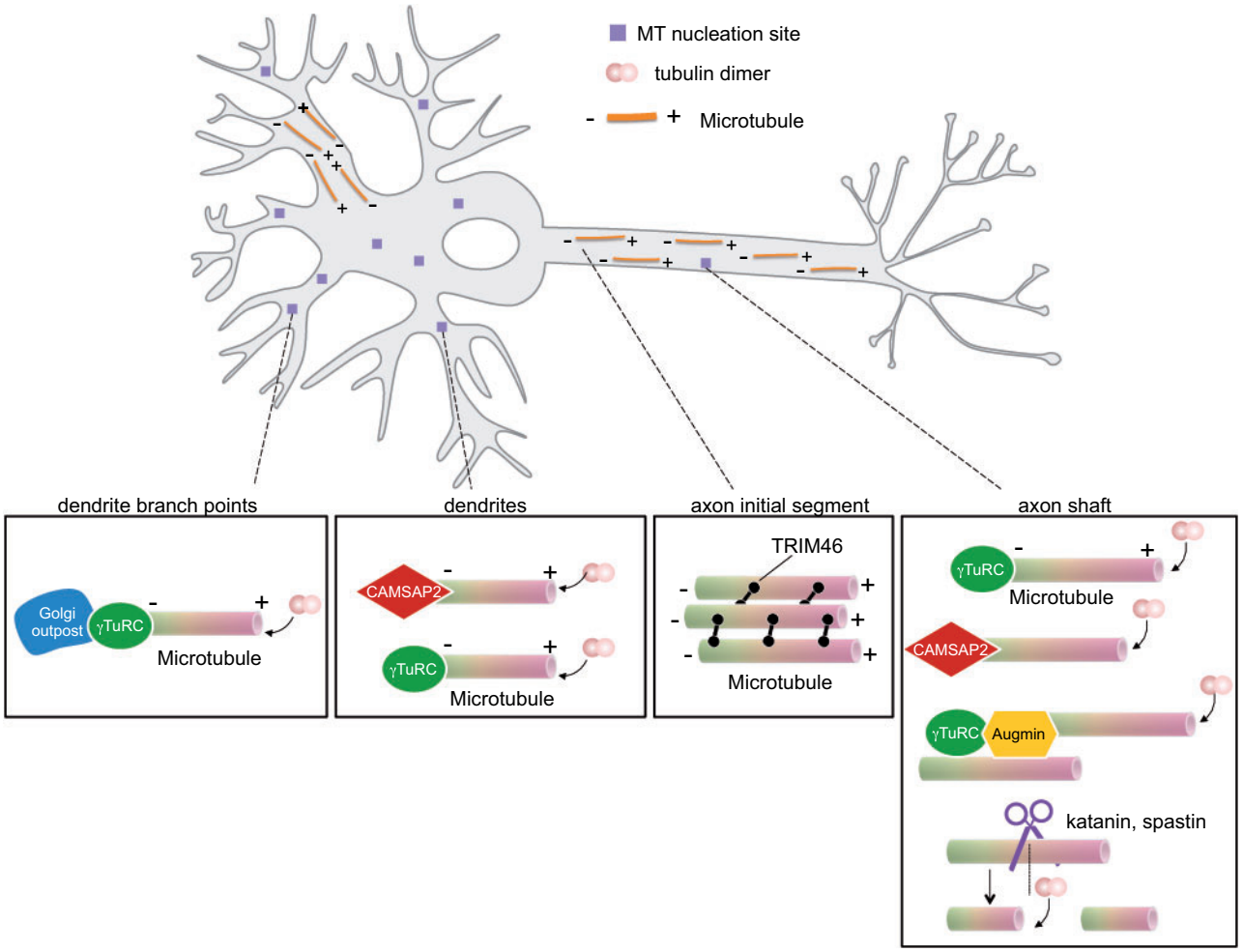

Fig. 4 Model for MT organization in matured neurons. The centrosome in neurons gradually loses its activity to nucleate MTs as they develop neuronal polarity. Instead, the role is replaced by non-centrosomal MTOCs, which distribute in the cell body, dendrites and axon. In the axon where MTs orient uniformly with plus-end-out polarity, CAMSAP2 and augmin are thought to facilitate *de novo* nucleation of non-centrosomal MTs together with γ-TuRC. These proteins, as well as TRIM46 which crosslinks MTs at the axon initial segment, appear to play important roles to ensure the uniform MT polarity in the axon. Katanin and spastin also contribute to increase of nucleation sites by severing preexisting MTs. In dendrites in which MTs orient in a mixed manner, MTs are shown to polymerize from Golgi outposts, and play important roles for dendrite branching.

general exhibit MT arrays along the apical–basal axis with their minus ends accumulated at the apical side (Fig. 3). Such non-centrosomal MT assembly is regulated by relocation of the γ-TuRC-binding MT anchoring proteins, such as ninein, from the centrosome to the apical region, as described above. In fact, ninein localizes to the apical non-centrosomal sites in inner pillar cells and to the adherens junctions in polarized MDCK cells (*35*). Relocation of ninein is associated with MT rearrangement from centrosomal to non-centrosomal pattern, which is important for mammalian epidermal cell differentiation (*52*). In *C. elegans* embryos, γ-TuRC and NOCA-1 organize non-centrosomal MTs at the epidermal junctions to ensure proper transport of the junctional remodelling factors, required for embryonic elongation (*53*) (Fig. 3). Recent studies have also shown that CAMSAP3, which binds to the minus ends of non-centrosomal MTs independent of γ-TuRC and protects them from depolymerization, is an important regulator of apical–basal MT array in epithelial cells. CAMSAP3 was originally identified in epithelial cell line Caco-2 as a binding partner of PLEKHA7, a component of the apical adherens junctions (zonula adherens), where a population of the MT-minus ends is anchored (*43*). CAMSAP3 appears to tether the MT-minus ends to the junctions and thereby regulates their integrity in Caco-2 cell layer. Interestingly, CAMSAP-3 disappeared from the junctions and instead accumulated at the apical cortical regions when Caco-2 cell layer becomes fully matured, whereas PLEKHA7 remains localized at the junctions after maturation (*54*). In agreement with the findings, CAMSAP3 is exclusively localized at the apical cortical regions, but not the adherence junctions, in mouse small intestinal epithelial cells *in vivo* (*54*). These observations indicate that localization and function of CAMSAP3 at the adherens junctions might be restricted to immature stages of epithelial cells.

Apical cortex of the epithelial cells contains actin meshwork, where CAMSAP3 is localized and tethers the MT-minus ends. In small intestinal epithelial cells

from *Camsap3*-mutant mice, that express truncated CAMSAP3 lacking the CKK (the MT-binding domain), apical tethering of the MT-minus ends is no longer observed, and most of the MTs become wavy and lose directionality (*54*). Furthermore, stereotypic arrangement of the nucleus and Golgi apparatus, which depends on proper MT arrays, is disturbed in these cells (*54*). Thus, CAMSAP3 plays an important role in apical–basal array of the non-centrosomal MTs and proper organelle arrangement, through tethering the MT-minus ends to the apical cortex in small intestinal epithelial cells (Fig. 3).

How is CAMSAP3 localized at the apical cortex? CAMSAP3 mutant lacking the CKK fails to localize at the apical surface (*54*). In addition, MT depolymerization, by using the drug nocodazole, results in the cytoplasmic distribution of CAMSAP3 (*54*, *55*), suggesting that apical localization of CAMSAP3 depends on its interaction with MTs via the CKK domain. Apical localization of CAMSAP3 has also been shown to require its CC1 domain and F-actin (*54*), suggesting a role of the CC1 domain in anchoring CAMSAP3 to the apical cortical F-actin in epithelial cells. A screen for CAMSAP3-interacters has revealed that ACF7 (also known as MACF1), a member of the spectraplakin family of proteins that crosslinks MTs and actin filaments, binds to CAMSAP3, but not CAMSAP2, through the region containing the CC1 domain (*55*, *56*). ACF7 has been suggested to act as a linker, connecting the non-centrosomal MTs to the actin-based circular plate localized at the apical regions in cochlear hair cells (*57*). In Caco-2 cell layer, ACF7 is partially colocalized with CAMSAP3 at the F-actin-rich apical surface, and knockdown of ACF7 perturbs apical accumulation of CAMSAP3 (*55*), indicating a critical role of ACF7 in apical recruitment of CAMSAP3 in epithelial cells. Furthermore, ACF7-depleted Caco-2 cells fail to form polarized cysts properly in 3D culture (*55*). Thus, ACF7–CAMSAP3 complex plays a key role in organizing the apical–basal array of non-centrosomal MTs by tethering their minus ends to the apical cortical F-actin for epithelial cell polarization (Fig. 3). Another study using subconfluent Caco-2 cells has further demonstrated an important role of ACF7–CAMSAP3 complex in regulating orientation of MTs relative to the cell edge by tethering their minus ends to F-actin at the edge, essential for cell migration (*56*). *Drosophila* homologues of ACF7 and CAMSAPs, Shortstop (Shot) and Patronin, respectively, colocalize at the apical regions of follicle cells through binding to Spectrin, which appears to be essential for polarization of non-centrosomal MTs along the apical–basal axis and maintenance of tissue integrity (*58*, *59*).

Roles of non-centrosomal MTs in neurons

Behaviours of neurons largely depend on the centrosome and centrosomal MTs until they finish differentiation and migration to positions where they start to polarize (*60*). In this early developmental stage, the centrosomes are essential for asymmetric division of neural stem cells and migration of immature neurons. This is the reason why mutations of the centrosome-related proteins result in brain malformations, such as lissencephaly or microcephaly (*61*).

On the other hand, early electron microscopic studies revealed that MTs in mature neurons do not anchor to the centrosome (*62*, *63*). Later studies using primary culture of rodent embryonic neurons have demonstrated that MTs gradually become non-centrosomal as neurons develop and establish their polarity (*64*, *65*). It has long been thought that MTs assemble at the centrosome even in matured neurons, and are released and conveyed along the axon by motor proteins (*66*, *67*). However, recent studies have demonstrated that the centrosome progressively loses its function as an MTOC during neuronal development *in vitro* and *in vivo* (*22*, *68*). Furthermore, laser ablation of the centrosome was found not to affect axonal growth even in young neurons whose centrosome still functions as an MTOC (*65*). These results are consistent with the findings that γ-tubulin and other PCM components are depleted from the centrosome or PCM during neuronal development (*22*, *40*, *65*, *69*). Because γ-tubulin is present throughout the axon and dendrites (*65*) and plays essential roles for MT nucleation (*22*, *40*), it is now widely appreciated that non-centrosomal MTOCs substitute the function of the centrosome in well-developed neurons (*61*) (Fig. 4). Consistently, studies with *Drosophila* da neurons have revealed that MTs nucleate from Golgi outposts in the dendrites depending on γ-tubulin and the *Drosophila* homologue of AKAP450, and play important roles in growth and stabilization of the dendritic branches (*70*). Although specific subcellular structures with which γ-tubulin associates have not been reported in the axon, a recent study using super-resolution microscopy clearly has detected free minus ends of MTs, decorated with CAMSAP2, which scatter throughout the cell body, axon and dendritic shaft as a variety of small clusters and punctuated stretches (*22*). This study has also elucidated that CAMSAP2 plays indispensable roles to establish and maintain the MT network in the developing and mature neurons downstream of γ-tubulin (Fig. 4). Recently, the augmin, which recruits γ-tubulin to MT lattices in mitotic spindle to nucleate MT branches (*71*), was shown to be required for MT nucleation in the axon (*40*) (Fig. 4). Severing of pre-existing MTs by katanin or spastin can also contribute to the *de novo* nucleation of non-centrosomal MTs in the axon (*72*, *73*) (Fig. 4).

MT nucleation from single MTOC (centrosome) might be incompatible with the complex organization of MTs specifically observed in developed neurons (*65*). In fact, it has been shown that non-centrosomal MTs in the axon exhibit uniform polarity with plus-end-out, whereas those in the dendrites show mixed polarity (*74*, *75*) (Fig. 4). Interestingly, a recent study based on live cell imaging has demonstrated that MTs in newly formed neurites of non-polarized neurons already possess mixed polarity both *in vitro* and *in vivo* (*76*). Although the findings are inconsistent with the long-favoured idea that the plus-end-out polarity is default for MTs in newly formed neurites of non-polarized neurons (*77*), they raise an intriguing possibility that regulation of non-centrosomal MTs might

play essential roles in axon specification from several indistinguishable neurites. In fact, suppressed expression of CAMSAP2 results in an impairment of axon specification both *in vitro* and *in vivo* (*22*). Considering that CAMSAP2 depletion completely blocks taxol-induced axon formation (*22*), local stabilization of non-centrosomal MTs by CAMSAP2 may be important for specifying axon with uniformly oriented MTs. Interestingly, the augmin–γ-TuRC complex was also demonstrated to control the orientation of MT nucleation, and ensure their uniform polarity in the axon (*40*). In addition, TRIM46, a newly identified MT cross-linking protein localizing at the axon initial segment was shown to be required for parallel MT array in the proximal axon and for axon specification (*78*). At present, a possible involvement of the centrosome and centrosomal MTs in neuronal polarization remains controversial (*79–81*). On the other hand, together with the report that mutant flies lacking the centrosome develop a largely normal nervous system (*82*), the above results appear to favour the idea that regulated organization of non-centrosomal MTs is also required for axon specification and neuronal polarization.

Future Directions

Non-centrosomal MTs appear to be assembled via at least two distinct modes: (i) direct MT nucleation, in which MT-minus end proteins are localized at non-centrosomal sites to nucleate, stabilize and anchor MTs there; and (ii) centrosomal MT relocation, in which the minus ends of centrosomal MTs are released and relocated to non-centrosomal sites. During these processes, the abilities of the centrosome to nucleate and/or anchor MTs are likely to be suppressed to potentiate the assembly of non-centrosomal MTs. In fact, some nucleation and/or anchoring factors are localized at both the centrosome and non-centrosomal sites, and release of these factors from the centrosome is associated with loss and gain of the MT nucleation activity at the centrosome and non-centrosomal sites, respectively. Furthermore, disruption of apical–basal MT arrays in epithelial cells enhances centrosomal MT assembly (*55*, *83*), indicating a reciprocal relationship between centrosomal and non-centrosomal MTs. Thus, assembly of centrosomal and non-centrosomal MTs seems to be regulated differently but coordinately in a cell type-dependent manner, although the basis for such regulation remains to be elucidated. The mechanisms by which the non-centrosomal MTOC sites can be specified, depending on the cell type or context, are also largely unknown. Further studies combining different biological approaches are necessary to obtain comprehensive understanding of the molecular basis for the assembly of non-centrosomal MTs and their fundamental cellular functions, as well as their implications in pathological conditions.

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Conflict of Interest
None declared.

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