Review

Interplay between microtubule dynamics and intracellular organization

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ABSTRACT

Microtubules are hollow tubes essential for many cellular functions such as cell polarization and migration, intracellular trafficking and cell division. They are polarized polymers composed of α and β tubulin that are, in most cells, nucleated at the centrosome at the center of the cell. Microtubule plus-ends are oriented towards the periphery of the cell and explore the cytoplasm in a very dynamic manner. Microtubule alternate between phases of growth and shrinkage in a manner described as dynamic instability. Their dynamics is highly regulated by multiple factors: tubulin post-translational modifications such as detyrosination or acetylation, and microtubule-associated proteins, among them the plus-tip tracking proteins. This regulation is necessary for microtubule functions in the cell. In this review, we will focus on the role of microtubules in intracellular organization. After an overview of the mechanisms responsible for the regulation of microtubule dynamics, the major roles of microtubules dynamics in organelle positioning and organization in interphase cells will be discussed. Conversely, the role of certain organelles, like the nucleus and the Golgi apparatus as microtubule organizing centers will be reviewed. We will then consider the role of microtubules in the establishment and maintenance of cell polarity using few examples of cell polarization: epithelial cells, neurons and migrating cells. In these cells, the microtubule network is reorganized and undergoes specific and local regulation events; microtubules also participate in the intracellular reorganization of different organelles to ensure proper cell differentiation.

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Contents

1. Introduction

Microtubules are essential components of the cytoskeleton that play a major role in many cellular functions such as cell migration and polarization, intracellular trafficking and cell division. Microtubules are hollow tubular structures constituted of heterodimers of α and β tubulin. In most vertebrate cells, they are nucleated at the centrosome that works as a MTOC (Microtubule Organizing Center) in the perinuclear region. It is constituted of two centrioles, each composed of nine triplets of microtubules, surrounded by peri-centriolar material that contains proteins implicated in microtubule nucleation and organization. Among them, the γ-tubulin associates with other proteins to form a ring complex, the γ-TuRC (Tubulin Ring Complex), onto which dimers of α and β tubulin are added to build a microtubule. Microtubules are thus polarized with a minus-end capped and anchored at the MTOC and a plus-end generally localized at the periphery of the cell.

Microtubule minus-ends can elongate *in vitro* but at lower speed than the plus-ends, and they are mostly stable or depolymerizing in cells. They are capped by the γ-TuRC (see Raynaud-Messina and Merdes, 2007). The plus-ends explore the cytoplasm in a very dynamic manner. Microtubules undergo phases of growth, pause and shrinkage, separated by rescue (transition from shrinkage to growth phase) or catastrophe (transition from growth phase to shrinkage) events. This dynamic behavior was termed "dynamic instability" by Mitchison and Kirschner (1984) (for a review see Desai and Mitchison, 1997). During microtubule polymerization, heterodimers of guanoside-triphosphate (GTP)-bound tubulin are added at the plus-end of microtubules. A slight delay between polymerization and hydrolysis of the GTP by β-tubulin creates a GTP-tubulin cap. The loss of this cap induces a rapid depolymerization of the microtubule. In this model, stochastic rescue events allow the microtubule to enter a new phase of polymerization. Another model suggests that rescue events might not be stochastic. Dimitrov et al. (2008) showed *in vivo*, using a conformation-sensitive antibody, that GTP-tubulin was found not only at the plus-ends of microtubules, but GTP-tubulin remnants were also identified in older parts of the polymer. Upon depolymerization of the microtubule, they would be exposed and behave as a GTP-cap to promote rescue events. Lattice defects or specific structures within the microtubule lattice could also play a role in regulation of microtubule dynamics.

2. Regulation of microtubule dynamics

Intrinsic processes such as the presence of the GTP-cap and GTP-islands thus regulate microtubule dynamics. Extrinsic regulation of microtubule is mostly due to the numerous MAPs (microtubule-associated proteins) that bind to microtubules, and especially to the family of proteins that bind to the plus-ends of microtubules. We will summarize in the first part the role of MAPs and plus-ends binding proteins and will discuss in the second part the role of microtubule post-translational modifications on the regulation of microtubule dynamics (see Fig. 1).

2.1. Microtubule-associated proteins

MAPs have been shown to play a crucial role in the regulation of microtubule dynamics. The most studied stabilizing MAPs are Tau, MAP2 and MAP4, the first two being strongly expressed in neurons. Other MAPs have a destabilizing effect on microtubules, either by severing microtubules or by inducing depolymerization. Three proteins, katanin, spastin and fidgetin regulate the number and length of microtubules through their severing activity (Zhang et al., 2007). In particular, they increase the number of
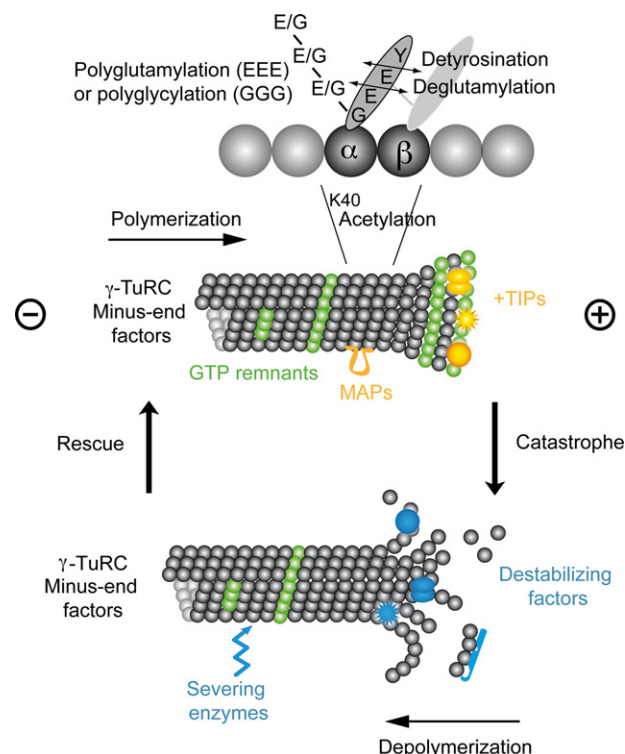
Fig. 1. Regulation of microtubule dynamics. Microtubules are highly dynamic structures that alternate between phases of growth, pause and shrinkage, separated by catastrophe and rescue events. The presence of GTP-bound β-tubulin subunits (in red) at the microtubule plus-end (GTP-cap) or along the microtubule (GTP-remnant) promotes stabilization. Microtubule dynamics is also regulated by external factors: stabilizing factors including +TIPs, MAPs and minus-end capping proteins, destabilizing factors and severing proteins. Microtubules can undergo multiple post-translational modifications that often correlate with stability. Most of them occur on the C-terminal tail of α and β-tubulin except detyrosination that only concerns the α-subunit, and the acetylation of Lysine40 which is located in the lumen of the microtubule. E, glutamate; G, glycine; K, lysine; Y, tyrosine; +TIP, plus-tip tracking protein; MAP, microtubule-associated protein; γ-TuRC, γ-tubulin ring complex.

microtubules, which is necessary for the formation of the mitotic spindle and in some polarized cells. Some MAPs induce depolymerization: stathmin binds to free tubulin dimers (Belmont et al., 1996) and favors GTP hydrolysis (Howell et al., 1999); proteins from the kinesin-13 family induce a conformational change of the tubulin dimers that triggers catastrophe events (reviewed in Ems-McClung and Walczak, 2010). Interestingly, some proteins may be involved both in microtubule nucleation and in the regulation of microtubule dynamics. Recent studies indeed showed that a fraction of the γ-TuRC localized along microtubules regulate microtubule dynamics by inducing pauses (Bouissou et al., 2009). More recently, Goodwin and Vale identified in Drosophila cells a minus-end-specific-protein "cap": Patronin protects the minus-ends of microtubule from depolymerization (Goodwin and Vale, 2010). In mammals, one of its homologue, Nezha binds to microtubule minus-ends *in vitro* and anchors microtubule minus-ends to adherens junctions in epithelial cells (Meng et al., 2008).

A important family of MAPs, the plus-end tracking proteins (+TIPs), dynamically track the growing plus-ends of microtubules (for reviews, see Akhmanova and Steinmetz, 2010; Schuyler and Pellman, 2001). Because of their localization, they play a major role in the regulation of microtubule dynamics. They also participate in the interactions of microtubules with the chromosomes during mitosis and with the cellular cortex both in interphase and mitosis. CLIP170 (cytoplasmic linker protein) was the first MAP to be identified as a +TIP (Perez et al., 1999). It was later shown

to play a role in rescue events: inactivation of CLIP170 induces a very low rescue frequency accompanied by the loss of p150Glued from microtubule plus-ends (Komarova et al., 2002). Normal microtubule dynamics was restored by expression of the CLIP170-head domain, whereas p150Glued did not rescue the phenotype. Initially, CLIP170 was described as a phospho-sensitive MAP (Rickard and Kreis, 1991). Since then, at least four different kinases were identified as regulators of CLIP170 interaction with microtubules. Among them, FKBP-Rapamycin-Associated Protein (FRAP) (Choi et al., 2002) enhances its binding to microtubules while AMP kinase (Nakano et al., 2010) and PKA (protein kinase A) (Lee et al., 2010) inhibit its binding.

Many other +TIPs have then been characterized. The EB (End-Binding) proteins are now considered as of major importance at the tips of microtubules. Dimers of EB1 promote persistent microtubule growth by suppressing catastrophes *in vitro* (Komarova et al., 2009). Unexpectedly, Mal3p, its homologue in *Schizosaccharomyces pombe*, induces rescue events. The reasons for such a difference are still a matter of debate. EBs dynamically bind to microtubule plus-ends, either recognizing the GTP-cap or a specific structural conformation of the protofilaments, where they recruit other +TIPs. EBs bind their partners via two different types of interactions. Proteins containing a CAP-Gly (cytoskeleton-associated protein-glycine-rich) domain such as CLIPs interact with the EEY/F motif of the EB C-terminal tail, which shares similarities with the tubulin tail (Komarova et al., 2005). Other partners such as CLASPs (CLIP-Associated Proteins) and MCAK (Mitotic Centromere-Associated Kinesin) associate with the hydrophobic cavity of EBs through their serine-rich region containing the SxIP motif (Honnappa et al., 2009). CLASPs promote rescue events by recruiting tubulin dimers at the plus-ends of microtubules (Al-Bassam et al., 2010; Maiato et al., 2005) and MCAK is a depolymerizing +TIP that induces catastrophes (Newton et al., 2004). Like for CLIP170, the activity and binding of other +TIPs are regulated by phosphorylation or degradation, both of which participate indirectly to regulation of microtubule dynamics. Ban et al. (2009) show that Aurora A kinase phosphorylates EB3 during the G2 phase to mitosis transition and induces its binding to SIAH1, an ubiquitin E3-ligase, and its degradation in late cytokinesis. Aurora B and GSK-3β (Glycogen Synthase Kinase 3β) kinases are involved in the phosphorylation of MCAK (Andrews et al., 2004; Lan et al., 2004) and of the SxIP motif of CLASP (Kumar et al., 2009), decreasing the affinity of these two +TIPs for EB1 and affecting their plus-ends tracking behavior.

In addition, two novel +TIPs, SLAIN2 in mammalian cells, and Sentin in Drosophila, were recently identified as EB-partners (Li et al., 2011; van der Vaart et al., 2011). SLAIN2 interacts with CLIPs, CLASPs and ch-TOG and promotes persistent microtubule growth. It is regulated by CDK1 phosphorylation: phosphorylated SLAIN2 has a low affinity for EBs and cannot recruit ch-TOG at microtubule plus-tips in mitotic cells. Another family of +TIPs is indeed the TOG proteins family, among them XMAP215 which induces the addition of tubulin dimers at the plus-ends of microtubules and thus promotes microtubule growth (Brouhard et al., 2008).

2.2. Tubulin modifications

Tubulin post-translational modifications are linked to the regulation of microtubule dynamics. These modifications seem to form a readable code on microtubules for MAPs or motors (reviewed in Janke and Kneussel, 2010 and in Wloga and Gaertig, 2010). Microtubule modifications such as detyrosination, glutamylation, glycylation and acetylation are almost all carried out on polymerized tubulin. They are mostly linked to microtubule stability but no direct role of tubulin modifications on microtubule dynamics

has been described yet. They participate in microtubule dynamics regulation by recruiting MAPs or by affecting the behavior of motors.

Tyrosination of tubulin is carried out by the tubulin-tyrosine ligase that catalyzes the re-addition of a tyrosine at the C-terminal tail of tubulin after it has been clipped-off by a carboxypeptidase. The presence of a carboxyterminal tyrosine has a positive effect on microtubules by recruiting stabilizing factors and affecting the binding of destabilizing MAPs. Tubulin tyrosination is for example necessary for the binding of CLIP170 and CLIP115 or p150Glued at the plus-tip of microtubules (Peris et al., 2006). Similarly, the removal of this tyrosine reduces the affinity of depolymerizing motors such as kinesin-13 family motors, MCAK or KIF2A (Peris et al., 2009) and promotes the accumulation of a stable subpopulation of microtubules that might play an important role in cellular differentiation.

Other enzymes related to the tubulin tyrosine-ligase direct microtubule modifications that are more specific of some cell types or cell structures and that also have an indirect role on microtubule dynamics. Glutamylation and glycylation are essentially found in cilia and flagella in mammalian cells whereas in neurons most microtubules are poly-glutamylated (reviewed in Janke and Kneussel, 2010). Poly-glutamylation is also found at centrioles and basal bodies and on some spindle microtubules. Data suggest that several neuronal MAPs and kinesins bind preferentially to poly-glutamylated tubulin (Bonnet et al., 2001). This modification also induces microtubule severing by recruitment of spastin (Lacroix et al., 2010).

A more ubiquitous tubulin modification, tubulin acetylation, also increases the binding of motors to microtubules. This is in particular true for KIF5A, KIF5B/kinesin-1 and dynein, for example in neurons where transport is stimulated. Friedman et al. (2010) also showed that microtubule acetylation enhances ER sliding, a mechanism where the extremity of an ER tubule binds to and slides along a microtubule. In addition, mitochondria, but not endosomes, bind preferentially to acetylated microtubules, a subpopulation of microtubules that would promote ER-mitochondria interactions. Mec17/Atat1 seems to play a major role in tubulin acetylation (Akella et al., 2010). It was shown to play a role in differentiation: Atat1 promotes the assembly of the primary cilia (Shida et al., 2010). The same study shows that it is required for touch sensation in mechanoreceptors in *Caenorhabditis elegans*. Several acetyltransferases were recently described (reviewed in Perdiz et al., 2011). For example, Elp1 and Elp3, proteins of the elongator complex, interact with tubulin in cortical projection neurons and promote their migration and differentiation during corticogenesis (Creppe et al., 2009); Gcn5 plays a role in myoblast differentiation (Conacci-Sorrell et al., 2010).

The dynamic behavior of microtubules results from a balance of many regulatory processes imprinted on the microtubule itself or due to its interactions with regulatory proteins. Membrane-bound organelles and trafficking routes will then use this dynamic network to organize and connect intracellular compartments.

3. Cross-regulation between microtubule dynamics and intracellular organization

In most animal cells in interphase, microtubules are nucleated and organized by the centrosome, the major MTOC. The centrosomal microtubules spread out in the whole cell and explore the cytoplasm, playing a role in positioning, organization and maintenance of different organelles (Fig. 2A). As detailed below, in some cells the nucleus and the Golgi apparatus can, in addition to the centrosome, be sites of microtubule nucleation and influence their own organization with a sub-population of specific microtubules.
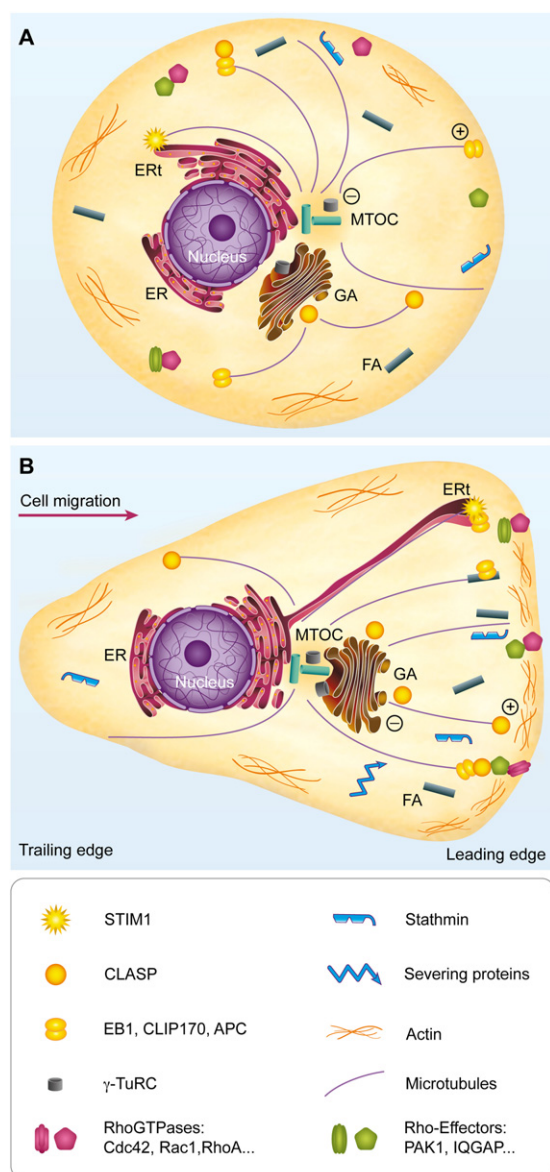
Fig. 2. Intracellular organization of non-polarized cell (A) or migrating cell (B). (A) The most important polarity established in a so-called "non-polarized" cell is the asymmetry between the center and the periphery of the cell. This intracellular asymmetry is established by the microtubule network. Minus-ends of microtubules are in general located at the cell center, bound to a Microtubule Organizing Center, and their plus-ends explore the cell periphery. +TIPs bind to the plus-ends of growing microtubules and participate in the regulation of their dynamics at the periphery of the cells. This asymmetry imposes a particular organization of the organelles like the Golgi apparatus, the ER or the endosomes (not shown here). (B). Strong cellular polarization, as observed during migration, imposes an additional axis, a front and a rear of the cell being differentiated in addition to the center and periphery. The reorganization of the MTOC and other organelles during polarization allows the formation of a leading edge, with the presence of focal adhesions sites, required for cell migration. Microtubules work together with another essential cytoskeleton structure, the microfilaments of actin, to establish cell polarity. A local regulation of +TIPs and small GTPases at the leading edge is necessary for the establishment of a polarized microtubule network and cell polarization. MTOC, Microtubule Organizing Center; γ-TuRC, γ-tubulin ring complex; ER, endoplasmic reticulum; ERt, endoplasmic reticulum tubule; GA, Golgi apparatus; FA, focal adhesions.

3.1. Nucleus

Microtubules are essential to ensure proper segregation of chromosomes during mitosis (for a review, see Tanaka, 2010) and are also directly implicated in nuclear envelope breakdown (Beaudouin et al., 2002). However, the nucleus, like smaller organelles in the cell, is dynamic and moves in the cytoplasm. The whole cytoskeleton, in particular the acto-myosin and microtubule networks, is involved in these movements.

The role of microtubules in nucleus positioning and maintenance of its position in interphase cells has mostly been described in *S. pombe* and *Aspergillus nidulans*. The importance of microtubule dynamics in the maintenance of nuclear position was shown by different mutations affecting microtubule behavior: Caron et al. (2001) showed that a mutation in α-tubulin may induce palmitoylation and affects astral microtubules dynamics in *Saccharomyces cerevisiae*. Another mutation, which suppresses microtubule dynamics by inhibition of the GTPase activity of the β-subunit, also affects nuclear positioning (Dougherty et al., 2001). In yeast, a γ-tubulin mutation induces microtubule stability and leads to microtubules that do not stop growing and wrap themselves around the cell. Such cells loose their polarity and show impaired nuclear positioning (Paluh et al., 2000).

Nuclear positioning depends mostly on centrosomal microtubules and proteins found at their plus-ends, among them some +TIPs, dynein and dynactin, a dynein activator complex. These proteins appear to interact with cortical proteins to generate forces to regulate nuclear positionning. For example in *S. pombe*, astral microtubules maintain the nucleus at the center of the cell by pushing forces against the ends of the cell (Tran et al., 2001). In animal cells, some additional proteins are particularly important: CLASPs, found on microtubules of the leading edge of migrating cells, or IQGAP1, an actin-binding protein, and APC (Adenomatous Polyposis Coli) involved in interactions between microtubules plus-ends and the cortex, may be involved in pushing or pulling forces to participate in nuclear positioning. Centrosomal microtubules also interact directly with the nuclear envelope (reviewed in Mellad et al., 2011). Linkers between the nucleoskeleton and the cytoskeleton (LINC) have been identified among them KASH and SUN that are essential for nuclear and centrosome positioning (reviewed in Razafsky and Hodzic, 2009).

In muscle cells, during skeletal muscle differentiation, the nucleus can also be a site of microtubule nucleation. Ninein, an anchorage protein localized at the centrosome in most cells, relocalizes at the nuclear envelope during differentiation. Pericentrin and γ-tubulin are then relocalized at the same site where nucleation of microtubules occurs (Bugnard et al., 2005).

3.2. Trafficking organelles

Microtubule dynamics play a central role in the positioning and function of organelles involved in intracellular trafficking: the endoplasmic reticulum (ER), the Golgi apparatus and the endosomes/lysosomes. Microtubules are also well known to accelerate intracellular motility of transport intermediates and are particularly important to establish and maintain cell polarity. However, we will not describe here the role of microtubule in trafficking but we will concentrate on their implication in intracellular organization.

3.2.1. Endoplasmic reticulum

The ER is composed of lamellar and tubular membranes that form a large interconnected network continuous with the nuclear envelope. It is involved in the synthesis of very diverse proteins destined for secretion, adhesion, signaling at the cell surface or of proteins involved in trafficking and endocytosis. It is also a place of lipid synthesis and a major site of calcium storage. It is very dynamic and continually remodels itself even in resting cells. It can quickly increase or decrease its volume depending on cell needs.

In animal cells, the microtubule-based motor KIF5B/kinesin-1 seems to be the major motor protein involved in ER positioning and movement. It was shown by Wozniak et al. (2009) that ER tubules movement is inhibited by a dominant-negative construct

of kinesin-1 in Vero cells. *In vitro* studies showed that inhibitors of kinesin-1 inhibit ER movement (Lane and Allan, 1999). However, a study in mouse embryo fibroblasts shows that knockout of kinesin heavy chain has no effect on ER positioning (Nakajima et al., 2002). This suggests that kinesin-1 is not the only mechanism involved in ER movement.

STIM1 (Stromal-Interaction Molecule 1) was shown to be a +TIP by Grigoriev et al. (2008), to localize at the ER and to play a major role in ER remodeling. Contrary to all other +TIPs identified so far, STIM1 is not a cytoplasmic protein but an integral protein of the ER. Comet-like structures positive for STIM1 and EB1 were shown to decorate the ER network. This was reminiscent of Tip Attachment Complexes (TACs) that were described earlier in an *in vitro* reconstitution experiment (Waterman-Storer et al., 1995). TACs enable ER tubules to attach to microtubule tips, and to grow and elongate together with EB-positive comets. Individual depletion of STIM1 or EB1 reduced the frequency of ER protrusions but had no effect on microtubule density or ER sliding on microtubules while overexpression of STIM1, but not of EB1, increased TAC-mediated ER tubules growth. This suggests a link between STIM1 present in the ER membrane and EB1 at the microtubule plus-end to sustain tubule formation dependent on microtubule polymerization. Another microtubule-binding protein, CLIMP63 (Cytoskeleton-Linking Protein 63), is localized to TACs and mediates static interactions between ER tubules and microtubules (Vedrenne and Hauri, 2006). Microtubules in animal cells are thus involved both in shaping the ER and in the formation of ER tubules.

ER morphology and movement are essential for its function in protein synthesis and trafficking to the Golgi. Kinesin-1 is also involved in positioning and motility of ER exit-sites and participates in ER to Golgi trafficking.

The comet-like behavior of STIM1 also seems to play a significant role in the regulation of calcium storage. A previous study shows that microtubule depolymerization is involved in calcium entry at the ER and that STIM1 localization and function are necessary. Inhibition of store-operated calcium entry (SOCE) by nocodazole was almost completely rescued by over-expression of YFP-STIM1 in HEK293 cells suggesting a role for both microtubules and STIM1 in SOCE regulation (Smyth et al., 2007). This further underlined the link that exists between intracellular organization and functions of the organelles.

3.2.2. Golgi apparatus

In most mammalian cells, the Golgi apparatus is shaped like a ribbon and is closely associated with the centrosome. The use of microtubule poisons, colchicine, nocodazole, vinblastine or taxol for example, revealed that the juxta-nuclear localization of the Golgi apparatus required the presence of microtubules (Thyberg et al., 1980; Wehland et al., 1983). Without microtubules, the Golgi apparatus was found to be dispersed in the form of mini-stacks. This was not only due to physical break down of long cisternae because Turner and Tartakoff showed in 1989 that this dispersion required energy (Turner and Tartakoff, 1989). Lippincott-Schwartz and colleagues later observed that in the absence of microtubules, Golgi mini-stacks formed at ER exit-sites and were unable to migrate to the cell center (Cole et al., 1996). Upon wash-out of nocodazole, these mini-stacks migrated again along reformed microtubules (Ho et al., 1989).

The molecular mechanisms responsible for microtubule-dependent Golgi organization have been studied extensively. As expected, microtubule-dependent molecular motors were found to be essential for Golgi organization and in particular the minus-end directed motor, cytoplasmic dynein and its regulatory proteins. When looking at Golgi organization, it is possible to pheno-copy the absence of microtubules by interfering with dynein activity. This was done either by perturbing the dynein regulatory complex dynactin (Burkhardt et al., 1997) or more directly by knocking-out cytoplasmic dynein in mice (Harada et al., 1998). The role of Bicaudal D was also investigated as it is localized at the Golgi in a Rab6-dependent manner. It was shown to recruit the dynein–dynactin complex to Rab6-positive membranes at the Golgi and on cytoplasmic vesicles (Hoogenraad et al., 2001, Matanis et al., 2002). The N-terminal domain of Bicaudal D induced minus-end directed transport mediated by dynein, in different molecular contexts (Hoogenraad et al., 2003). Plus-end directed motors are also involved in Golgi organization. A fraction of KIF5B/kinesin-1 for example is localized at the Golgi and its depletion induces the compaction of the Golgi apparatus (reviewed in Allan et al., 2002).

Non-motor microtubule binding proteins also participate in Golgi assembly and dynamics (for a review, see Rios and Bornens, 2003). For example, cells depleted of CLASP show defects in Golgi morphology and a radial microtubule array (Miller et al., 2009). CLASPs participate in the maintenance of a polarized Golgi ribbon, probably through its effect on microtubule nucleation at the Golgi apparatus. SCG10, a stathmin-like protein, seems also involved (Gavet et al., 1998) as are Hook-3 (Walenta et al., 2001) and CLIPR59, a CLIP170-related protein (Lallemand-Breitenbach et al., 2004).

The interactions between the Golgi apparatus and the centrosome also play a part in the regulation of Golgi positioning and organization in most interphase cells. Kodani and Sütterlin (2008) showed that GM130, a protein of the *cis*-Golgi, was involved in this interaction. Cells depleted of GM130 display aberrant centrosome morphology in interphase and multipolar spindle in mitosis. They also have defects in microtubule acetylation that is rescued by GRASP65, a GM130-interacting protein. Interestingly, GRASP65 cannot rescue the aberrant spindle phenotype. GM130 controls the activation of Cdc42, a small GTPase of the Rho family, by facilitating its interaction with its guanine exchange factor, Tuba. Moreover, the effect of GM130 depletion on centrosome in interphase cells is rescued by a constitutively active form of Cdc42 (Kodani et al., 2009). Another Golgi matrix protein, GMAP210, seems to play an essential role. In addition to its role in trafficking (Drin et al., 2008), GMAP210 interacts with γ-tubulin and is involved in the organization of Golgi microtubules (Rios et al., 2004). Golgi-nucleated microtubules indeed play a key role in Golgi organization. Purified rat liver Golgi membranes are able to nucleate microtubules *in vitro* (Chabin-Brion et al., 2001). These non-centrosomal microtubules are shown *in vivo* to be nucleated very early after nocodazole wash out, in the vicinity of dispersed Golgi fragments. In non-treated cells, it was observed that Golgi membranes nucleate microtubules in a CLASP-dependent manner (Efimov et al., 2007). This microtubule sub-population is stable and acetylated. As discussed by Chabin-Brion and colleagues, Golgi membranes could specifically stabilize a microtubule subset upon exit from mitosis that would be involved in the organization of Golgi mini-stacks into bigger structures to reform the Golgi ribbon in the centrosomal area. Finally, a long scaffolding protein, AKAP450 was recently found to be an essential linker between the centrosome and the Golgi apparatus. This involves the γ-TuRC complex recruited by AKAP450 on Golgi membranes. AKAP450 localization is GM130 dependent: cells depleted from GM130 show a disorganization of AKAP450 and an impaired nucleation of Golgi microtubules. The same study confirms the role of CLASP on Golgi-dependent stabilization of these newly formed microtubules (Rivero et al., 2009). In the absence of proper AKAP450 activity, the Golgi complex can be unlinked from the centrosome, inducing defects in cell polarity (Hurtado et al., 2011).

The important role of Golgi-derived microtubules in the regulation of Golgi apparatus formation and maintenance suggests that some organelles are not only passively arranged by cytoskeletal elements but also actively participate in cellular and

cytoskeleton organization. Although we focused here on the main secretory organelles, it is important to stress that the endocytic compartment localization also strongly depends on microtubules even though the role of microtubule dynamics in this localization is less clear. It is however worth mentioning that localization of endosomes is finely regulated and highly correlated with their function (Collinet et al., 2010).

3.3. Mitochondria

Mitochondria form a dynamic tubular network in cells and are essential for cell metabolism, cell growth and cell survival. In mammalian cells, microtubules are essential for mitochondria positioning and transport. Mitochondria transport has been studied especially in axons, in neuronal cells, but also in non-polarized cells and it has been observed that several motors, such as Kif1B, transport mitochondria as cargos along microtubules (Nangaku et al., 1994). Microtubule dynamics itself, in particular in the yeast *S. pombe*, is involved in mitochondria organization. Yaffe et al. (2003) have shown that mitochondrial tubules grow and shrink along with dynamic microtubules, and that immobile mitochondria are captured by plus-ends of growing microtubules. Two other studies identified microtubule-binding proteins that interact with mitochondria: peg1p, homologue of mammalian CLASP, is the first +TIP shown to be necessary for mitochondrial distribution (Chiron et al., 2008) and mmb1p binds microtubules and attenuates microtubule dynamics to enhance mitochondria-microtubule interactions (Fu et al., 2011).

4. Role of microtubules in cell polarization

As discussed above, microtubules in non-polarized cells are mostly organized by the centrosome with the growing plus-tips spread out at the periphery of the cell (Fig. 2A). During establishment of polarity, the microtubule network undergoes profound changes and reorientation, along with a whole intracellular reorganization. Both establishment and maintenance of polarity require the actin and microtubule cytoskeletons. We will discuss here the role of microtubules in polarization and take three examples of polarized cells: (a) epithelial cells: they are highly polarized with an apical pole usually directed to the lumen of the tissue or the organ and a basal pole in contact with the underlying basal membrane. Cell–cell and cell-to-matrix junctions are necessary for the homeostasis of the tissue and are mediated by cadherin and integrins adhesions respectively. Proteins specific to tight junctions like ZO-1, claudin or occludin also play an essential role in epithelium function and organization. (b) Neuronal cells: their polarity is crucial for the activity of the whole neuronal network. Neurons differentiate from round cells that gradually acquire polarity, from a multipolarity stage with many neurites to the formation of one single axon and multiple dendrites (reviewed in Tahirovic and Bradke, 2009). (c) Migrating cells (Fig. 2B): in metazoan, migration is involved in development, tissue repair or immunological surveillance for example and is essential to ensure survival of many unicellular organisms. Migrating cells respond to various stimuli and undergo polarization with a complete reorganization of intracellular components (Vinogradova et al., 2009). A classical example is the mesenchymal-like movement where cells strongly polarize with a leading edge and lamellipodia at the front, and a trailing edge at the rear. During this type of migration, cells usually bind to the extracellular matrix in an integrin-dependent manner at focal adhesions that serve as anchors onto which the cells push or pull themselves in order to migrate (reviewed in Friedl and Wolf, 2010). Contrary to fibroblasts or macrophages, fast migrating cells including keratocytes and neutrophils do not require microtubules for

migration (reviewed in Wittmann and Waterman-Storer, 2001). Microtubule disruption induces neutrophil polarity and migration (Niggli, 2003).

4.1. Reorganization of the microtubule network

The establishment of polarity in higher eukaryotes is a microtubule-dependent process and often starts with the reorganization of the whole microtubule network. In neurons, the reorientation of the centrosome usually takes place in the multipolarized stage 2-cell, before axonal growth, and correlates with a reorganization of the Golgi apparatus (de Anda et al., 2010). The centrosomal protein Cep120 was shown to be involved in this reorientation. It has long been thought that the centrosome position was determining the neurite that will eventually become the axon, but this model has now been challenged (for a review, see Stiess and Bradke, 2011). To generate a polarized microtubule array, microtubules are released from the centrosome and transported in the axon in a dynein/dynactin-dependent manner (Ahmad and Baas, 1995; Ahmad et al., 1998). Moreover, the severing enzyme katanin is required in both steps of this process (Ahmad et al., 1999). Interestingly, in the axon, all the plus-ends face towards the growth cone, whereas in dendrites, microtubules have mixed orientations (Baas et al., 1988).

In epithelial cells, microtubules are reorganized in non-centrosomal arrays with a meshwork at the apical and basal poles, and a longitudinal array organized parallel to the apical-basal axis of the cell (reviewed in Bartolini and Gundersen, 2006). Like in neurons, microtubule minus-ends are released from the centrosome (Keating et al., 1997) but minus-ends binding factors such as ninein and Nezha/PLEKHA7 anchor microtubules either at cell–cell adhesive junctions or at the apical pole. In both cases, most microtubules are directed towards the basal pole and to cadherin-based cell–cell contacts (Ligon and Holzbaur, 2007; Stehbens et al., 2006). For example, Nezha, first identified as a PLEKHA7 partner at cell junctions, was found to be a microtubule-binding protein and to promote microtubule nucleation (Meng et al., 2008).

In mesenchymal-like migrating cells, a microtubule-array oriented in the direction of cell movement is established: microtubules target focal adhesions at the leading edge and are required for their rapid turnover (reviewed in Small et al., 2002 and Efimov et al., 2008). They also participate in actin polymerization and polarized trafficking of components of the leading edge. Reorientation of the microtubule array is coupled to the orientation of the Golgi apparatus at the front of the cell. Golgi-derived microtubules are also organized and grow mostly towards the cell leading edge in a polarized manner (Efimov et al., 2007). Like in axons, the reorganization of the microtubule network involves the release of microtubules from the centrosome and microtubules transport within the cell in different regions (Yvon and Wadsworth, 2000). Several studies describe alternative mechanisms that participate in MTOC reorientation. For eaxample, a recent study shows a MTOC reorientation dependent on dynein and Cdc42, which would pull the MTOC from the leading edge (Manneville et al., 2010). Interestingly, Gomes et al. (2005) show that MTOC reorientation in 3T3 fibroblasts is organized by acto-myosin-dependent nucleus movement, whereas the centrosome remains stationary.

4.2. Regulation of microtubule dynamics in polarized cells

Post-translational modifications of microtubules, MAPs and +TIPs play an important role in cell polarization. In neurons, microtubule stability is involved in axon formation (Witte et al., 2008). A high ratio of acetylated/tyrosinated microtubules is observed in the neurite that will become the axon, as compared to other neurites that have more dynamic microtubules. Relocalization of the

centrosome has been proposed to create a local gradient of acetylated stable microtubules, which promote axon formation. Witte et al. (2008) also showed an accumulation of detyrosinated microtubules in the axon.

In epithelial cells, post-translational modifications appear gradually with the establishment of polarity. Studying dog kidney epithelial cell (MDCK), Quinones et al. (2011) showed that before polarization, detyrosinated microtubules are numerous as compared to acetylated tubulin. After polarization, detyrosinated microtubules are only found in the primary cilia at the apical pole, and acetylated microtubules become more abundant at the apical pole but also on lateral bundles. This study highlights the existence of sub-populations of microtubules that are differently modified and localized. Functions for these modifications are not yet clear and will need further investigation. They may be involved in polarized trafficking, as are the MAPs and +TIPs, enhancing microtubule stability. APC is the major +TIP involved in polarization of epithelial cells, together with EB1 and CLIP170, forming puncta at the cell cortex that are essential for microtubule re-organization, attachment and capture (Bellett et al., 2009; Mogensen et al., 2002; Reilein and Nelson, 2005). Septins, filamentous GTPases, have been shown to suppress MT catastrophes and participate in microtubule guidance during polymerization. Septins seem to modulate microtubule growth and shrinkage and influence the directionality of microtubule movement, which might be important for the positioning of the microtubule network during cell polarization (Bowen et al., 2011).

In migrating cells, microtubule dynamics is required to maintain a rapid turnover of focal adhesions, which allows rapid and efficient migration. Microtubules target focal adhesions, which disassemble when microtubules depolymerize. ER tubules are also involved in this rapid turnover, promoting focal adhesion assembly through the kinesin–kinectin complex (Zhang et al., 2010) or other ER proteins such as calnexin and the protein tyrosine-phosphatase B (Hernandez et al., 2006). Cells treated with an inhibitor of histone deacetylase 6 accumulate stable hyperacetylated microtubules and show a decrease in focal adhesion turnover with a reduction of cell migration (Tran et al., 2007). Microtubule stability is locally up-regulated in migrating cells. For example, it has been reported that there is a strong accumulation of detyrosinated microtubules at the leading edge (Gundersen and Bulinski, 1988). This accumulation depends on the RhoA effector, mDia (Palazzo et al., 2001). A very precise regulation of microtubule dynamics is therefore necessary at the leading edge and a feedback loop is established. Rho-like GTPases are activated by microtubules and, in turn, they participate in the local control of microtubule dynamics. Three main Rho-like GTPases Rac1, Cdc42 and RhoA, and their effectors interact with MAPs or +TIPs and participate in microtubule targeting of focal adhesions (Watanabe et al., 2005). For example, PAK1, an effector of Rac1 and Cdc42, directly phosphorylates stathmin and inhibits its depolymerization activity (Daub et al., 2001; Wittmann et al., 2004). Moreover, stathmin is also accumulated at the trailing edge and locally increases microtubule catastrophe frequency (Niethammer et al., 2004). Cdc42 is considered to be the main Rho-like GTPase involved in the establishment of polarization in migrating cells (reviewed in Etienne-Manneville, 2004). Its main effector, IQGAP binds to the +TIPs CLIP170, CLASPs and APC. These +TIPs promote respectively microtubule capture (Fukata et al., 2002; Watanabe et al., 2009) and growth at the cortex (Kita et al., 2006). EB1 and CLASP are also found at the leading edge (Wen et al., 2004). CLASP is locally regulated by Rac1 and GSK3β (Akhmanova et al., 2001; Wittmann and Waterman-Storer, 2005), was shown to stabilize the polarized microtubule array that is involved in persistent migration (Drabek et al., 2006). It also facilitates the attachment of microtubule plus-ends to focal adhesions (Mimori-Kiyosue et al., 2005).

In the axon of hippocampal neurons, the Rac activator DOCK7 (Dedicator of Cytokinesis 7) promotes microtubule stabilization by reducing stathmin activity (Watabe-Uchida et al., 2006). In addition, Collapsin Response Mediator-2 (CRMP-2) binds to polymerized tubulin dimers and increases microtubule assembly (Fukata et al., 2002). GSK3β allows a local regulation of APC, but also of MAP1B and CRMP-2, increasing their affinity for microtubule plus-ends. CLIP170 is accumulated in neurite growth cone and participates to axonal specification. Interestingly, Nakata and Hirokawa (2003) reported the presence of EB1 in the initial segment of the axon where it is believed to have a role in selective sorting and where it appears to bind to the microtubule lattice and not to the plus-ends specifically. More recently, it has been revealed that the presence of GTP-tubulin itself along the microtubule plays a direct role on neuron polarization. Nakata et al. (2011) indeed showed that the GTP-tubulin remnants increase the activity of a molecular motor (kinesin-1/KIF5B), hence influencing axonal organization.

5. Conclusion

The microtubule cytoskeleton has, for a long time, been considered as polarized rails used by the cell for shape maintenance and motor tracks for trafficking. It is now clear that microtubule dynamics itself is used and finely regulated to organize the intracellular space and that, in turn, organelles influence microtubule organization and dynamics. Integrative studies will thus be necessary to assemble in common networks proteins involved in the control of microtubule dynamics, proteins regulating organelle organization and function and cell shape and polarity.

Acknowledgements

References

Ahmad FJ, Baas PW. Microtubules released from the neuronal centrosome are transported into the axon. J Cell Sci 1995;108(Pt 8):2761–9.

Ahmad FJ, Echeverri CJ, Vallee RB, Baas PW. Cytoplasmic dynein and dynactin are required for the transport of microtubules into the axon. J Cell Biol 1998;140:391–401.

Ahmad FJ, Yu W, McNally FJ, Baas PW. An essential role for katanin in severing microtubules in the neuron. J Cell Biol 1999;145:305–15.

Akella JS, Wloga D, Kim J, Starostina NG, Lyons-Abbott S, Morrissette NS, et al. MEC-17 is an alpha-tubulin acetyltransferase. Nature 2010;467:218–22.

Akhmanova A, Hoogenraad CC, Drabek K, Stepanova T, Dortland B, Verkerk T, et al. Clasps are CLIP-115 and -170 associating proteins involved in the regional regulation of microtubule dynamics in motile fibroblasts. Cell 2001;104:923–35.

Akhmanova A, Steinmetz MO. Microtubule +TIPs at a glance. J Cell Sci 2010;123:3415–9.

Al-Bassam J, Kim H, Brouhard G, van Oijen A, Harrison SC, Chang F. CLASP promotes microtubule rescue by recruiting tubulin dimers to the microtubule. Dev Cell 2010;19:245–58.

Allan VJ, Thompson HM, McNiven MA. Motoring around the Golgi. Nat Cell Biol 2002;4:E236–42.

Andrews PD, Ovechkina Y, Morrice N, Wagenbach M, Duncan K, Wordeman L, et al. Aurora B regulates MCAK at the mitotic centromere. Dev Cell 2004;6:253–68.

Baas PW, Deitch JS, Black MM, Banker GA. Polarity orientation of microtubules in hippocampal neurons: uniformity in the axon and nonuniformity in the dendrite. Proc Natl Acad Sci U S A 1988;85:8335–9.

Ban R, Matsuzaki H, Akashi T, Sakashita G, Taniguchi H, Park S-Y, et al. Mitotic regulation of the stability of microtubule plus-end tracking protein EB3 by ubiquitin ligase SIAH-1 and Aurora mitotic kinases. J Biol Chem 2009;284:28367–81.

Bartolini F, Gundersen GG. Generation of noncentrosomal microtubule arrays. J Cell Sci 2006;119:4155–63.

Beaudouin JL, Gerlich D, Daigle N, Eils R, Ellenberg J. Nuclear envelope breakdown proceeds by microtubule-induced tearing of the lamina. Cell 2002;108:83–96.

Bellett G, Carter JM, Keynton J, Goldspink D, James C, Moss DK, et al. Microtubule plus-end and minus-end capture at adherens junctions is involved in the assembly of apico-basal arrays in polarised epithelial cells. Cell Motil Cytoskeleton 2009;66:893–908.

Belmont L, Mitchison T, Deacon HW. Catastrophic revelations about Op18/stathmin. Trends Biochem Sci 1996;21:197–8.

Bonnet C, Boucher D, Lazereg S, Pedrotti B, Islam K, Denoulet P, et al. Differential binding regulation of microtubule-associated proteins MAP1A, MAP1B, and MAP2 by tubulin polyglutamylation. J Biol Chem 2001;276:12839–48.

Bouissou A, Vérollet C, Sousa A, Sampaio P, Wright M, Sunkel CE, et al. {gamma}-Tubulin ring complexes regulate microtubule plus end dynamics. J Cell Biol 2009;187:327–34.

Bowen JR, Hwang D, Bai X, Roy D, Spiliotis ET. Septin GTPases spatially guide microtubule organization and plus end dynamics in polarizing epithelia. J Cell Biol 2011;194:187–97.

Brouhard GJ, Stear JH, Noetzel TL, Al-Bassam J, Kinoshita K, Harrison SC, et al. XMAP215 is a processive microtubule polymerase. Cell 2008;132:79–88.

Bugnard E, Zaal KJ, Ralston E. Reorganization of microtubule nucleation during muscle differentiation. Cell Motil Cytoskeleton 2005;60:1–13.

Burkhardt JK, Echeverri CJ, Nilsson T, Vallee RB. Overexpression of the dynamitin (p50) subunit of the dynactin complex disrupts dynein-dependent maintenance of membrane organelle distribution. J Cell Biol 1997;139:469–84.

Caron JM, Vega LR, Fleming J, Bishop R, Solomon F. Single site alpha-tubulin mutation affects astral microtubules and nuclear positioning during anaphase in *Saccharomyces cerevisiae*: possible role for palmitoylation of alpha-tubulin. Mol Biol Cell 2001;12:2672–87.

Chabin-Brion K, Marceiller J, Perez F, Settegrana C, Drechou A, Durand G, et al. The Golgi complex is a microtubule-organizing organelle. Mol Biol Cell 2001;12:2047–60.

Chiron S, Bobkova A, Zhou H, Yaffe M. CLASP regulates mitochondrial distribution in *Schizosaccharomyces pombe*. J Cell Biol 2008;182(1):41–9.

Choi JH, Bertram PG, Drenan R, Carvalho J, Zhou HH, Zheng XFS. The FKBP12-rapamycin-associated protein (FRAP) is a CLIP-170 kinase. EMBO Rep 2002;3:988–94.

Cole NB, Sciaky N, Marotta A, Song J, Lippincott-Schwartz J. Golgi dispersal during microtubule disruption: regeneration of Golgi stacks at peripheral endoplasmic reticulum exit sites. Mol Biol Cell 1996;7:631–50.

Collinet C, Stoter M, Bradshaw CR, Samusik N, Rink JC, Kenski D, et al. Systems survey of endocytosis by multiparametric image analysis. Nature 2010;464:243–9.

Conacci-Sorrell M, Ngouenet C, Eisenman RN. Myc-nick: a cytoplasmic cleavage product of Myc that promotes alpha-tubulin acetylation and cell differentiation. Cell 2010;142:480–93.

Creppe C, Malinouskaya L, Volvert ML, Gillard M, Close P, Malaise O, et al. Elongator controls the migration and differentiation of cortical neurons thrhough acetylation of alpha-tubulin. Cell 2009;136(3):551–64.

Daub H, Gevaert K, Vandekerckhove J, Sobel A, Hall A. Rac/Cdc42 and p65PAK regulate the microtubule-destabilizing protein stathmin through phosphorylation at serine 16. J Biol Chem 2001;276:1677–80.

de Anda FC, Meletis K, Ge X, Rei D, Tsai L-H. Centrosome motility is essential for initial axon formation in the neocortex. J Neurosci 2010;30:10391–406.

Desai A, Mitchison TJ. Microtubule polymerization dynamics. Annu Rev Cell Dev Biol 1997;13:83–117.

Dimitrov A, Quesnoit MI, Moutel S, Cantaloube I, Poüs C, Perez F. Detection of GTP-tubulin conformation in vivo reveals a role for GTP remnants in microtubule rescues. Science 2008;322:1353–6.

Dougherty CA, Sage CR, Davis A, Farrell KW. Mutation in the beta-tubulin signature motif suppresses microtubule GTPase activity and dynamics, and slows mitosis. Biochemistry 2001;40:15725–32.

Drabek K, van Ham M, Stepanova T, Draegenstein K, van Horssen R, Sayas CL, et al. Role of CLASP2 in microtubule stabilization and the regulation of persistent motility. Curr Biol 2006;16(22):2259–64.

Drin G, Morello V, Casella JF, Gounon P, Antonny B. Asymetric tethering of flat and curved lipid membranes by a golgin. Science 2008;320(5876):670–3.

Efimov A, Kharitonov A, Efimova N, Loncarek J, Miller PM, Andreyeva N, et al. Asymmetric CLASP-dependent nucleation of noncentrosomal microtubules at the trans-Golgi network. Dev Cell 2007;12:917–30.

Efimov A, Schiefermeier N, Grigoriev I, Ohi R, Brown MC, Turner CE, et al. Paxillin-dependent stimulation of microtubule catastrophes at focal adhesion sites. J Cell Sci 2008;121(Pt 2):196–204.

Ems-McClung SC, Walczak CE. Kinesin-13s in mitosis: key players in the spatial and temporal organization of spindle microtubules. Semin Cell Dev Biol 2010;21:276–82.

Etienne-Manneville S. Cdc42 – the centre of polarity. J Cell Sci 2004;117:1291–300.

Friedl P, Wolf K. Plasticity of cell migration: a multiscale tuning model. J Cell Biol 2010;188(1):11–9.

Friedman JR, Webster B, Mastronarde D, Verhey K, Voeltz G. ER sliding dynamics and ER-mitochondrial contacts occur on acetylated microtubules. J Cell Biol 2010;190(3):363–75.

Fu C, Jain D, Costa J, Velve-Casquillas G. Tran PT. mmb1p binds mitochondria to dynamic microtubules. Curr Biol 2011;21(17):143–9.

Fukata M, Watanabe T, Noritake J, Nakagawa M, Yamaga M, Kuroda S, et al. Rac1 and Cdc42 capture microtubules through IQGAP1 and CLIP-170. Cell 2002;109:873–85.

Gavet O, Ozon S, Manceau V, Lawler S, Curmi P, Sobel A. The stathmin phosphoprotein family: intracellular localization and effects on the microtubule network. J Cell Sci 1998;111(Pt 22):3333–46.

Goodwin SS, Vale RD. Patronin regulates the microtubule network by protecting minus-ends. Cell 2010;143(2):263–74.

Gomes ER, Jani S, Gundersen GG. Nuclear movement regulated by Cdc42, MRCK, myosin, and actin flow establishes MTOC polarization in migrating cells. Cell 2005;121:451–63.

Grigoriev I, Gouveia SM, van der Vaart B, Demmers J, Smyth JT, Honnappa S, et al. STIM1 is a MT-plus-end-tracking protein involved in remodeling of the ER. Curr Biol 2008;18:177–82.

Gundersen GG, Bulinski JC. Selective stabilization of microtubules oriented toward the direction of cell migration. Proc Natl Acad Sci U S A 1988;85:5946–50.

Harada A, Takei Y, Kanai Y, Tanaka Y, Nonaka S, Hirokawa N. Golgi vesiculation and lysosome dispersion in cells lacking cytoplasmic dynein. J Cell Biol 1998;141:51–9.

Hernandez MV, Sala MG, Balsamo J, Lilien J, Arregui CO. ER-bound PTP1B is targeted to newly forming cell-matrix adhesions. J Cell Sci 2006;119:1233–43.

Ho WC, Allan VJ, van Meer G, Berger EG, Kreis TE. Reclustering of scattered Golgi elements occurs along microtubules. Eur J Cell Biol 1989;48:250–63.

Honnappa S, Gouveia SM, Weisbrich A, Damberger FF, Bhavesh NS, Jawhari H, et al. An EB1-binding motif acts as a microtubule tip localization signal. Cell 2009;138:366–76.

Hoogenraad CC, Akhmanova A, Howell SA, Dortland BR, De Zeeuw CI, Willemsen R, et al. Mammalian Golgi-associated Bicaudal-D2 functions in the dynein–dynactin pathway by interacting with these complexes. EMBO J 2001;20:4041–54.

Hoogenraad CC, Wulf P, Schiefermeier N, Stepanova T, Galjart N, Small JV, et al. Bicaudal D induces selective dynein-mediated microtubule minus end-directed transport. EMBO J 2003;22:6004–15.

Howell B, Deacon H, Cassimeris L. Decreasing oncoprotein 18/stathmin levels reduces microtubule catastrophes and increases microtubule polymer in vivo. J Cell Sci 1999;112(Pt 21):3713–22.

Hurtado L, Caballero C, Gavilan MP, Cardenas J, Bornens M, Rios RM. Disconnecting the Golgi ribbon from the centrosome prevents directional cell migration and ciliogenesis. J Cell Biol 2011;193:917–33.

Janke C, Kneussel M. Tubulin post-translational modifications: encoding functions on the neuronal microtubule cytoskeleton. Trends Neurosci 2010;33:362–72.

Keating TJ, Peloquin JG, Rodionov VI, Momcilovic D, Borisy GG. Microtubule release from the centrosome. Proc Natl Acad Sci U S A 1997;94:5078–83.

Kita K, Wittmann T, Näthke IS, Waterman-Storer CM. Adenomatous polyposis coli on microtubule plus ends in cell extensions can promote microtubule net growth with or without EB1. Mol Biol Cell 2006;17:2331–45.

Kodani A, Kristensen I, Huang L, Sütterlin C. GM130-dependent control of Cdc42 activity at the Golgi regulates centrosome organization. Mol Biol Cell 2009;20:1192–200.

Kodani A, Sütterlin C. The Golgi protein GM130 regulates centrosome morphology and function. Mol Biol Cell 2008;19:745–53.

Komarova Y, De Groot CO, Grigoriev I, Gouveia SM, Munteanu EL, Schober JM, et al. Mammalian end binding proteins control persistent microtubule growth. J Cell Biol 2009;184:691–706.

Komarova Y, Lansbergen G, Galjart N, Grosveld F, Borisy GG, Akhmanova A. EB1 and EB3 control CLIP dissociation from the ends of growing microtubules. Mol Biol Cell 2005;16:5334–45.

Komarova YA, Akhmanova AS, Kojima S-I, Galjart N, Borisy GG. Cytoplasmic linker proteins promote microtubule rescue in vivo. J Cell Biol 2002;159:589–99.

Kumar P, Lyle KS, Gierke S, Matov A, Danuser G, Wittmann T. GSK3beta phosphorylation modulates CLASP-microtubule association and lamella microtubule attachment. J Cell Biol 2009;184:895–908.

Lacroix B, van Dijk J, Gold ND, Guizetti J, Aldrian-Herrada G, Rogowski K, et al. Tubulin polyglutamylation stimulates spastin-mediated microtubule severing. J Cell Biol 2010;189:945–54.

Lallemand-Breitenbach Vr, Quesnoit MI, Braun V, El Marjou A, Poüs C, Goud B, et al. CLIPR-59 is a lipid raft-associated protein containing a cytoskeleton-associated protein glycine-rich domain (CAP-Gly) that perturbs microtubule dynamics. J Biol Chem 2004;279:41168–78.

Lan W, Zhang X, Kline-Smith SL, Rosasco SE, Barrett-Wilt GA, Shabanowitz J, et al. Aurora B phosphorylates centromeric MCAK and regulates its localization and microtubule depolymerization activity. Curr Biol 2004;14:273–86.

Lane JD, Allan VJ. Microtubule-based endoplasmic reticulum motility in *Xenopus laevis*: activation of membrane-associated kinesin during development. Mol Biol Cell 1999;10:1909–22.

Lee H-S, Komarova YA, Nadezhdina ES, Anjum R, Peloquin JG, Schober JM, et al. Phosphorylation controls autoinhibition of cytoplasmic linker protein-170. Mol Biol Cell 2010;21:2661–73.

Li W, Miki T, Watanabe T, Kakeno M, Sugiyama I, Kaibuchi K, et al. EB1 promotes microtubule dynamics by recruiting Sentin in Drosophila cells. J Cell Biol 2011;193:973–83.

Ligon LA, Holzbaur ELF. Microtubules tethered at epithelial cell junctions by dynein facilitate efficient junction assembly. Traffic 2007;8:808–19.

Maiato H, Khodjakov A, Rieder CL. Drosophila CLASP is required for the incorporation of microtubule subunits into fluxing kinetochore fibres. Nat Cell Biol 2005;7:42–7.

Manneville J-B, Jehanno M, Etienne-Manneville S. Dlg1 binds GKAP to control dynein association with microtubules, centrosome positioning, and cell polarity. J Cell Biol 2010;191:585–98.

Matanis T, Akhmanova A, Wulf P, Del Nery E, Weide T, Stepanova T, et al. Bicaudal-D regulates COPI-independent Golgi-ER transport by recruiting the dynein–dynactin motor complex. Nat Cell Biol 2002;4:986–92.

Mellad JA, Warren DT, Shanahan CM. Nesprins LINC the nucleus and cytoskeleton. Curr Opin Cell Biol 2011;23:47–54.

Meng W, Mushika Y, Ichii T, Takeichi M. Anchorage of microtubule minus ends to adherens junctions regulates epithelial cell–cell contacts. Cell 2008;135:948–59.

Miller PM, Folkmann AW, Maia ARR, Efimova N, Efimov A, Kaverina I. Golgi-derived CLASP-dependent microtubules control Golgi organization and polarized trafficking in motile cells. Nat Cell Biol 2009;11:1069–80.

Mimori-Kiyosue Y, Grigoriev I, Lansbergen G, Sasaki H, Matsui C, Severin F, et al. CLASP1 and CLASP2 bind to EB1 and regulate microtubule plus-end dynamics at the cell cortex. J Cell Biol 2005;168:141–53.

Mitchison T, Kirschner M. Dynamic instability of microtubule growth. Nature 1984;312:237–42.

Mogensen MM, Tucker JB, Mackie JB, Prescott AR, Näthke IS. The adenomatous polyposis coli protein unambiguously localizes to microtubule plus ends and is involved in establishing parallel arrays of microtubule bundles in highly polarized epithelial cells. J Cell Biol 2002;157:1041–8.

Nakajima K, Takei Y, Tanaka Y, Nakagawa T, Nakata T, Noda Y, et al. Molecular motor KIF1C is not essential for mouse survival and motor-dependent retrograde Golgi apparatus-to-endoplasmic reticulum transport. Mol Cell Biol 2002;22:866–73.

Nakano A, Kato H, Watanabe T, Min KD, Yamazaki S, Asano Y, et al. AMPK controls the speed of microtubule polymerization and directional cell migration through CLIP170 phosphorylation. Nat Cell Biol 2010;12:583–90.

Nakata T, Hirokawa N. Microtubules provide directional cues for polarized axonal transport through interaction with kinesin motor head. J Cell Biol 2003;162:1045–55.

Nakata T, Niwa S, Okada Y, Perez F, Hirokawa N. Preferential binding of a kinesin-1 motor to GTP-tubulin-rich microtubules underlies polarized vesicle transport. J Cell Biol 2011;194:245–55.

Nangaku M, Sato-Yoshitake R, Okada Y, Noda Y, Takemura R, Yamazaki H, et al. KIF1B: a novel microtubule plus end-directed monomeric motor protein for transport of mitochondria. Cell 1994;79(7):1209–20.

Newton CN, Wagenbach M, Ovechkina Y, Wordeman L, Wilson L. MCAK, a Kin I kinesin, increases the catastrophe frequency of steady-state HeLa cell microtubules in an ATP-dependent manner in vitro. FEBS Lett 2004;572:80–4.

Niethammer P, Bastiaens P, Karsenti E. Stathmin-tubulin interaction gradients in motile and mitotic cells. Science 2004;303:1862–6.

Niggli V. Microtubule-disruption-induces and chemotactic-peptide-induced migration of human neutrophils: implications for differential sets of signalling pathways. J Cell Sci 2003;166(Pt5):813–22.

Palazzo AF, Joseph HL, Chen YJ, Dujardin DL, Alberts AS, Pfister KK, et al. Cdc42, dynein, and dynactin regulate MTOC reorientation independent of Rho-regulated microtubule stabilization. Curr Biol 2001;11:1536–41.

Paluh JL, Nogales E, Oakley BR, McDonald K, Pidoux AL, Cande WZ. A mutation in gamma-tubulin alters microtubule dynamics and organization and is synthetically lethal with the kinesin-like protein pkl1p. Mol Biol Cell 2000;11:1225–39.

Perdiz D, Mackeh R, Poüs C, Baillet A. The ins and outs of tubulin acetylation: more than just a post-translational modification? Cell Signal 2011;23:763–71.

Perez F, Diamantopoulos GS, Stalder R, Kreis TE. CLIP-170 highlights growing microtubule ends in vivo. Cell 1999;96:517–27.

Peris L, Thery M, Faure J, Saoudi Y, Lafanechère L, Chilton JK, et al. Tubulin tyrosination is a major factor affecting the recruitment of CAP-Gly proteins at microtubule plus ends. J Cell Biol 2006;174:839–49.

Peris L, Wagenbach M, Lafanechère L, Brocard J, Moore AT, Kozielski F, et al. Motor-dependent microtubule disassembly driven by tubulin tyrosination. J Cell Biol 2009;185:1159–66.

Quinones GB, Danowski BA, Devaraj A, Singh V, Ligon LA. The posttranslational modification of tubulin undergoes a switch from detyrosination to acetylation as epithelial cells become polarized. Mol Biol Cell 2011;22:1045–57.

Raynaud-Messina B, Merdes A. Gamma-tubulin complexes and microtubule organization. Curr Opin Cell Biol 2007;19:24–30.

Razafsky D, Hodzic D. Bringing KASH under the SUN: the many faces of nucleo-cytoskeletal connections. J Cell Biol 2009;186:461–72.

Reilein A, Nelson WJ. APC is a component of an organizing template for cortical microtubule networks. Nat Cell Biol 2005;7:463–73.

Rickard JE, Kreis TE. Binding of pp170 to microtubules is regulated by phosphorylation. J Biol Chem 1991;266:17597–605.

Rios RM, Bornens M. The Golgi apparatus at the cell centre. Curr Opin Cell Biol 2003;15:60–6.

Rios RM, Sanchis A, Tassin AM, Fedriani C, Bornens M. GMAP-210 recruits gamma-tubulin complexes to cis-Golgi membranes and is required for Golgi ribbon formation. Cell 2004;118:323–35.

Rivero S, Cardenas J, Bornens M, Rios RM. Microtubule nucleation at the cis-side of the Golgi apparatus requires AKAP450 and GM130. EMBO J 2009;28:1016–28.

Schuyler SC, Pellman D. Microtubule "plus-end-tracking proteins": the end is just the beginning. Cell 2001;105:421–4.

Shida T, Cueva JG, Xu Z, Goodman MB, Nachury MV. The major alpha-tubulin K40 acetylatransferase alphaTAT1 promotes rapid ciliogenesis and efficient mechanosensation. Proc Natl Acad Sci U S A 2010;107(50):21517–22.

Small JV, Geiger B, Kaverina I, Bershadsky A. How do microtubules guide migrating cells? Nat Rev Mol Cell Biol 2002;3(12):957–64.

Smyth JT, DeHaven WI, Bird GS, Putney Jr JW. Role of the microtubule cytoskeleton in the function of the store-operated Ca^{2+} channel activator STIM1. J Cell Sci 2007;120:3762–71.

Stehbens SJ, Paterson AD, Crampton MS, Shewan AM, Ferguson C, Akhmanova A, et al. Dynamic microtubules regulate the local concentration of E-cadherin at cell–cell contacts. J Cell Sci 2006;119:1801–11.

Stiess M, Bradke F. Neuronal polarization: the cytoskeleton leads the way. Dev Neurobiol 2011;71:430–44.

Tahirovic S, Bradke F. Neuronal polarity. Cold Spring Harb Perspect Biol 2009;1:a001644.

Tanaka TU. Kinetochore-microtubule interactions: steps towards bi-orientation. EMBO J 2010;29:4070–82.

Thyberg J, Piasek A, Moskalewski S. Effects of colchicine on the Golgi complex and GERL of cultured rat peritoneal macrophages and epiphyseal chondrocytes. J Cell Sci 1980;45:41–58.

Tran AD-A, Marmo TP, Salam AA, Che S, Finkelstein E, Kabarriti R, et al. HDAC6 deacetylation of tubulin modulates dynamics of cellular adhesions. J Cell Sci 2007;120:1469–79.

Tran PT, Marsh L, Doye V, Inoué S, Chang F. A mechanism for nuclear positioning in fission yeast based on microtubule pushing. J Cell Biol 2001;153:397–411.

Turner JR, Tartakoff AM. The response of the Golgi complex to microtubule alterations: the roles of metabolic energy and membrane traffic in Golgi complex organization. J Cell Biol 1989;109:2081–8.

van der Vaart B, Manatschal C, Grigoriev I, Olieric V, Gouveia SM, Bjelic S, et al. SLAIN2 links microtubule plus end-tracking proteins and controls microtubule growth in interphase. J Cell Biol 2011;193:1083–99.

Vedrenne C, Hauri H-P. Morphogenesis of the endoplasmic reticulum: beyond active membrane expansion. Traffic 2006;7:639–46.

Vinogradova T, Miller PM, Kaverina I. Microtubule network asymmetry in motile cells: role of Golgi-derived array. Cell Cycle 2009;8:2168–74.

Walenta JH, Didier AJ, Liu X, Krämer H. The Golgi-associated hook3 protein is a member of a novel family of microtubule-binding proteins. J Cell Biol 2001;152:923–34.

Watabe-Uchida, John KA, Janas JA, Newey SE, Van Aelst L. The Rac activator DOCK7 regulates neuronal polarity through local phosphorylation of stathmin/Op18. Neuron 2006;51(6):727–39.

Watanabe T, Noritake J, Kakeno M, Matsui T, Harada T, Wang S, et al. Phosphorylation of CLASP2 by GSK-3beta regulates its interaction with IQGAP1, EB1 and microtubules. J Cell Sci 2009;122(Pt16):2969–79.

Watanabe T, Noritake J, Kaibuchi K. Regulation of microtubules in cell migration. Trends Cell Biol 2005;15:76–83.

Waterman-Storer CM, Gregory J, Parsons SF, Salmon ED. Membrane/microtubule tip attachment complexes (TACs) allow the assembly dynamics of plus ends to push and pull membranes into tubulovesicular networks in interphase Xenopus egg extracts. J Cell Biol 1995;130:1161–9.

Wehland J, Henkart M, Klausner R, Sandoval IV. Role of microtubules in the distribution of the Golgi apparatus: effect of taxol and microinjected anti-alpha-tubulin antibodies. Proc Natl Acad Sci U S A 1983;80:4286–90.

Wen Y, Eng CH, Schmoranzer J, Cabrera-Poch N, Morris EJS, Chen M, et al. EB1 and APC bind to mDia to stabilize microtubules downstream of Rho and promote cell migration. Nat Cell Biol 2004;6:820–30.

Witte H, Neukirchen D, Bradke F. Microtubule stabilization specifies initial neuronal polarization. J Cell Biol 2008;180:619–32.

Wittmann T, Bokoch GM, Waterman-Storer CM. Regulation of microtubule destabilizing activity of Op18/stathmin downstream of Rac1. J Biol Chem 2004;279:6196–203.

Wittmann T, Waterman-Storer CM. Cell motility: can Rho GTPases and microtubules point the way? J Cell Sci 2001;114(Pt 21):3795–803.

Wittmann T, Waterman-Storer CM. Spatial regulation of CLASP affinity for microtubules by Rac1 and GSK3beta in migrating epithelial cells. J Cell Biol 2005;169:929–39.

Wloga D, Gaertig J. Post-translational modifications of microtubules. J Cell Sci 2010;123:3447–55.

Wozniak MJ, Bola B, Brownhill K, Yang Y-C, Levakova V, Allan VJ. Role of kinesin-1 and cytoplasmic dynein in endoplasmic reticulum movement in VERO cells. J Cell Sci 2009;122:1979–89.

Yaffe M, Stuurman N, Vale R. Mitochondrial positioning in fission yeast is driven by association with dynamic microtubules and mitotic spindle poles. Proc Natl Acad Sci U S A 2003;100:11424–8.

Yvon AM, Wadsworth P. Region-specific microtubule transport in motile cells. J Cell Biol 2000;151:1003–12.

Zhang D, Rogers GC, Buster DW, Sharp DJ. Three microtubule severing enzymes contribute to the Pacman-flux machinery that moves chromosomes. J Cell Biol 2007;177:231–42.

Zhang X, Tee YH, Heng JK, Zhu Y, Hu X, Margadant F, et al. Kinectin-mediated endoplasmic reticulum dynamics supports focal adhesion growth in the cellular lamella. J Cell Sci 2010;123:3901–12.