Trends in Cell Biology

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REVIEWS

Review

Coming into Focus: Mechanisms of Microtubule Minus-End Organization

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Microtubule organization has a crucial role in regulating cell architecture. The geometry of microtubule arrays strongly depends on the distribution of sites responsible for microtubule nucleation and minus-end attachment. In cycling animal cells, the centrosome often represents a dominant microtubule-organizing center (MTOC). However, even in cells with a radial microtubule system, many microtubules are not anchored at the centrosome, but are instead linked to the Golgi apparatus or other structures. Non-centrosomal microtubules predominate in many types of differentiated cell and in mitotic spindles. In this review, we discuss recent advances in understanding how the organization of centrosomal and non-centrosomal microtubule networks is controlled by proteins involved in microtubule nucleation and specific factors that recognize free microtubule minus ends and regulate their localization and dynamics.

Highlights

Microtubule minus-end organization in interphase and mitotic animal cells should be envisioned beyond the centrosome-centered view.

MTOC activity can be separated into a nucleating and an anchoring function, which require some common and distinct factors.

Microtubule minus-end organization in spindles depends on their nucleation, stabilization, and motor-based microtubule sliding.

The MTOC activities of the centrosome and the Golgi share common regulators and compete with each other.

An increasing number of regulatory proteins recognize free, uncapped microtubule minus ends and regulate their dynamics.

The interprotofilament interface is an important site for specific microtubule end recognition.

Microtubule-Organizing Centers

Microtubules are dynamic polymeric filaments, which drive chromosome separation during cell division, serve as rails for intracellular transport and organelle positioning, and regulate cell polarity and motility. They are formed by the addition of asymmetric building blocks (tubulin dimers) to their two ends: the fast-growing plus end and the slow-growing minus end (Figure I in Box 1). In cells, the plus ends represent the major sites of microtubule elongation, while the minus ends are often stabilized and anchored, and their distribution determines the overall geometry of the microtubule network in a given cell type.

The textbook view of microtubule organization in interphase animal cells, such as fibroblasts or cancer cells, is a radial, aster-like microtubule array, which is transformed into two asters that form the mitotic spindle during cell division. In this view, the **centrosome** (see Glossary), as the major MTOC, nucleates most microtubules and anchors their minus ends. However, even a casual inspection of the microtubule network in almost any interphase or mitotic cultured animal cell of an epithelial or mesenchymal origin shows that, in fact, microtubules, even when they form an aster, do not all converge in a single spot (Figure 1). This means that, while microtubule minus ends are concentrated in the central part of the cell or the spindle poles, they are not all attached to the centrosome, and thus additional mechanisms are responsible for their organization. Therefore, the centrosome-centered view represents a strong oversimplification of the actual microtubule arrangement. However, this view has strongly dominated the thinking about how microtubule arrays are formed and maintained, and, as a result, relatively little effort was invested, until recently, into investigating the dynamics and organization of microtubule minus ends in cultured animal cells. Studies of microtubule minus-end organization were also strongly hampered by the high microtubule density in the central part of the cell, making it difficult to discern individual microtubules. As a result, our knowledge of how microtubule minus ends are organized and how their dynamics is controlled is strongly underdeveloped compared with our current understanding of microtubule plus-end behavior [1].

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Recent studies showed that microtubule minus ends, similar to the plus ends, display interesting dynamics that is relevant for understanding their organization, and that they associate with different dedicated regulatory factors (reviewed in [1]). Advances in microscopy and the advent of new pharmacological and genetic tools made it easier to probe the organization and function of the centrosome and other MTOCs, thus providing a more balanced view of the relative importance of different factors controlling microtubule minus ends. In this review, we discuss the mechanisms responsible for organizing microtubule minus ends in cycling animal cells. We focus on the structures where microtubule minus ends are concentrated in such cells: the centrosomes, the Golgi apparatus, and the mitotic spindle poles.

Microtubule Nucleation and Anchoring at the Centrosome

The centrosome is a spherical structure comprising the mother and the daughter **centriole** surrounded by pericentriolar material (PCM) [2,3]. The major function of PCM is to concentrate proteins responsible for microtubule nucleation, outgrowth, stabilization, and anchoring. One of the main players in this process is the **γ-tubulin ring complex** (γ-TuRC), which is generally regarded as the template for microtubule nucleation and is recruited to the centrosome by PCM components. These include the coiled coil adaptor proteins pericentrin and AKAP450, as well as their interacting partners CDK5RAP2 (also known as centrosomin in flies) and its ortholog myomegalin (reviewed in [3]; Figure 2). All these proteins can interact with γ-TuRC, and some share specific motifs, such as centrosomin motif 1 (CM1), which is involved in binding to γ-TuRC ([4–7], reviewed in [8]). NEDD1 is another centrosomal γ-TuRC adaptor, which is structurally unrelated to those mentioned above [9,10] and which is recruited to the centrosomes by the PCM adaptor protein CEP192 (reviewed in [3]). Centrosomal γ-TuRC-binding proteins display some degree of redundancy, and their functional significance varies between species (reviewed in [2,8]). Importantly, although γ-TuRC can promote microtubule nucleation, in yeast, its activation requires conformational changes [11]. There are indications that also in mammalian cells γ-TuRC activity is regulated. For example, a fragment of CDK5RAP2 that contains the CM1 domain (γ-TUNA) appears to promote γ-TuRC activity [12]). A small protein,

Box 1. Microtubule Plus and Minus Ends and the Mechanisms of Microtubule End Recognition

Microtubules are built of dimers of α- and β-tubulin, which are attached in a head-to-tail fashion, resulting in a polarized structure, with α-tubulin protruding at the slow-growing minus end and β-tubulin at the fast-growing plus end (Figure I). Recognition of microtubule ends can be based on binding to tubulin surfaces, which are only exposed at the ends and occluded within the microtubule lattice due to longitudinal interactions between tubulin dimers. γ-TuRC uses this mechanism at the minus end, whereas the centriolar protein CPAP has a domain that uses this principle at the plus end [114]. All other structurally characterized microtubule end-binding proteins interact with the lateral microtubule surface. End Binding family (EB) proteins bind to growing plus and minus ends by recognizing the stabilizing cap [106]. The globular calponin homology (CH) domain of EBs binds between protofilaments, at the corners of four tubulin dimers, and recognizes a tubulin conformation associated with GTP hydrolysis [107]. TPX2 appears to show a preference for the GTP-bound state of the microtubule lattice; it contains two short sequence elements, 'ridge' and 'wedge', which bind across longitudinal and lateral tubulin interfaces [17]. The microtubule-binding domain of doublecortin binds to the same site as the CH domain of EB [108]. Doublecortin showed preference for microtubule ends *in vitro* [109] and was reported to be sensitive to microtubule bending and the spacing between adjacent tubulin dimers [110]. The globular C-terminal domain common to CAMSAP1, KIAA1078, and KIAA1543 (CKK) domain of CAMSAPs also binds between protofilaments, but at the site between two tubulin dimers, and, thus, is not sensitive to the GTP state of tubulin [111]. CKK shows highest affinity to a region located behind the outermost minus end, which corresponds to the transition between straight and curved protofilaments. The interprotofilament space at this transition region will have a different shape at the plus and minus end, and the current model suggests that it is this difference that is recognized by CKK [111].

Protofilament curvature at microtubule ends is proposed to be recognized by other MAPs preferentially associating with microtubule ends, such as NuMA, katanin, doublecortin, and kinesin-13, but direct structural data supporting this notion exist only for kinesin-13 ([115] and references therein). Finally, motile motor proteins can pile up at microtubule ends if their dissociation from the ends is slow due to the presence of microtubule-binding domains or additional cofactors.

Glossary

Centriole: a cylindrical cell structure containing nine symmetrically arranged triplets of stable microtubules, which is present within centrosomes and also serves as a basal body for cilia.
Centrosome: a globular structure comprising two centrioles and pericentriolar material, which organizes microtubules in many types of mammalian cell.
Cilium: a sensory or motile organelle extending from the surface of most mammalian cells; contains an array of nine symmetrically arranged microtubule doublets anchored at the basal body.
Dynein: a motor protein complex that uses ATP hydrolysis to move along microtubules towards their minus ends. Dynein transports a variety of intracellular cargos and can also slide microtubules. Cytoplasmic dynein participates in intracellular transport and microtubule organization, while ciliary dyneins are responsible for the movement of cilia.
γ-tubulin ring complex (γ-TURC): a ring-shaped protein complex of approximately 2 MDa that serves as a template for microtubule nucleation and as a microtubule minus-end cap. It comprises γ-tubulin molecules, which directly bind to microtubule minus ends and several additional proteins.
Intraflagellar transport: a process of transport of different macromolecules in and out of cilia and flagella.
Kinesin: a motor protein that uses ATP hydrolysis to move along microtubules. Kinesins participate in cargo transport and regulation of microtubule organization and dynamics. Many kinesins walk towards microtubule plus ends, while kinesin-14s move towards the minus ends, and kinesin-13s interact with microtubule ends and induce their disassembly.
Kinetochore: a protein structure on chromosomes where the spindle microtubules attach during cell division to pull sister chromatids apart.
Microtubule lattice: the structure formed by lateral association of 10 to 15 protofilaments, rows of α/β-tubulin heterodimers, that together form a hollow tube.

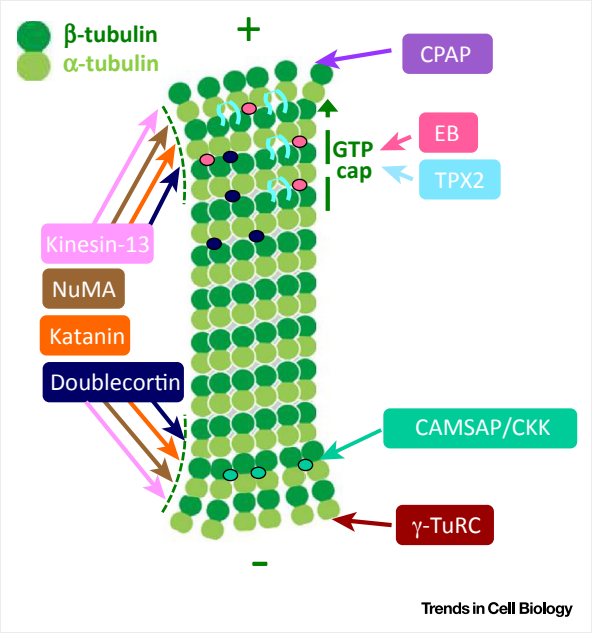

Figure I. Schematic Representation of the Microtubule Structure and the Position of the Binding Sites for Proteins Interacting with Microtubule Ends. The domains binding to the interprotofilament interface are illustrated by different symbols, while the binding sites for other proteins are shown by arrows. The broken lines represent the curvature of the microtubule protofilaments at the ends, whereas the broken arrow indicates the GTP-cap region of growing microtubule plus ends. Abbreviations: γ-TuRC, γ-tubulin ring complex; CKK, C-terminal domain common to CAMSAP1, KIAA1078, and KIAA1543; EB, End Binding.

Ran-GTP (Ras-related nuclear protein): a small GTPase involved in transport into and out of the cell nucleus during interphase; also participates in the organization of the cell division apparatus.

MOZART1, interacts with several γ-TuRC components and might also participate in γ-TuRC activation directly or indirectly [13,14].

Microtubule nucleation sites in cells not only recruit γ-TuRC, but also concentrate factors promoting microtubule growth. Microtubule outgrowth from a template, be it a γ-TuRC or a piece of a pre-existing microtubule, is distinct from the elongation of an already growing microtubule end, because it requires a higher tubulin concentration *in vitro* [15]. Templated microtubule nucleation is stimulated by factors promoting microtubule elongation and stability [15]. These include the microtubule polymerase XMAP215/chTOG, which accelerates tubulin dimer addition to microtubule plus ends [16], and mitotic spindle assembly factor TPX2, which contains separate microtubule protofilament cross-bridging and γ-TUNA-related domains [17,18]. Interestingly, when present in the same *in vitro* assay, chTOG and TPX2 can induce microtubule nucleation in the absence of any templates [19]. Furthermore, *in vitro* experiments using crowding agents showed that a combination of several centrosomal components from *Caenorhabditis elegans*, including a self-assembling scaffold SPD-5 together with the worm orthologs of chTOG and TPX2, can form phase-separated condensates that concentrate tubulin dimers and promote template-independent nucleation of microtubule asters [20]. Whether such a mechanism could operate *in vivo*, particularly in human cells, is currently unknown. The potential relevance of this mechanism is supported by the observation that, in some systems, γ-TuRC depletion does not completely block microtubule nucleation and sometimes can even have surprisingly mild effects (reviewed in [21]). For example, mitotic centrosomal asters still assembled in worm embryos in which γ-tubulin function was strongly compromised [22]. Interestingly, this assembly process did not require the worm homolog of

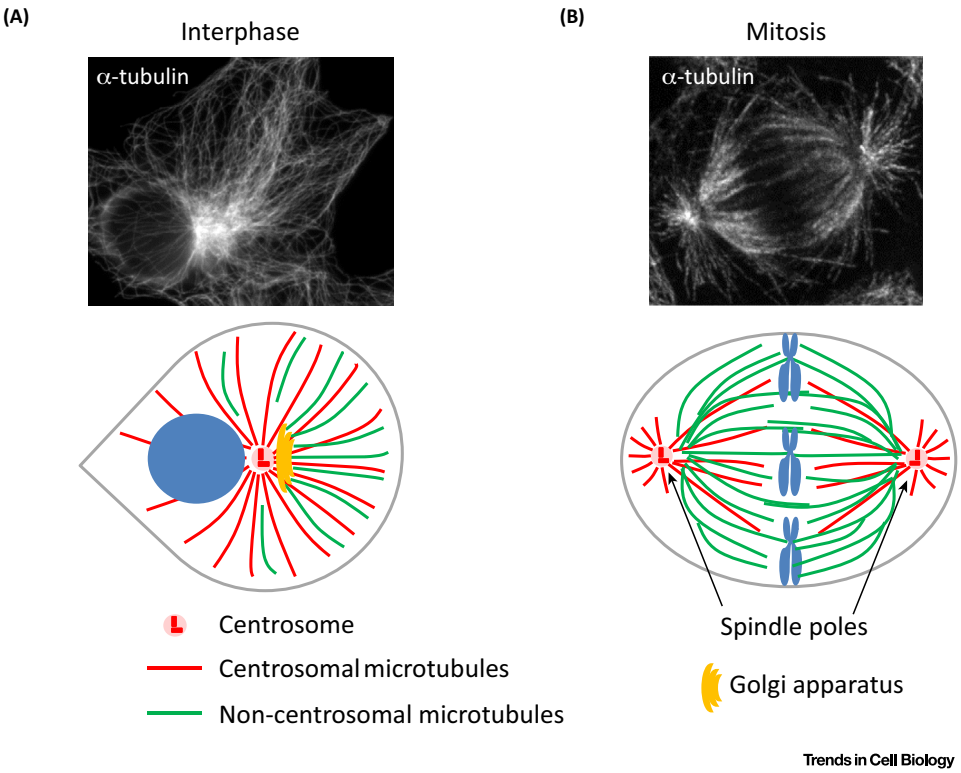

Figure 1. Microtubule Organization during Interphase and Mitosis. In addition to the radial centrosome-anchored microtubule network (red), most cultured animal cells contain microtubules that do not emanate from the centrosome (green). During interphase [(A) RPE1 cell], a large fraction of non-centrosomal microtubules is enriched at the Golgi apparatus and helps to support polarity. In cells undergoing mitosis [(B) HeLa cell], non-centrosomal microtubules are distributed throughout the spindle, converge at the spindle poles, and increase microtubule density in the spindle to allow efficient chromosome capture and separation. Reproduced from [55] (A) and [88] (B).

chTOG [22] and, therefore, the involvement of this microtubule polymerase in microtubule nucleation in cells needs to be examined further.

Microtubule nucleation is only one part of the microtubule-organizing function of the centrosome: to form an aster, microtubule minus ends must also be stabilized and anchored at the centrosome. The biochemical activities responsible for this function are still obscure, but γ-TuRC is likely to have a role. In fission yeast, the presence of γ-TuRC at microtubule minus ends promotes their stability even when the γ-TuRC adaptor required for microtubule nucleation is mutated [23]. In mammalian cells, the presence of γ-TuRC is by itself insufficient for microtubule minus-end anchoring: for example, γ-TUNA from CDK5RAP2 can recruit γ-TuRC to ectopic sites and nucleate microtubules, but cannot anchor them in the absence of NEDD1 [24]. Thus, NEDD1 represents an interesting candidate for a factor anchoring γ-TuRC-associated microtubules. Another potential candidate is ninein, a coiled coil protein that might bind to γ-TuRC directly or indirectly and is concentrated at the so-called 'subdistal appendages', structures extending from the surface of the mother centriole [25]. Whether subdistal appendages or the PCM itself are responsible for microtubule anchoring is not clear and might differ between cell types, because the protein ODF2 (also known as cenexin) is essential for appendage formation but not for centrosomal microtubule organization [26]. Ninein and its invertebrate counterparts can also cooperate with γ-TuRC to organize non-centrosomal

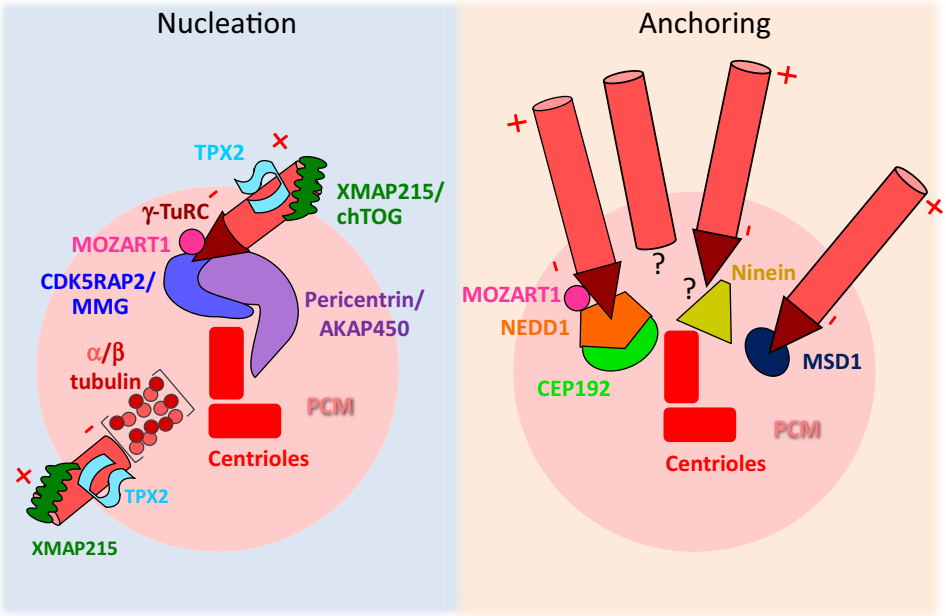

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Figure 2. Microtubule Organization at the Centrosome. Microtubule organization at the centrosome requires nucleating (A) and anchoring and/or stabilizing (B) activities. The γ-tubulin ring complex (γ-TuRC), the template of microtubule nucleation, is recruited and regulated by various centrosomal adaptors. Among them are pericentrin, AKAP450, and CDK5RAP2 and its ortholog myomegalin (MMG). CDK5RAP2 and a small γ-TuRC-binding protein MOZART1 have been implicated in γ-TuRC activation. It has also been proposed that microtubule nucleation at centrosomes can occur without a template, due to concentration of tubulin dimers. The microtubule-binding protein TPX2 and the microtubule polymerase XMAP215 (chTOG in mammals) stimulate microtubule nucleation and elongation. The anchoring function associated with the centrosome appears to be separate, at least to some extent, from microtubule nucleation: for example, the γ-TuRC-binding protein NEDD1 is dispensable for nucleating microtubules, but is required for their centrosomal tethering. CEP192, another pericentriolar material (PCM) protein important for γ-TURC recruitment, binds to NEDD1. Ninein and MSD1 were also shown to participate in microtubule attachment at centrosomes; whether ninein can directly bind to γ-TURC or anchors microtubules due to its microtubule lattice affinity requires further elucidation (question mark). Whether all centrosomal microtubule minus are capped by γ-TuRC is also unclear.

microtubule minus ends in differentiated cells, including neurons [27] and cortical microtubule arrays in epithelia [28,29].

Microtubule anchoring at the centrosome provides a positive feedback loop: minus-end-directed motor cytoplasmic **dynein** uses centrosome-attached microtubules to concentrate microtubule-anchoring factors at the centrosome. Some of these, such as ninein, bind to dynein directly [30], while others are part of centriolar satellites, cytoplasmic granules that mediate protein delivery to centrosomes and **cilia** and include components that are involved in microtubule minus-end attachment, such as the centriolar satellite component Msd1/SSX2IP (reviewed in [31]). Depletion of centriolar satellite components as well as dynein affects centrosomal microtubule anchoring, but the direct effects related to microtubule binding are not easy to separate from indirect ones, which are related to insufficient PCM accumulation at the centrosome.

Due to the large number of microtubule-associated proteins (MAPs) present at the centrosome, it is possible that centrosomal microtubule minus-end tethering depends on multiple weak

interactions. From this point of view, PCM clustering is by itself an important factor. It is driven by the oligomerization of centrosomal scaffolds, such as SPD-5 or centrosomin [20,32–34], and could be regarded as centriole-catalyzed phase separation [35]. During interphase, the assembly of PCM is triggered around the centriole wall and proceeds in a highly orderly manner, while an expanded and more amorphous PCM structure is formed during mitosis [36–38]. Interestingly, PCM can also self-assemble without centrioles: for example, acentriolar MTOCs drive microtubule organization during meiosis and early development in some animal species [39,40], and formation of acentriolar MTOCs can be triggered by phosphorylation and increased self-association of certain PCM components [33]. When centriole duplication is blocked and centrioles are lost (Box 2), a single PCM cluster, albeit an abnormally shaped one, can still form and support radial microtubule organization, provided that other pathways of microtubule minus-end stabilization are inactivated [6]. Thus, the centriole pair appears to serve not as a completely essential component of centrosome formation but rather as a catalyst of PCM assembly, enabling the centrosome to outcompete other pathways of microtubule minus-end organization. When animal cells differentiate, in many cases such as epithelial, muscle or neuronal cells, the centrosome is inactivated and cells acquire non-centrosomal microtubule systems with different geometries. Such cells often employ and amplify the pathways of non-centrosomal microtubule formation and stabilization that already exist in cycling cells, and also switch on specific factors, which have been reviewed in detail recently [41,42].

Golgi Apparatus as a Major Alternative MTOC in Mammalian Cells

Genetic studies have shown that the presence of centrioles and centriole-organized centrosomes is not strictly required for many aspects of animal development, although centrioles are essential for the formation of cilia and all cilia-dependent processes. Flies lacking centrioles develop relatively normally but die due to the lack of cilia [43], and mouse embryos lacking an essential microtubule-binding protein involved in centriole duplication, CPAP, survive until midgestation [44]. In mammalian interphase cells, the major alternative MTOC responsible for the formation of a radial microtubule array is the Golgi apparatus (reviewed in [45,46]). Golgi membranes can both nucleate and anchor microtubules. Importantly, Golgi-derived microtubule networks are asymmetric and have optimal organization to control secretory trafficking to and from the Golgi apparatus; for these reasons, Golgi-derived microtubules are important for cell polarity and migration in different settings [6,7,47,48].

Box 2. Experimental Approaches to Eliminate the Centrosome

Given the prominent role of the centrosome as a major MTOC, many research efforts have focused on centrosome removal. The first group of approaches relies on the physical elimination of the centrosome, for example, by using laser irradiation of the centrosomal area in early experiments [116], which suggested that the centrosome is important for cell motility. Later, precise ablation of fluorescently labeled centrosome by laser microsurgery was used to show that cells in which centrosomes were destroyed in prophase can still form a bipolar spindle, but the poles do not contain centrioles and are not enriched in γ-tubulin [117]. Centrosome ablation was also used to study the relative importance of the centrosome and the Golgi in microtubule organization during interphase [47,118]. The inherent property of this method is that it acutely reduces the pool of microtubule-nucleating and stabilizing factors, which are bound to the centrosome.

Another set of approaches makes use of the fact that centrosomes are assembled around centrioles and, thus, blocking centriole duplication inhibits centrosome formation, without eliminating pericentriolar material (PCM) components. Centriole duplication can be prevented by mutating essential centriole proteins, such as, for example, CPAP/SAS-4, which is essential for the assembly of centriole microtubules. These experiments demonstrated that many steps of animal development do not require centrosomes [43,44]. In addition to knockout of structural centriole proteins, centriole duplication can also be inhibited by eliminating the activity of Plk4, the kinase that initiates centriole duplication. Recently, a specific cell permeable Plk4 inhibitor, centrinone, was developed [72] that represents a powerful tool to study the importance of centrosome in different processes without the need for complex genetic manipulations.

The pathways responsible for organizing Golgi-derived microtubules display similarities and differences to the centrosomal ones. The major player in microtubule organization at the Golgi is a large scaffolding protein AKAP450, which is also present at the centrosome but is not essential for centrosome function (Figure 3). This protein is targeted to *cis*-Golgi through an interaction with the Golgi matrix protein GM130 [48]; IFT20, a protein which is located at the Golgi and is also involved in **intraflagellar transport**, might also participate in the formation of the GM130-AKAP450 complex [49]. AKAP450 mediates microtubule nucleation from the Golgi by directly binding γ-TuRC and by recruiting CDK5RAP2 and myomegalin (Figure 3); however, only AKAP450 itself is essential for this process [6,7,50,51]. Interestingly, microtubule nucleation sites at the Golgi are distributed unevenly, possibly because of clustering or local activation of γ-TuRC-binding proteins [52].

To maintain stable microtubule minus ends at the Golgi, another factor is essential, a microtubule minus-end-binding protein CAMSAP2. CAMSAP2 is a member of a protein family that contains three homologs in mammals (CAMSAP1, CAMSAP2, and CAMSAP3, also known as Nezha) and a single homolog, Patronin, in invertebrates. These proteins associate with and decorate growing, uncapped microtubule minus ends, thus forming stretches of stabilized **microtubule lattice** [53,54]. In mammalian cells, CAMSAPs are not localized to the centrosomes, and their recruitment to the centrosome vicinity correlates with microtubule release from the centrosome [53]. CAMSAP-decorated microtubule minus ends are laterally attached to Golgi membranes by binding to the complex of AKAP450 and myomegalin [6] (Figure 3). However, this connection is not sufficient, because a second link between microtubule minus end and Golgi membranes is provided by unexpected players: the members of the End Binding family, EB1 and EB3. These proteins bind with high affinity to freshly polymerized plus and minus ends (Box 1) and also show weaker interactions with microtubule shafts. EB1 and EB3

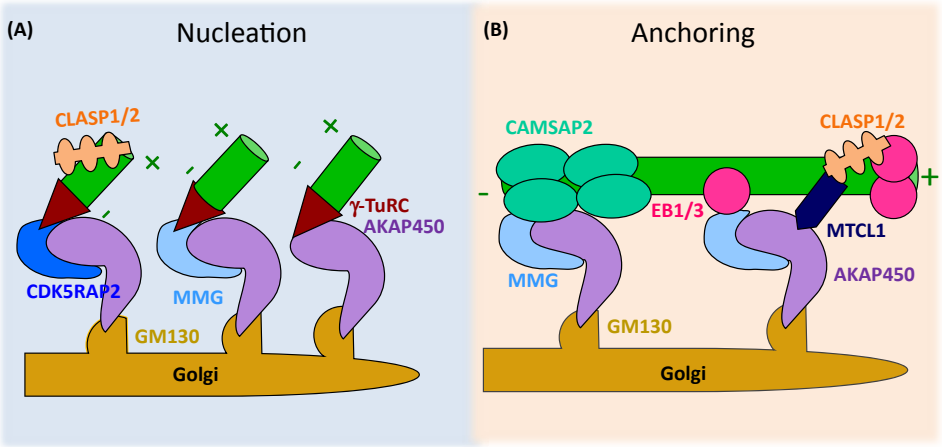

Figure 3. Microtubule Organization at the Golgi Apparatus. The scaffold protein AKAP450, which is recruited to *cis*-Golgi by GM130, has a central role in both the nucleation (A) and anchoring (B) of Golgi-derived microtubules. It targets γ-tubulin ring complex (γ-TuRC) to the Golgi directly and also recruits two other γ-TuRC-binding factors: CDK5RAP2 and myomegalin (MMG). Together with MMG, AKAP450 also participates in the Golgi anchoring of free microtubule minus ends stabilized by CAMSAP2. MMG is required for an additional pathway responsible for tethering of CAMSAP2-decorated microtubules to the Golgi through recruitment of EB1 and EB3. Other microtubule-binding proteins, such as CLASP1/2 and MTCL1, contribute to microtubule nucleation and/or anchoring at the Golgi. The recruitment of CLASPs to the Golgi membranes by the *trans*-Golgi protein GCC185 contributes to Golgi microtubule organization (not depicted because *trans*-Golgi is not included in the scheme).

directly bind to myomegalin [7,51], and the complex of myomegalin with EBs provides a second essential link between the Golgi and microtubule minus ends [55] (Figure 3). Importantly, microtubule nucleation from the Golgi in nocodazole washout assays is completely normal in cells lacking myomegalin, CAMSAP2, or EB1/3 [6,55]. However, in the absence of myomegalin or EB1 and EB3, non-centrosomal CAMSAP2-stabilized microtubule minus ends are detached from the Golgi and, in the absence of CAMSAPs, most non-centrosomal microtubules, including Golgi-derived ones, are absent [53,56]. These data demonstrate that microtubule organization at the Golgi depends on two molecularly and functionally distinct steps: nucleation and tethering. These data also illustrate that assays investigating microtubule recovery after depolymerization, which are easy and popular in the field, are insufficient to study processes responsible for microtubule organization and can provide a skewed picture when not accompanied by studies of unperturbed cells.

Several additional MAPs contribute to microtubule organization at the Golgi. These include microtubule stabilization and rescue factors named CLASPs, which promote microtubule nucleation at the Golgi [47] and regulate the formation or stability of CAMSAP2-decorated microtubule stretches [6]. CLASPs can be anchored at the Golgi directly, by binding to the *trans*-Golgi-associated protein GCC185 [47], but this connection does not appear to be essential to link microtubule minus ends to the Golgi [6]. Another MAP, MTCL1, which binds to AKAP450 and CLASPs, also contributes to microtubule tethering to the Golgi [57]. The cooperation of multiple MAPs in microtubule organization at the Golgi suggests that this function requires multiple weak interactions between Golgi membranes and microtubules. Furthermore, similar to centrosomal microtubule asters, the organization of Golgi-derived microtubules strongly depends on cytoplasmic dynein, which promotes the clustering of Golgi membranes that in turn use Golgi-anchored microtubules for efficient self-assembly [58].

The centrosomal and Golgi dependent pathways of microtubule organization compete with each other: the depletion of AKAP450, which completely abrogates the formation of Golgi-derived microtubules, was suggested to increase γ-TuRC accumulation at the centrosome [59]. Conversely, when centrosomes are depleted, γ-TuRC and pericentrin relocalize to the Golgi in an AKAP450-dependent manner, and Golgi-dependent microtubule nucleation is increased [6,59]. The latter experiments might reflect what happens in differentiated cells, in which the centrosome is inactivated whereas Golgi maintains its microtubule-organizing functions. For example, the Golgi apparatus, together with the nuclear envelope, is responsible for microtubule organization in muscle cells [60] and, interestingly, recent work demonstrated the crucial role of AKAP450 in microtubule minus-end organization by nuclei in muscle fibers [61]. Furthermore, Golgi-derived microtubules dependent on the orthologs of AKAP450 and CDK5RAP2 were observed in fly neurons [62,63]. However, experimentally induced repositioning of Golgi membranes did not affect the localization of γ-tubulin in fly neurons, suggesting that the Golgi is not an important site of neuronal microtubule nucleation in these cells [64]. Furthermore, CAMSAPs and Patronin are required for the formation of non-centrosomal neuronal microtubule networks as well as for cortical microtubule minus-end attachment in epithelia and fly embryos (reviewed in [42]). These data illustrate that different microtubule geometries can be generated by recruiting the same factors to different cellular locations.

Microtubule Minus-End Organization in Spindles

When cells enter mitosis, many interphase MAPs are inactivated [65]. By contrast, mitotic MAPs become available for the interaction with microtubules due to nuclear envelope breakdown, and their activity can also be regulated by **Ran-GTP**, which releases these MAPs from nuclear import receptors [66]. At the onset of mitosis, PCM recruitment to the centrosome is

dramatically increased [3], while microtubule nucleation from the Golgi is strongly reduced [67]. However, Golgi membranes still appear to have a role in microtubule formation by controlling the activity of the spindle assembly factor TPX2 through GM130 [68]. Mitotic spindle assembly is a highly complex process, which has been reviewed recently [69,70]; therefore, here we focus only on recent work directly related to the regulation of the dynamics and positioning of microtubule minus ends.

During cell division, the organization of the minus ends depends not only on the localization of the sites of microtubule nucleation and stabilization, but also on motor-based microtubule sliding. When present, centrosomes typically have a dominant role in spindle formation as the major microtubule nucleation sites. However, cell division can proceed in the absence of centrosomes. In flies, centrioles are essential for the first rounds of cell divisions, but are dispensable for the rest of development [43]; removing centrosomes (Box 2) does not prevent the formation of bipolar spindles, although it does slow down cell division and makes it less precise [43,71,72]. Spindle microtubules can be nucleated by different centrosome-independent pathways, which can involve acentriolar MTOCs (e.g., in meiosis), as well as chromosome arms and **kinetochores**. Some players responsible for centrosomal microtubule nucleation, such as γ-TuRC and NEDD1 [73], and Ran-GTP-controlled spindle assembly factors, such as TPX2, participate in chromosome-dependent microtubule assembly (reviewed in [69,70]). Another important pathway of spindle microtubule generation depends on microtubule nucleation from the sides of other microtubules by the eight-subunit complex augmin (HAUS in human cells). This complex nucleates microtubule 'branches' that emanate from pre-existing microtubules at shallow angles, thus amplifying parallel microtubule arrays, and requires TPX2 and γ-TuRC for its activity (see [18] and references therein). Motor proteins organize microtubules nucleated through different pathways into a spindle with two poles, where minus ends are clustered [69,70] (Figure 4). However, a significant proportion of the minus ends is distributed throughout the spindle [74–76].

Microtubule minus-end dynamics within spindles is fundamentally different from interphase asters, because spindle microtubules are not stably anchored but rather slowly translocate towards spindle poles through a motor-dependent process and disassemble at their minus ends at the poles (a process termed 'poleward spindle flux'). The speed and functional importance of the spindle flux vary between cell types (reviewed in [77]). The centrosomes are physically linked to spindle poles, but this coupling can be perturbed by the loss of different pole proteins, resulting in centrosome detachment, which can compromise spindle integrity and endanger proper centrosome inheritance (reviewed in [78]).

Microtubule minus ends can converge at spindle poles due to activities of the minus-end-directed motors, cytoplasmic dynein and **kinesin**-14 family members, such as the human KIFC1/HSET, with the relative importance of these motors varying between different cell types (reviewed in [79–81]; Figure 4). Kinesin-14 can focus minus ends autonomously [82], while dynein typically cooperates with different cofactors, such as the multisubunit complex dynactin and a large microtubule-binding protein, NuMA, which can regulate its processivity and microtubule binding ([83,84] and references therein). Another microtubule-binding protein strongly involved in spindle pole organization is ASPM (ASP in flies). In *Drosophila*, ASP is required for spindle pole focusing and centrosome attachment to the pole ([85–87] and references therein). In mammalian cells, ASPM is not essential for spindle pole organization, but participates in regulating minus-end dynamics and spindle flux together with the microtubule-severing protein katanin [88]. ASPM function in spindle pole focusing is redundant with that of CDK5RAP2 [89]. CDK5RAP2 also promotes the attachment between spindle poles and centrosomes through an interaction with

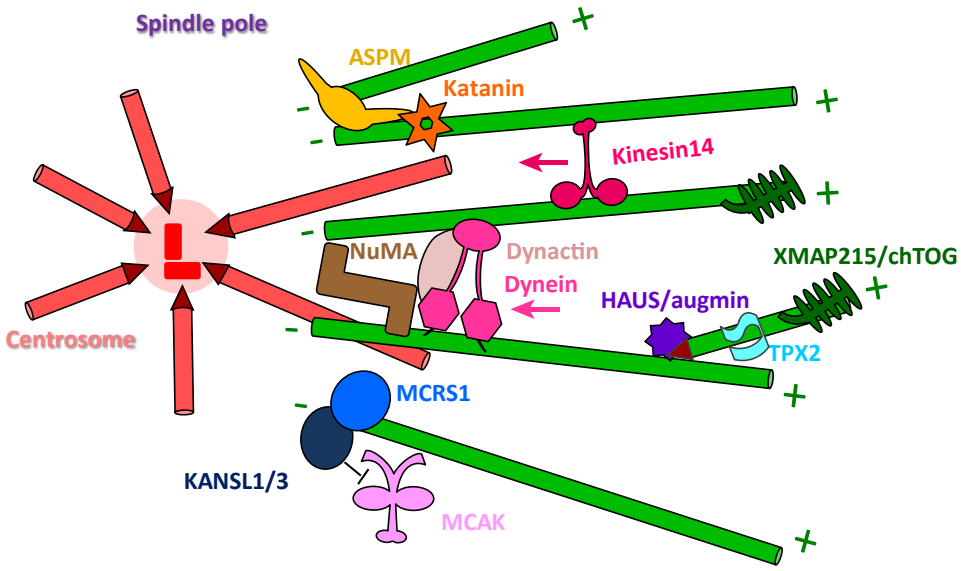

Figure 4. Microtubule Organization at Mitotic Spindle Poles. During mitosis, the organization of non-centrosomal microtubule minus ends is crucial for the formation of a functional spindle. The spindle poles represent the sites where spindle microtubule minus ends are focused and bundled, and where the proteins controlling microtubule minus-end dynamics are concentrated. Microtubules are nucleated at the centrosomes and throughout the spindle; the latter function depends on augmin, which promotes γ-TuRC- and TPX2-dependent microtubule nucleation from the lateral surfaces of other microtubules. XMAP215/chTOG also strongly contributes to microtubule formation in the spindle. ASPM is an autonomous microtubule minus-end-binding protein that slows down minus-end growth and interacts with the microtubule-severing protein katanin to regulate spindle flux. Through its microtubule lattice binding, ASPM can also participate in microtubule cross-linking at the poles. Minus-end-directed microtubule motor cytoplasmic dynein together with its cofactor dynactin is important for organizing the poles. It cooperates with NuMA, which has microtubule crosslinking activity and can be recruited to microtubule minus ends in a dynein-independent manner. Another important minus-end-directed motor involved in focusing microtubules at the poles is kinesin-14 (HSET). The microtubule minus-end-binding proteins KANSL1 and -3 interact with MCRS1 to protect microtubule minus ends from kinesin-13 (MCAK)-mediated depolymerization.

HSET [90], but since no defects in pole focusing were found in cells lacking both ASPM and HSET [89], CDK5RAP2 must also have additional roles in spindle pole organization. ASPM can bind autonomously to microtubule minus ends and lattices [86,88] and could participate in crosslinking microtubules at the pole directly. ASPM is also required for spindle pole localization of NuMA and dynein homologs in invertebrates [87,91], although not in mammals [88], and, thus, can promote dynein-mediated spindle pole assembly.

The members of the CAMSAP/Patronin family also differ between species with respect to their importance in cell division. In flies, Patronin inhibits minus-end disassembly at the poles and controls spindle length and symmetry by antagonizing the activity of microtubule depolymerase kinesin-13 [92–94]. By contrast, in mammals, CAMSAP2 and CAMSAP3 are removed from microtubules in mitosis by phosphorylation [53,65], whereas CAMSAP1, which dynamically tracks microtubule minus ends, has only a mild effect on the spindle length [84]. In vertebrates, the minus ends of kinetochore-attached microtubules are regulated by the proteins KANSL1/KANSL3, which in interphase are part of a chromatin regulator complex, and their binding partner MCRS1, which, similar to fly Patronin, can counteract the kinesin-13 depolymerase MCAK [95,96] (Figure 4). Interestingly, MCRS1 can also participate in crosstalk with the dynein pathway and PCM recruitment to the centrosome [97].

In addition to the factors mentioned above, multiple MAPs present at spindle poles can help to preserve their integrity and withstand forces exerted on the poles during chromosome alignment and separation (reviewed in [81]). Perturbation of various MAPs, including CLASPs and ninein [98], or centrosome abnormalities, such as premature disengagement of the mother and daughter centrioles, can cause pole splitting and lead to cell division defects [81]. Furthermore, although centrosomes are not essential for spindle assembly, they are required for the formation of astral microtubules, which, in turn, control spindle positioning (reviewed in [99]). Interestingly, genetic defects affecting many of the factors discussed above lead to microcephaly, a developmental disorder associated with small brain and intellectual disability [100]. Although the molecular mechanisms underlying this disorder are still unclear, mitotic delays and spindle positioning defects associated with centrosome and spindle pole abnormalities could be a contributing factor and, therefore, understanding the molecular basis of spindle pole formation might help to shed light on human brain development.

Molecular Mechanisms of Microtubule Minus-End Recognition and Regulation

Mechanisms of microtubule minus-end organization critically depend on proteins that can specifically recognize these ends (Box 1). The most conceptually simple mechanism of minus-end binding is its capping by γ-TuRC [101]. Both the nucleating and minus-end stabilizing activities of γ-TuRC are thought to be modulated by its various binding partners, but whether any of them have some affinity for minus ends is unknown. The fly ninein-related protein was shown to bind to microtubules *in vitro* and the same might be the case for the worm homolog [28,102]; however, no minus-end preference has been demonstrated for these proteins. Ninein also forms a triple complex with dynein and dynactin and serves as a dynein activator [30], but whether this activity directly relates to its microtubule-anchoring function is unclear. Augmin complex and its microtubule-binding ability have been reconstituted *in vitro* [103], and it is known that it binds along microtubules and also interacts with γ-TuRC by binding to NEDD1 [104,105], but it is still unclear how all these proteins cooperate for microtubule nucleation. TPX2 is an important catalyst of microtubule nucleation: this MAP can not only bind along microtubules, but also preferentially accumulate at their ends [19]. The mechanism of interaction between TPX2 and microtubules involves two small domains that can bind across longitudinal and lateral tubulin interfaces between protofilaments [17]. Interprotofilament interface is also recognized by two other protein domains, the calponin homology (CH) domain of EB1 and its homologs [106,107] and by the microtubule-binding domain of doublecortin [108]. The former recognizes polymerizing microtubule plus and minus ends (Box 1), while the latter binds along microtubule shafts in cells but can display microtubule end preference *in vitro* [109,110].

CAMSAPs and Patronin family members autonomously recognize microtubule minus ends due to the presence of a highly conserved domain, C-terminal domain common to CAMSAP1, KIAA1078, and KIAA1543 (CKK) [53,111]. This globular domain binds at the interprotofilament interface at a site distinct from that of EB1 and doublecortin (Box 1); the preference of CKK for microtubule minus ends is likely based on specific recognition of the microtubule region where the straight microtubule lattice switches into outwardly curved protofilament structures. The CKK domain alone dynamically tracks growing but not depolymerizing microtubule ends [111]. The ability of full-length CAMSAP2 and CAMSAP3 to decorate and stabilize free, growing microtubule minus ends depends on additional microtubule-binding regions, which by themselves show no preference for the minus ends but are likely to stabilize and enhance the microtubule interaction initiated by the CKK [53,111]. Since CAMSAPs bind to the lateral surface of microtubules, they do not cap them and, in fact, microtubule minus-end

polymerization is a prerequisite for the efficient CAMSAP minus-end loading, which is thus incompatible with γ-TuRC-mediated end capping. Another autonomous microtubule minus end-binding protein is KANSL3, which participates in kinetochore fiber stabilization [95], but the underlying biochemical mechanism still needs to be dissected. KANSL3 possibly acts by controlling minus-end recruitment of stabilizing MAPs, MCRS1 and TPX2, which by themselves do not show a minus-end preference [95,96].

ASPM, as well as its fly and worm homologs, can autonomously track free microtubule minus ends in a manner dependent on the conserved CH domains [88]. Human ASPM directly binds to the microtubule-severing enzyme katanin [88], a heterodimer comprising the catalytic subunit p60, which can form a hexameric ring and carry out microtubule severing, and a regulatory subunit p80. The binding between p60 and p80 depends on formation of a bundle of α-helices, which has its own affinity for microtubules and can specifically accumulate at polymerizing microtubule plus and minus ends [88]. The end-recognition mechanism of this katanin domain likely depends on protofilament curvature, because katanin can bend and break growing microtubule ends in an ATPase-independent manner. Together, ASPM and katanin form a complex that can block microtubule minus-end growth and sever microtubule lattices [88]. These processes, together with the microtubule-bundling activity of ASPM [86], likely contribute to the regulation of microtubule minus-end dynamics at the pole. Interestingly, in interphase cells, katanin interacts with CAMSAP2 and CAMSAP3 and limits the length of CAMSAP2/3-stabilized microtubule segments [53]. Strikingly, the interaction modes of katanin with CAMSAP and ASPM are similar [112], suggesting that katanin cooperates with these two microtubule minus-end regulators through similar mechanisms.

Minus-end-directed microtubule motors, dynein and kinesin-14s, can in principle accumulate at the minus ends if they detach slowly upon reaching the end, and this feature was recently demonstrated *in vitro* for active dynein–dynactin complexes [83]; these motors can also concentrate other factors at the minus ends. It has long been assumed that NuMA accumulates at microtubule minus ends due to dynein-based transport; however, recent work showed that this assumption is incorrect: NuMA rapidly binds to freshly generated minus ends in the spindle, and this process does not require the activities of dynein, γ-TuRC, or other known autonomous minus end-binding spindle MAPs [84]. A purified fragment of NuMA can bind to both plus and minus ends [113], but the underlying mechanism, as well as the origin of minus-end specificity of NuMA within the spindle, still need to be deciphered. Altogether, it is becoming increasingly clear that multiple proteins can autonomously recognize one or both microtubule ends to control their dynamics and subcellular localization.

Concluding Remarks and Future Perspectives

While the known complexity of microtubule plus end-binding proteins resembles a jungle [1], the realm of microtubule minus-end regulators for a long time looked like a desert dominated by one major player, γ-TuRC. This situation is now rapidly changing, with an increasing number of specific microtubule minus end-binding proteins and their partners being discovered. These studies provide molecular tools to investigate and control microtubule organization from the minus end. Improved microscopy techniques and optical manipulation approaches allow studying individual microtubule ends within dense cytoskeletal networks. Sophisticated *in vitro* reconstitution assays with multiple purified proteins allow dissection of complex systems that might rely on weak interactions and phase separation, such as the centrosome. Finally, improved structural approaches provide insights into the mechanisms responsible for the recognition of short-lived conformations of microtubule plus and minus ends. The combination of these approaches will make it feasible to address many questions on microtubule minus-end

Outstanding Questions

What is the relative importance of γ-tubulin-dependent and -independent microtubule nucleation pathways in different systems? Can local concentration of tubulin dimers in the absence of nucleation templates be sufficient for microtubule nucleation in cells?

How many types of microtubule minus-end-binding proteins are present in different cells and what are the mechanisms underlying minus-end recognition by different protein families?

Are the protection against kinesin-13 depolymerases and cooperation with severing enzymes general mechanisms of action of different minus end-stabilizing proteins?

What is the proportion of free and γ-TuRC-capped microtubule minus ends in different systems and what is their relative importance in controlling the architecture of microtubule networks?

What are the biochemical mechanisms of microtubule minus-end anchoring at the centrosome? Do the proteins responsible for this function stabilize the link between the γ-tubulin ring complex and microtubule minus end?

Are there additional MTOC types to be discovered? What determines the relative activity of different MTOCs, such as the centrosome and the Golgi, in different cell types?

organization and function (see Outstanding Questions). These studies will help to achieve a thorough and quantitative molecular description of cytoskeletal networks governing cell architecture in health and disease.

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