JCB Journal of Cell Biology

REVIEW

Slow but steady: Molecular mechanisms controlling microtubule growth in centrioles and cilia

Saishree S. Iyer[1] and Anna Akhmanova[1]

Microtubules are cytoskeletal filaments that form dynamic cytoplasmic arrays and stable cores of centrioles and cilia. Microtubule dynamics depend on GTP hydrolysis by tubulin: addition of GTP-tubulin creates a stabilizing cap, whereas cap loss triggers depolymerization. In structures with very slowly growing microtubules, such as centrioles and cilia, long GTP caps cannot form; instead, specialized protein complexes stabilize microtubule plus ends and support their slow elongation. Recent studies showed that although centrioles and cilia use distinct proteins to control their microtubule plus ends, the underlying mechanisms are similar. Both rely on complexes combining microtubule growth inhibitors with polymerases that counteract inhibition, which jointly stabilize the plus ends and drive their gradual extension. Both centriolar and ciliary microtubule tip regulators form assemblies that span inner and outer microtubule surfaces and reduce protofilament peeling. Because many centriole and ciliary tip proteins are mutated in human disorders, including microcephaly and ciliopathies, these findings provide insight into the molecular basis of such diseases.

Introduction

Microtubules are dynamic cytoskeletal polymers required for intracellular transport and organelle positioning, cell polarity, motility, and division (Desai and Mitchison, 1997; Goodson and Jonasson, 2018) (Fig. 1 A). Microtubules also form the stable cores of centrioles and cilia. Centrioles are cylindrical organelles with a wall composed of nine radially arranged microtubule triplets (Banterle and Gönczy, 2017; Loncarek and Bettencourt-Dias, 2018) (Fig. 1 B). Centrioles serve as a scaffold for microtubule-organizing centers, centrosomes, or as basal bodies for cilia, elongated structures that protrude from the cell surface and have motile, sensory, or signaling functions (see Mill et al. [2023]; Zhu [2025] for recent reviews). Ciliary cores, axonemes, are composed of microtubule doublets that extend from the distal centriole end and are arranged in a ninefold radial symmetry; furthermore, motile cilia also contain two centrally positioned microtubules, called the central pair (Klena and Pigino, 2022) (Fig. 1 C).

The dynamic instability of microtubules (Mitchison and Kirschner, 1984) depends on GTP hydrolysis by tubulin: the addition of GTP-tubulin leads to the formation of a stabilizing GTP cap that promotes growth, whereas GTP hydrolysis within the microtubule lattice destabilizes the structure, and the loss of the GTP cap triggers rapid depolymerization (Carlier and Pantaloni, 1981; David-Pfeuty et al., 1977; Desai and Mitchison, 1997). The exact length of the GTP cap and its effects on microtubule growth and stability have been a matter of extensive studies, discussion, and modeling (reviewed in Brouhard and Sept [2012]; Cleary and Hancock [2021]; Farmer and Zanic [2023]). The discovery that end-binding (EB) proteins report on the length of the GTP cap (Maurer et al., 2012; Roostalu et al., 2020; Zhang et al., 2015) allowed estimating its length in cells. Cytoplasmic microtubules can grow fast, with a rate of up to 20–25 µm/min, and, based on the length of EB-positive regions at microtubule plus ends, have stabilizing GTP caps of ∼1–2 µm (Akhmanova and Steinmetz, 2008; Seetapun et al., 2012). In contrast, centriolar microtubules typically grow at a rate of only tens of nanometers per hour (Aydogan et al., 2018; Chrétien et al., 1997; Kuriyama and Borisy, 1981). Cilia can grow more rapidly, up to 0.05–0.4 µm/min (observed in regenerating flagella in the single-celled green alga *Chlamydomonas* (Abbühl et al., 2025; Marshall et al., 2005; Rosenbaum and Child, 1967; Rosenbaum et al., 1969)). Still, in both centrioles and cilia, the rate of tubulin addition will typically be insufficient to form a long stabilizing GTP cap, yet the centrioles and ciliary axonemes are very stable.

Extensive genetic, proteomics, and cell biological studies identified numerous proteins that stabilize centriolar and ciliary microtubules and control their length (Banterle and Gönczy, 2017; Deretic et al., 2023; Loncarek and Bettencourt-Dias, 2018). Here, we review the biochemical activities of these factors and their effects on microtubule tip structure and dynamics, focusing on emerging common mechanistic principles of slow microtubule growth.

---

[1] Cell Biology, Neurobiology and Biophysics, Department of Biology, Faculty of Science, Utrecht University, Utrecht, the Netherlands.

Correspondence to Anna Akhmanova: a.akhmanova@uu.nl.

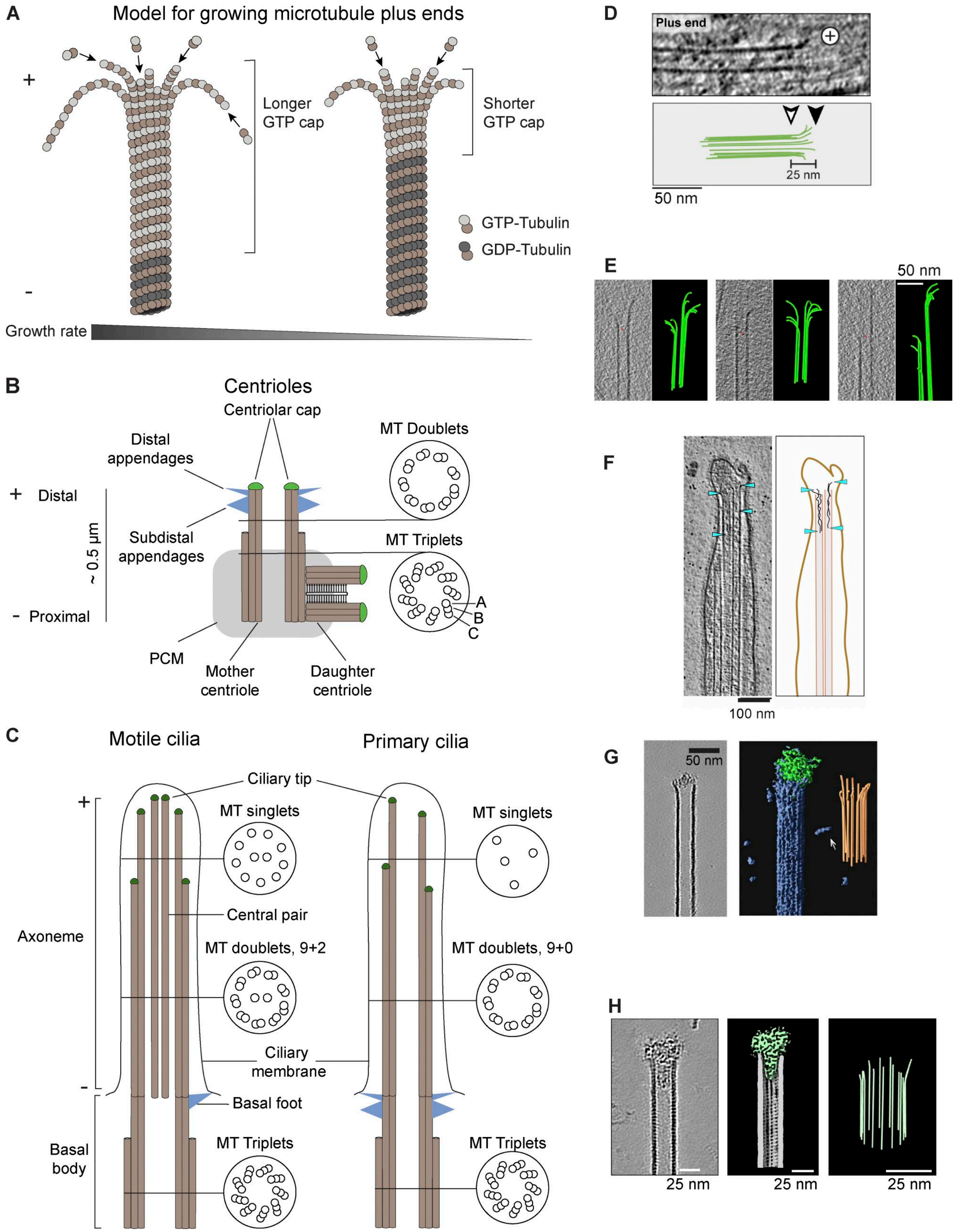

Figure 1. **Structures of microtubule plus ends and the associated caps and plugs in centrioles and cilia. (A)** Scheme of the growing plus end of a microtubule, characterized by flared protofilaments of varying lengths, and a longer or shorter stabilizing GTP cap (light gray tubulin). **(B)** Illustration of a mother centriole–daughter centriole pair surrounded by PCM. The fully elongated mother centriole is ~0.5 µm in length and has triplet microtubules arranged in radial symmetry in the proximal two thirds. A triplet has an A-tubule with 13 protofilaments, a B-tubule, and a C-tubule with 10 protofilaments each. The

C-tubule terminates growing, and thus, the distal one third is composed of doublet microtubules (just A- and B-tubules). The centriolar cap complex (green) is present on the distal end of both mother and daughter centrioles. **(C)** Illustration of motile and primary cilia. The basal body, composed of triplet microtubules, acts as a template for the axoneme, which is the core of the cilium. The axoneme has doublet microtubules arranged in radial symmetry (9 + 0 arrangement) and surrounded by the ciliary membrane. Motile cilia have an additional pair of singlet microtubules in the center called the central pair (9 + 2 arrangement). The doublet microtubules transition into singlet microtubules in the distal ciliary compartment. The ciliary tip complex (green) is present on the distal tip of the ciliary microtubules. **(D)** Tomogram slice and corresponding 3D model depicting the flared protofilaments of a microtubule plus end in the dorsal root ganglion neurons, from Foster et al. (2022). **(E)** Tomograms and corresponding 3D models depicting the variable structure of flared plus ends of microtubules grown *in vitro*. Modified from McIntosh et al. (2018). **(F)** Tomogram slice and the corresponding diagram highlighting what is shown in the tomogram, illustrating filamentous proteins extending into the central pair microtubules in cilia of *Tetrahymena thermophila*. Modified from Legal et al. (2023). **(G)** Tomogram and associated 3D model showing the electron-dense cap structure found on the plus end of microtubules grown *in vitro* in the presence of centriolar proteins (from Iyer et al. [2025]). **(H)** Cork-like electron-dense structures found on the plus end of microtubules grown in the presence of ciliary proteins *in vitro* (from Saunders et al. [2025]). The plus ends in G and H look relatively blunt compared to the control microtubules in E.

Microtubule plus-end architecture

Microtubules are cylindrical polymers built of tubulin dimers, which bind to each other in a head-to-tail fashion to form protofilaments. Within the tube, laterally associated protofilaments are straight, forming a uniform lattice. In contrast, microtubule ends have intrinsically variable structures, where individual protofilaments of different length and curvature can bend away from the tube axis (Gudimchuk and McIntosh, 2021) (Fig. 1 A). Early electron microscopy (EM)–based analyses, based on 2D images, suggested that growing microtubule plus ends terminate with straight protofilaments or tubulin sheets, whereas depolymerizing ends have protofilament curls, resembling ram's horns (Chrétien et al., 1995; Mandelkow et al., 1991; Simon and Salmon, 1990). However, recent 3D analyses using cryo-electron tomography (cryo-ET) suggested that both growing and shrinking microtubules have flared ends (Atherton et al., 2018; McIntosh et al., 2018; Gudimchuk et al., 2020; Foster et al., 2022) (Fig. 1, D and E). How protofilament end structures relate to the presence and length of the GTP cap is still a matter of debate. Since tubulin dimers have a bent shape both in the GDP and the GTP form (reviewed in Brouhard and Rice [2014]), it is thought that the subunits likely first assemble longitudinally into curved protofilaments, and these protofilaments subsequently undergo lateral interactions and straighten, resulting in tube closure (Gudimchuk and McIntosh, 2021; Gudimchuk et al., 2020). A recent model suggests that during microtubule growth, curved protofilaments form discrete laterally attached clusters, which represent short-lived intermediates in the transition to the straight microtubule lattice, and that this process is enhanced by GTP-bound tubulin (Kalutskii et al., 2025).

Mildly curved or flared protofilament ends have also been described at the tips of motile cilia (Legal et al., 2023), and open ends with outwardly curved extensions have been observed at the plus ends in growing centrioles (Guichard et al., 2010). A distinctive feature of centrioles and cilia is the presence of the electron-dense material associated with their ends. Already, early EM work described caps on the distal ends of elongating and resorbing axonemes in *Chlamydomonas* flagella (Dentler and Rosenbaum, 1977). The central pair microtubules have prominent caps, while the A-tubules contain filamentous plugs that extend into their lumen. Subsequent ET studies have shown the presence of caps and filamentous plugs at axonemal microtubule plus ends in cilia from different species and also documented significant variability in these structures (Höög et al., 2014; Legal et al., 2023; Leung et al., 2021; Louka et al., 2018; Reynolds et al., 2018; Zabeo et al., 2018) (Fig. 1F). In some cases, filaments from the plugs extend into the ciliary membrane, potentially anchoring the membrane to the axoneme. Interestingly, cryo-ET of microtubules polymerized *in vitro* in the presence of centriolar or ciliary tip regulators revealed electron-dense plug- or cork-shaped structures at their plus ends (Iyer et al., 2025; Saunders et al., 2025) (Fig. 1, G and H). This indicates that certain aspects of centriolar and ciliary microtubule regulation can be reproduced in a cell-free system, even when using single microtubules composed of brain tubulin rather than the native tubulin isotypes with their specific posttranslational modifications. In the following sections, we first summarize our current knowledge on the properties and functions of individual centriolar cap and ciliary tip components that have been studied *in vitro* and then discuss how they might work together to control microtubule growth and stability.

The centriolar cap

Centriole biogenesis is a complex process involving numerous proteins that have been extensively reviewed (Banterle and Gönczy, 2017; Loncarek and Bettencourt-Dias, 2018; Nigg and Stearns, 2011). While centriole cores are built of microtubule triplets, consisting of a complete, 13-protofilament A-tubule and incomplete, 10-protofilament B- and C-tubules, distal centriolar ends typically miss the C-tubule (Fig. 1 B). Centrioles are decorated by numerous specific proteins, which control centriole elongation and stability, and also regulate their outgrowth during ciliogenesis. The major players in centriole elongation are centrosomal P4.1–associated protein (CPAP), CP110, and CEP97, as well as several factors that also modulate the dynamics of cytoplasmic microtubules, as discussed below.

Centrosomal P4.1–associated protein

CPAP (also called CENPJ in mammals and Sas-4 in invertebrates) is a microtubule- and tubulin-binding protein essential for the elongation of the centriolar microtubules during biogenesis (Table S1). It was initially identified as a centrosomal interaction partner of the erythrocyte protein 4.1R-135 (Hung et al., 2000). Later, CPAP was recognized as one of the key players in centriole biogenesis through an RNAi screen in *Caenorhabditis elegans* (Leidel and Gönczy, 2003). CPAP is essential for centriole formation and maintenance (Basto et al., 2006; Vásquez-Limeta et al., 2022, reviewed in Banterle and Gönczy [2017]; Loncarek and Bettencourt-Dias [2018]). CPAP was also proposed to contribute to the recruitment of pericentriolar material (PCM) to the centrosome through a mechanism that is suppressed by the interaction of CPAP with free tubulin (Gopalakrishnan et al., 2011; Mariappan et al., 2019). However, the role of CPAP in PCM organization has been disputed (Conduit et al., 2015; Vásquez-Limeta et al., 2022). In humans, missense mutations in the CPAP-encoding gene can lead to microcephaly and dwarfism (Bond et al., 2005; Jaiswal et al., 2023), consistent with its role in the formation of centrosomes and ciliary basal bodies. The overexpression of CPAP produces distinct phenotypes in a cell-type and species-specific manner. In mammalian cells, CPAP overexpression does not alter centriole number but causes centriole overelongation, with the distal end displaying splayed microtubules of varying lengths (Kohlmaier et al., 2009; Schmidt et al., 2009; Tang et al., 2009). In contrast, the overexpression of Sas-4 in the *Drosophila* wing disk does not lead to centriole overelongation or distal end protrusions (Franz et al., 2013).

CPAP is a dimeric protein (Zhao et al., 2010) with several helical domains and a glycine-rich C-terminal β sheet domain called the G-box (Hatzopoulos et al., 2013) (Table S1). The first helical domain binds to CP110 (Iyer et al., 2025) (Fig. 2), another protein regulating centriolar length (see below). The helical domains in the C-terminal part of CPAP mediate dimerization, and, along with the G-box, can form higher order oligomers and interact with multiple centriolar partners (Comartin et al., 2013; Cutts et al., 2015; Hatzopoulos et al., 2013; Lin et al., 2013a; Lin et al., 2013b).

The middle part of CPAP contains a PN2-3 domain, which can bind and sequester free tubulin dimers, and an unstructured, positively charged microtubule-binding domain (MBD) (Cormier et al., 2009; Hsu et al., 2008; Hung et al., 2004; Sharma et al., 2016). Such a combination of the tubulin-binding domain and MBD is reminiscent of the microtubule polymerase XMAP215/ch-TOG (Brouhard et al., 2008), which can associate with the growing microtubule plus ends and speed up their growth by promoting tubulin recruitment (Ayaz et al., 2014; Brouhard et al., 2008). The microtubule-binding part of CPAP (PN2-3 together with MBD) also autonomously recognizes and tracks growing microtubule plus ends; however, it does not accelerate but rather slows down microtubule polymerization and, at the same time, prevents catastrophes (Sharma et al., 2016). This behavior depends on a short region within the PN2-3 domain, which forms a dynamic “lid” that can inhibit tubulin addition and removal (see below) (Campanacci et al., 2022; Sharma et al., 2016; Zheng et al., 2016) (Fig. 2). Through its MBD, CPAP promotes rescues, and overall, it imparts slow but processive microtubule growth—an effect opposite to that of XMAP215, which promotes rapid growth, but can also trigger catastrophes (Brouhard et al., 2008; Farmer et al., 2021).

CP110 and CEP97

CP110 and CEP97 together form an inhibitory cap at the distal end of the centriole, which prevents excessive elongation of centriolar microtubules (Kohlmaier et al., 2009; Schmidt et al., 2009) and suppresses cilium formation (Spektor et al., 2007). CP110 was initially identified as a cyclin/CDK substrate, while CEP97 was discovered as a CP110-interacting protein (Chen et al., 2002; Spektor et al., 2007). During centriole growth, the CP110-CEP97 cap is continuously present at the distal end (Kleylein-Sohn et al., 2007). Ciliogenesis requires the removal of the CP110 cap, a process regulated by numerous factors (Spektor et al. [2007], reviewed in Xie et al. [2024]). Knockdown of CP110 and CEP97 in mammalian cells results in centriole overelongation, similar to CPAP overexpression (Kohlmaier et al., 2009; Schmidt et al., 2009; Tang et al., 2009). Effects of the loss of CP110 in *Drosophila* are cell-specific: in S2 cells, it causes centriole shortening and destabilization (Delgehyr et al., 2012), but in the wing disk, it causes a mild increase in centriolar length and overextension of the plus ends of centriolar microtubules, whereas CP110 overexpression causes a mild centriole shortening (Franz et al., 2013). CP110 counteracts the effects of both positive and negative regulators of centriole elongation, including a kinesin-13 depolymerase Klp10A and the microtubule growth-promoting factor CLASP (see below) (Delgehyr et al., 2012; Franz et al., 2013; Shoda et al., 2021). In contrast, CEP97 is essential for the formation of normal centrioles and has some CP110-independent functions, which seem to involve cooperation with microtubule acetylating enzymes through a currently unclear mechanism (Dobbelaere et al., 2020).

CP110 has no conserved domains, except for two helical regions. The N-terminal part of CP110 interacts with the C terminus of CEP97, while its C-terminal helical domain can either mediate homodimerization or bind to CPAP by forming a heterodimeric coiled coil (Iyer et al., 2025). CEP97 has a leucine-rich repeat domain at its N terminus for protein interactions and an IQ domain for calmodulin binding (Fig. 2 and Table S1).

In vitro experiments showed that whereas CEP97 does not bind to microtubules, CP110 specifically binds to microtubule plus ends through its C-terminal part and blocks their elongation (Iyer et al., 2025). At low concentrations, CP110 induces transient pauses in microtubule polymerization, whereas at higher concentrations, it completely blocks growth. This activity can be robustly recapitulated using a fusion protein containing the N-terminal part of CEP97 and the C-terminal part of CP110, and leaving out the domains through which the two proteins interact (Iyer et al., 2025). The growth inhibition imposed by CEP97-CP110 chimera on microtubules *in vitro* can be relieved by CPAP, resulting in very slow microtubule polymerization, with both CPAP and CP110 tracking the slowly growing plus ends (Iyer et al., 2025). The ability of CPAP to bind to the C-terminal coiled-coil domain of CP110 and disrupt its homodimerization may constitute a part of the mechanism allowing CPAP to overcome CP110-driven polymerization block (Fig. 2). In line with this idea, direct CPAP–CP110 interaction is required for efficient centriole elongation throughout the S and G2 phases of the cell cycle (Iyer et al., 2025). This explains why CP110 and CPAP antagonize each other in controlling the length of centriolar microtubules (Kohlmaier et al., 2009; Schmidt et al., 2009; Tang et al., 2009). Interestingly, centrioles can also elongate during mitosis (Kong et al., 2020), and the underlying

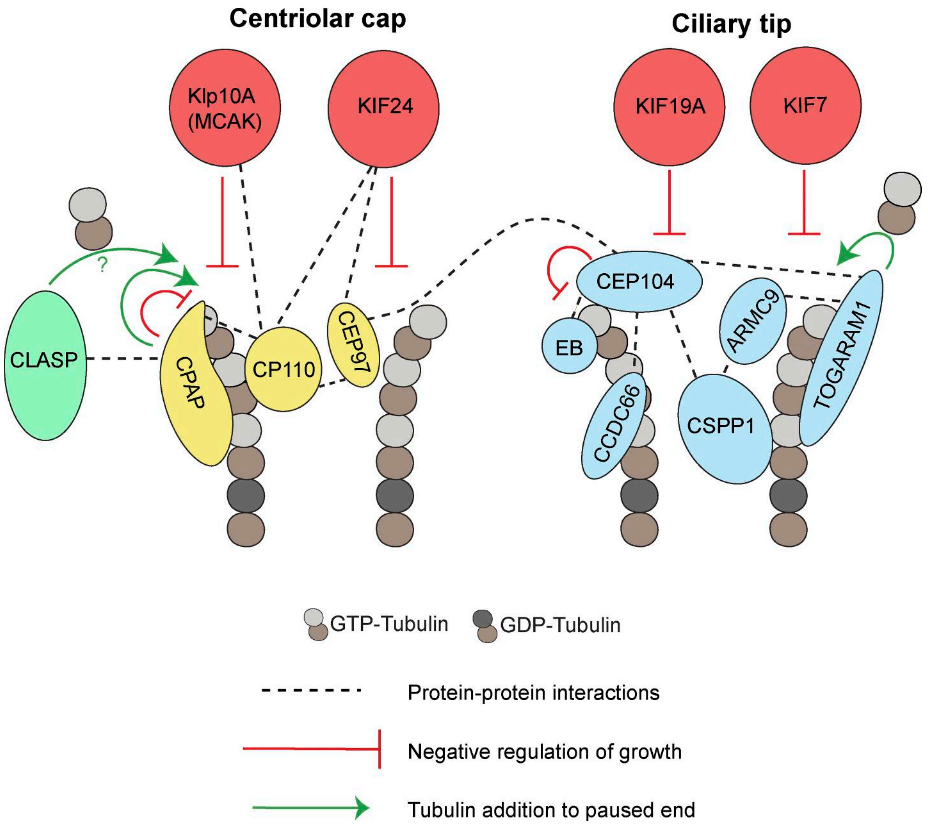

Figure 2. **Interactions of the centriolar cap and the ciliary tip proteins.** Scheme of protein–protein interactions and plus-end growth regulation in the centriolar cap and the ciliary tip. The scheme comprises the following proteins: Orbit/Mast/CLASP, KIF24, Klp10A of the kinesin-13 family, CPAP, CP110, CEP97, CCDC66, CEP104, EB3, ARMC9, CSPP1, TOGARAM1, KIF7, and KIF19A. CLASP, cytoplasmic linker–associated protein; KIF24, kinesin family member 24; Klp10A, kinesin-like protein at 10A; CP110, centriolar protein of 110 kDa; CEP97, centrosomal protein 97; CEP104, centrosomal protein 104; EB3, end-binding protein 3; TOGARAM1, TOG array regulator of axonemal microtubules 1; KIF7, kinesin family member 7; KIF19A, kinesin family member 19A.

mechanism is at least partly different because it does not depend on the CPAP–CP110 interaction (Iyer et al., 2025).

Other microtubule regulators

In addition to specific factors, fine-tuning of centriolar microtubule dynamics also involves general microtubule regulators. In *Drosophila*, control of centriole length can be most easily studied in spermatocytes before meiotic onset, when centrioles dramatically overelongate (Riparbelli et al., 2012). CLASP/Orbit/Mast, an extensively studied TOG domain–containing factor that inhibits catastrophes and promotes microtubule rescue, repair, and nucleation (Aher et al., 2018; Aher et al., 2020; Lawrence et al., 2020; Moriwaki and Goshima, 2016) (Table S1), localizes to the distal spermatocyte centriole ends, is required for their elongation, and induces their overextension upon overexpression (Shoda et al., 2021).

In contrast, microtubule depolymerase Klp10A causes centriole shortening: its loss induces centriole elongation in fly spermatocytes and cultured cells (Delgehyr et al., 2012; Shoda et al., 2021). Klp10A belongs to the kinesin-13 family, which comprises immotile kinesins with the motor domain located in the middle of the molecule; these proteins increase the outward curling of the protofilaments and depolymerize the GTP cap, triggering catastrophes (Friel and Welburn, 2018; Moriwaki and Goshima, 2016) (Table S1). Klp10A binds to CP110 in flies (Delgehyr et al., 2012; Ryniawec et al., 2023), whereas mammalian CLASPs were reported to bind to CPAP (Maffini et al., 2009) (Fig. 2), suggesting that stabilizing and destabilizing factors can work as a part of the same protein complex. In mammalian cells, a specialized kinesin-13, KIF24, interacts with CP110 and CEP97 (Kobayashi et al., 2011) (Fig. 2). KIF24 can cause microtubule disassembly *in vitro*; however, in cells, it does not seem to be involved in restricting centriole length but instead participates in ciliogenesis (Kobayashi et al., 2011). *In vitro* reconstitution of the activities of all these factors together with CPAP and CP110 would be needed to understand their joint mechanisms of action.

The ciliary tip

Axonemal microtubule plus ends participate in organizing the ciliary tip compartment, which has several important functions, including control of ciliary growth and shortening, conversion between anterograde and retrograde intraflagellar transport trains, and organization of signaling complexes, for example, in the Hedgehog pathway (Klena and Pigino, 2022; Mill et al., 2023; Nachury and Mick, 2019). Axonemes are formed by microtubule doublets, but typically only the complete A-tubules extend to the very end, whereas the incomplete B-tubules terminate earlier (Klena and Pigino, 2022). As mentioned above, motile but not primary cilia also contain two complete central pair microtubules that extend to the outermost tip of the cilium and are connected to the plasma membrane by the ciliary cap (Dentler and Rosenbaum, 1977). The lengths of axonemal microtubules vary across ciliary types and species—for example, in multiciliated cells of the Xenopus embryo epidermis, the central pair microtubules are significantly longer than the outer doublets, a structural feature that depends on specific microtubule-binding proteins (Hong et al., 2025) (Fig. 1 C).

Several ciliary tip regulators, collectively called "ciliary tip module," form an interaction network and, when mutated in vertebrates, cause formation of shorter primary cilia and different ciliopathies, such as the neurodevelopmental disorder called Joubert syndrome (Badano et al., 2006; Hildebrandt et al., 2011; Latour et al., 2020; Saunders et al., 2025). Here, we will focus on the proteins directly implicated in the regulation of axonemal microtubule growth, including CEP104, TOGARAM1, centrosome/spindle pole-associated protein (CSPP1), armadillo repeat–containing 9 (ARMC9), coiled-coil domain–containing protein 66 (CCDC66), kinesin-4 KIF7, and kinesin-8 KIF19A, and their effects on single, complete microtubules. The effects of these factors on incomplete B-tubules within doublets still need to be explored.

CEP104

CEP104 (FAP256 in ciliates and green algae) is a centriolar and ciliary protein that localizes to the distal tip of motile and primary cilia across species, including *Chlamydomonas*, *Tetrahymena,* and mammalian cells. It was first identified as a ciliary component in a proteomics screen for interacting partners of EB proteins, which can recruit CEP104 to growing microtubule ends (Jiang et al., 2012). In nonciliated mammalian cells, CEP104 localizes to the distal ends of both the mother and the daughter centriole, where it binds to CEP97 and CP110 (Jiang et al., 2012) (Fig. 2). CEP104 depletion has no effect on mammalian centrioles (Jiang et al., 2012), but the loss of its counterpart in insect cells makes centrioles slightly shorter, though this does not apply to all somatic *Drosophila* cell types (Ryniawec et al., 2023). The major function of CEP104/FAP256 is during ciliogenesis: its loss impairs formation of primary and motile cilia and results in shorter cilia and other ciliary defects in multiple species (Frikstad et al., 2019; Louka et al., 2018; Satish Tammana et al., 2013; Yamazoe et al., 2020). Mutations in the CEP104-encoding gene cause Joubert syndrome (Glass et al., 1993; Srour et al., 2015). In motile cilia, CEP104 is typically enriched at the ciliary tip during both ciliary growth and resorption (Satish Tammana et al., 2013); it localizes to the ends of both the outer doublets and the central pair (Hong et al., 2025). It promotes elongation of A-tubules (Louka et al., 2018), controls the length of the central pair tubules, and is required for the formation of the ciliary cap (Legal et al., 2023).

CEP104 has a distinct domain structure with well-defined functions. It consists of an N-terminal Jelly roll domain primarily involved in protein–protein interactions, a coiled-coil domain for dimerization, a TOG domain that binds to soluble tubulin, and a C-terminal zinc-finger domain also implicated in partner binding (Al-Jassar et al., 2017; Rezabkova et al., 2016) (Table S1). Based on the presence of a canonical tubulin-binding TOG domain, which by itself can promote tubulin polymerization *in vitro*, CEP104 was proposed to be a microtubule polymerase (Yamazoe et al., 2020). Surprisingly, experiments with purified full-length protein showed that CEP104 blocks growth of microtubule plus ends and can also inhibit their shortening and thus stabilize microtubules (Saunders et al., 2025). The affinity of CEP104 for microtubule plus ends *in vitro* is not high and can be enhanced by microtubule-binding interaction partners, including EB proteins, CSPP1, and CCDC66 (Saunders et al., 2025) (Fig. 2). The ability to stabilize depolymerizing microtubule plus ends might explain why CEP104 promotes rather than inhibits ciliary elongation; moreover, it is likely to cooperate with other ciliary proteins. In agreement with this view, CEP104 binds to another TOG domain protein, TOGARAM1, which can overcome the CEP104-imposed growth block (Saunders et al., 2025) (see the next section).

TOGARAM1/Crescerin1

TOGARAM1 (also known as Crescerin1, SHF1 [short flagella mutant in *Chlamydomonas*], or CHE-12 [chemotaxis mutant in *C. elegans*]) is a TOG domain–containing protein from the FAM179 family, which includes TOGARAM1/Crescerin1, found in both vertebrates and invertebrates, and TOGARAM2/Crescerin2, found exclusively in vertebrates (Bacaj et al., 2008; Das et al., 2015; Perlaza et al., 2022). Like CSPP1, TOGARAM1 is also a ciliopathy-associated protein, with mutations in the TOG domains linked to ciliopathies such as Joubert syndrome (Glass et al., 1993; Latour et al., 2020).

TOGARAM1 is a highly conserved protein found in ciliated organisms, but absent in nonciliated eukaryotes, such as yeast, underscoring its functional specificity for cilia (Das et al., 2015). It localizes to the basal body and along the cilium in multiple systems, including *C. elegans* sensory cilia and primary cilia in cultured mammalian cells (Das et al., 2015). In motile cilia of *Tetrahymena,* the TOGARAM1 ortholog localizes specifically to the tips of the axonemal B-tubules (Louka et al., 2018). In all studied systems, TOGARAM1 promotes proper ciliary development and elongation, and its activity depends on the functional TOG domains (Bacaj et al., 2008; Das et al., 2015; Latour et al., 2020; Louka et al., 2018; Perlaza et al., 2022).

Mammalian TOGARAM1 contains two pairs of TOG domains connected by linker regions (Table S1). Two of these domains, TOG2 and TOG4, structurally resemble those in XMAP215 and CEP104, can bind to soluble tubulin, and stimulate microtubule polymerization *in vitro* (Das et al., 2015). In contrast, the effect of full-length TOGARAM1 on microtubule dynamics is unremarkable: it mildly reduces microtubule rate and promotes rescues (Saunders et al., 2025). However, TOGARAM1 interacts with other ciliary proteins, including CEP104, and can overcome growth inhibition imposed by CEP104 and another ciliary tip protein, CSPP1 (see below), leading to slow but processive growth (Saunders et al., 2025) (Fig. 2). This helps to explain the importance of TOGARAM1 for axoneme elongation in different systems.

Centrosome/spindle pole-associated protein

CSPP1 is a ciliary protein, with mutations linked to ciliopathies such as Joubert syndrome and Meckel–Gruber syndrome, which affects the development of multiple organs (Akizu et al., 2014; Ben-Omran et al., 2015; Patzke et al., 2005; Patzke et al., 2006; Shaheen et al., 2014; Tuz et al., 2014). In addition to cilia, CSPP1 localizes to the centrosome and centriolar satellites in interphase cells and to spindle poles and the central spindle during mitosis (Frikstad et al., 2019; Patzke et al., 2005; Patzke et al., 2010). Depletion or mutations in CSPP1 impair ciliogenesis, leading to the formation of fewer and shorter cilia (Frikstad et al., 2019; Patzke et al., 2010). Conversely, the overexpression of CSPP1 in cultured mammalian cells increases axonemal length in primary cilia (Patzke et al., 2010).

CSPP1 contains several predicted short α-helical domains, which include a MBD in the middle and a centrosome-targeting region at the C terminus (Patzke et al., 2006) (Table S1). *In vitro* studies showed that CSPP1 directly binds and stabilizes microtubules at both the plus and the minus end by decreasing the growth rate, inducing pauses and suppressing catastrophes (van den Berg et al., 2023; Wang et al., 2024). Interestingly, CSPP1 binds to microtubules through a short α-helical domain that is sufficient for luminal targeting (van den Berg et al., 2023). *In vitro*, CSPP1 accumulates along the whole microtubule lumen, but at endogenous expression levels in cells, it localizes to the basal body, the ciliary transition zone, and the ciliary tip (Frikstad et al., 2019; Patzke et al., 2010), rather than along the entire axoneme, likely due to interactions with other ciliary proteins.

Coiled-coil domain–containing protein 66

CCDC66 is an α-helical protein (Table S1), which was identified in animal models as a gene for which the mutation causes degeneration and dysfunction of the retina and defects in the olfactory system, two organs that require specialized sensory cilia for their function (Dekomien et al., 2010; Gerding et al., 2011; Schreiber et al., 2018). However, until now, mutations in CCDC66 have not been reported in ciliopathy patients.

Similar to CSPP1, CCDC66 localizes to the centrosome and centriolar satellites through its interaction with the canonical satellite protein PCM1 (Conkar et al., 2019; Conkar et al., 2017). CCDC66 also regulates spindle assembly and orientation, as its depletion results in fewer microtubules in the spindle apparatus (Batman et al., 2022). In nondividing cells, CCDC66 localizes to the ciliary axoneme and is important for primary cilium formation and stability, since its depletion reduces ciliation frequency and ciliary length, and causes length fluctuations (Deretic et al., 2025; Odabasi et al., 2023). CCDC66 robustly binds along the entire microtubule lattice both in cells (Batman et al., 2022) and *in vitro*, but has little autonomous impact on microtubule dynamics: it marginally decreases the elongation rate (Saunders et al., 2025). Therefore, its impact on microtubules in different settings likely depends on its partners. Indeed, CCDC66 interacts with other ciliary tip module proteins, such as CEP104, and can recruit it to microtubules (Latour et al., 2020; Saunders et al., 2025) (Fig. 2), suggesting that it might primarily act as a scaffold.

Armadillo repeat–containing 9

ARMC9 is another scaffolding component of the ciliary tip module, implicated in Joubert syndrome and other ciliopathies (Kar et al., 2018; Latour et al., 2020; Van De Weghe et al., 2017). ARMC9 is conserved from ciliates to mammals and can localize to the basal bodies and along the cilium; in motile *Tetrahymena* cilia, it is present at the tips of the axonemal B-tubules (Latour et al., 2020; Louka et al., 2018; Van De Weghe et al., 2017). Mutations in the ARMC9-encoding gene disrupt ciliary assembly and function; interestingly, while ARMC9 impairment causes shortening of primary cilia in mammals (Latour et al., 2020), in motile cilia, ARMC9 localizes to the ends of the doublets (Hong et al., 2025), and the ARMC9 ortholog in *Tetrahymena* serves as a negative regulator of B-tubules (Louka et al., 2018).

ARMC9 has an N-terminal LisH domain (a short α-helical dimerization domain), a predicted coiled-coil domain, and armadillo repeats, which form a solenoid-like α-helical structure (Andrade et al., 2001). Biochemical characterization showed that ARMC9 can dimerize through its central coiled-coil domain, and these dimers can further multimerize (Saunders et al., 2025) (Table S1).ARMC9 does not bind to microtubules autonomously *in vitro* (Saunders et al., 2025). However, it can interact with TOGARAM1 and CSPP1 (Fig. 2), which can recruit it to microtubules, and enhance their effects on microtubule dynamics: it increases the frequency of rescues triggered by TOGARAM1 and the frequency of pauses induced by CSPP1 (Saunders et al., 2025). These data, together with the fact that ARMC9 can oligomerize, suggest that it might act as a scaffold to coordinate the activities of other ciliary tip proteins.

Collective activity of ciliary tip module proteins

Based on a recent *in vitro* reconstitution study (Saunders et al., 2025), two of the five investigated ciliary tip module proteins, CEP104 and CSPP1, can stabilize growing and shrinking microtubule plus ends and inhibit microtubule growth. The third one, TOGARAM1, can overcome this growth block, inducing slow polymerization. The remaining two, CCDC66 and ARMC9, promote binding of the others to microtubules and to each other, making slow growth robust (see below). EB proteins also appear to be part of the complex, since they have been reported to be present at the ciliary tip or along the cilium (Kiesel et al., 2020; Pedersen et al., 2003; Schrøder et al., 2011) and can directly bind to CEP104 (Jiang et al., 2012). EBs can serve as scaffolds for recruiting other proteins to the GTP cap (Akhmanova and Steinmetz, 2008; Roostalu et al., 2020), and can also accelerate cap maturation (Maurer et al., 2014). The relevance of these activities for cilium formation is currently unclear.

Ciliary tip regulation by kinesin family members

Similar to centrioles, ciliary length can be restricted by microtubule-depolymerizing kinesins. These include a kinesin-13 family member that can negatively control flagellar length in *Leishmania* (Blaineau et al., 2007), the kinesin-8 KIF19A in multiciliated mammalian cells (Niwa et al., 2012), and the kinesin-4 KIF7 (ortholog of *Drosophila* Costal-2), a human ciliopathy protein that helps to shape the tip compartment and organize Hedgehog signaling components in primary cilia (Glass et al., 1993; He et al., 2014). The activities of purified motor domains of KIF19A and KIF7 have been investigated. The motor domain of KIF19A preferentially depolymerizes microtubule plus ends *in vitro* (Niwa et al., 2012), but its motility on microtubules was not analyzed. The dimeric motor domain of KIF7 is immotile, which is unusual for kinesin-4 family members, most of which are plus-end–directed motors (He et al., 2014). Moreover, KIF7 has an unconventional mechanochemical cycle, as its affinity for microtubules is only weakly coupled to ATP hydrolysis (Jiang et al., 2019; Yue et al., 2018). KIF7 was reported to preferentially bind to GTP- versus GDP-tubulin in the microtubule lattice (Jiang et al., 2019), though this preference is extremely sensitive to the assay conditions (Blasius et al., 2021). Such a preference might help to explain the accumulation of KIF7 at the axonemal plus ends, where it promotes catastrophes and slows down polymerization, and thus restricts ciliary elongation and controls ciliary tip architecture (Blasius et al., 2021; He et al., 2014). No *in vitro* data are currently available for the full-length kinesins, but there are clear indications that kinesin tails could strongly modulate their behavior. For example, KIF7 is autoinhibited by a coiled-coil domain in its C terminus, and this inhibitory domain is required for the ciliary tip localization (Blasius et al., 2021) (Table S1). The interplay between inhibitory kinesins and the microtubule-stabilizing and growth-promoting ciliary factors described above is likely to contribute to controlling slow elongation of ciliary microtubules (Fig. 2).

Molecular mechanisms of regulation of slow microtubule growth

How slow yet processive microtubule growth is achieved has been an ongoing fundamental question. Two *in vitro* reconstitutions with nonoverlapping sets of centriole and ciliary tip regulators provided interesting clues, as they showed some striking similarities in biochemical activities involved (Iyer et al., 2025; Saunders et al., 2025). Both the complex of CPAP with CEP97-CP110 chimera and the six-component assembly containing CEP104, TOGARAM1, CSPP1, CCDC66, ARMC9, and EB3 were shown to combine three key features: (1) microtubule tip stabilization and catastrophe suppression; (2) inhibition of tubulin addition at the outermost plus end and pause induction; and (3) facilitation of slow tubulin incorporation (Fig. 3).

Microtubule tip stabilization

For microtubules to grow slowly yet processively, catastrophes must be strongly suppressed. Potential mechanisms to stabilize microtubule ends and prevent their depolymerization are by enhancing lateral interactions between neighboring protofilaments or by making them straighter, to prevent peeling. In both cases, one would expect to see a decrease in protofilament flaring, resulting in blunter plus ends (Fig. 3).

In the study of centriolar cap proteins, cryo-ET analysis showed that the CEP97-CP110 chimera specifically binds to the luminal side of the microtubule plus end, forming pluglike densities that inhibit plus-end growth and shrinkage, and reduce the length of protruding protofilaments, though not their curvature, making the ends appear more blunt (Iyer et al., 2025). Precisely, which structural feature of the plus-end lumen is recognized by CP110 remains unknown. CP110 does not bind to the solvent-exposed longitudinal surface of the terminal β-tubulin, as it does not compete with proteins that bind there, such as DARPin (Ahmad et al., 2016). Instead, CP110 may interact with the grooves between protofilaments, recognizing the flared geometry of the β-tubulin at the plus end, an idea supported by the fact that CP110 binds to the microtubule plus end but does not accumulate along the lumen and does not bind to soluble tubulin, suggesting that its interaction site involves at least two, specifically positioned tubulin dimers. CP110 may act as a "molecular glue" that stabilizes the plus ends by enhancing protofilament clustering and maintaining them at an angle that impedes proper tube closure and elongation. Such a mechanism would be analogous to the one proposed for the CKK domain of CAMSAPs, which specifically stabilize microtubule minus ends and inhibit their growth by binding to a site on the outer microtubule surface between two tubulin dimers (Atherton et al., 2017).

Ciliary tip proteins also form cork-like accumulations at microtubule plus ends and inhibit protofilament flaring (Saunders et al., 2025). Within the ciliary tip module, CSPP1 displays some similarities to CP110, as it binds to the microtubule lumen and prevents microtubule depolymerization (van den Berg et al., 2023). CSPP1 can trigger pauses in microtubule growth *in vitro* and stabilize nondynamic ends in cells. *In vitro*, every pause induced by CSPP1 is followed by polymerization, which suggests that CSPP1 does not occlude the outermost protofilament ends. In agreement with this notion, CSPP1 is never detected at incomplete microtubule ends or tubulin sheets. Unlike CP110, CSPP1 does not recognize plus-end features such as the geometry of the exposed β-tubulin: it can enter the microtubule through both plus and minus ends, or even through lattice defects, and accumulate in the lumen along the whole length of the microtubule (van den Berg et al., 2023; Wang et al., 2024). However, unlike many axonemal microtubule inner proteins (Leung et al., 2025; Ma et al., 2019), CSPP1 does not exhibit periodicity in its localization pattern (van den Berg et al., 2023). The accumulation of CSPP1 on microtubule ends is slow and cooperative, and this might explain why it preferentially binds and stabilizes microtubule tips in a precatastrophe state, when polymerization slows down.

CEP104 is another ciliary module component that can stabilize depolymerizing microtubule ends. This activity depends on its tubulin-binding TOG domain, but the underlying mechanism is unclear and might involve interactions with other microtubule-binding proteins that allow CEP104 to span both the outer and the inner microtubule surface (see below) (Saunders et al., 2025).

Microtubule growth inhibition

The second feature of the slow-growing microtubule state is a partial suppression of the longitudinal interactions between β-tubulin exposed at the plus end and free tubulin dimers. For this mechanism to be operational, not all protofilament ends need to be occluded (Fig. 3). A study using a fluorescent version of the microtubule-targeting drug eribulin, which binds to a

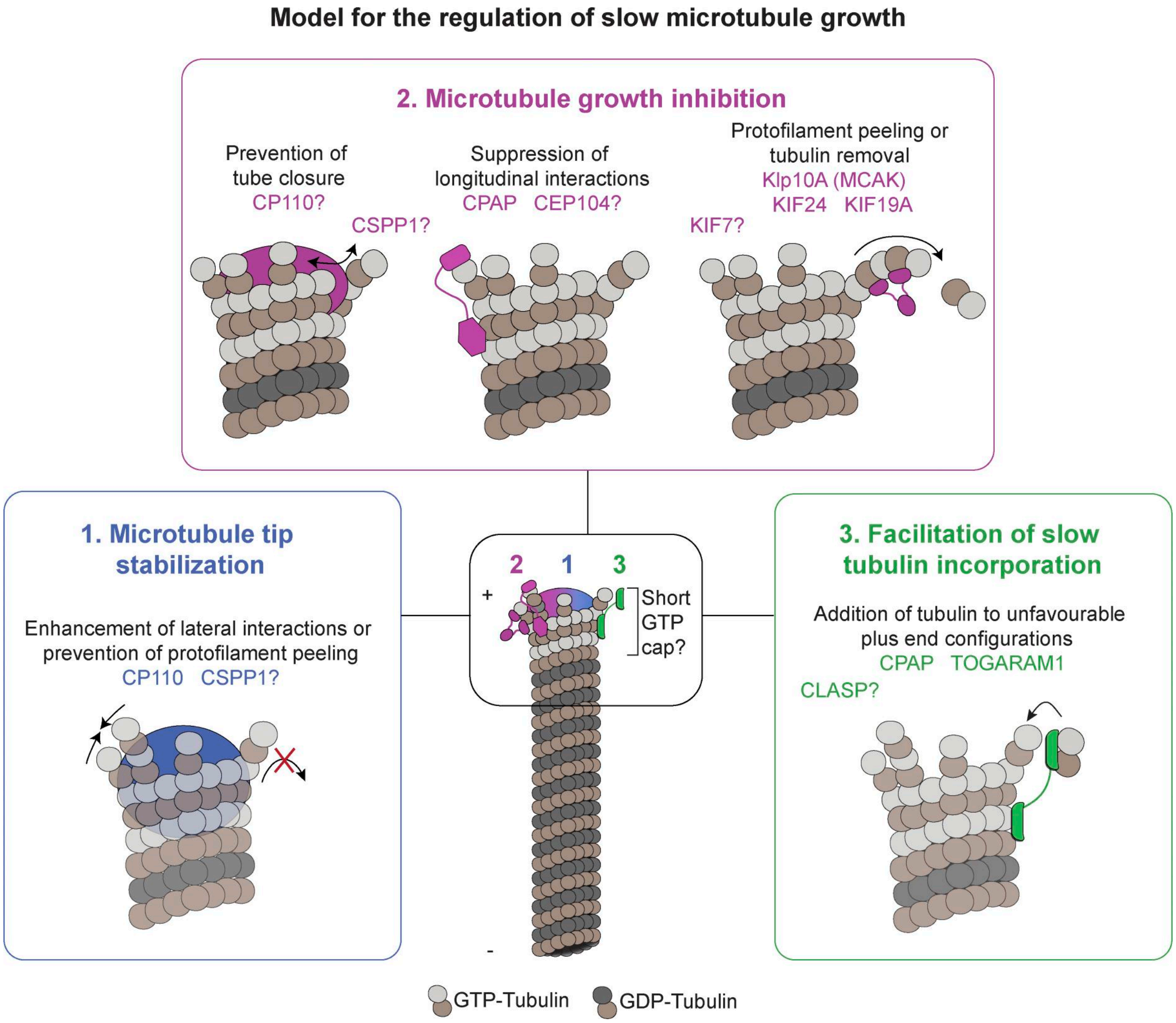

Figure 3. **Model for the regulation of slow growth of centriolar and ciliary microtubules.** Proposed model for regulation of slow-growing microtubule plus ends with three essential features: (1) stabilization of slowly growing plus ends by proteins like CP110 and CSPP (blue) that bind to the luminal side of the microtubule and may enhance lateral interactions between neighboring protofilaments (horizontal black arrows) or make the protofilaments straighter, preventing them from peeling (black arrow with a red cross). (2) Inhibition of growth by proteins such as CEP104 and the LID domain of CPAP (magenta) that may occlude the solvent-exposed surface of β-tubulin and partially suppress longitudinal tubulin interactions, by proteins such as CP110 that may inhibit growth by preventing tube closure, and by depolymerizing kinesins such as Klp10A (KIF2C/MCAK) and KIF24 that promote protofilament peeling at the microtubule plus end (curved black arrow) and KIF19A and KIF7 that can inhibit growth or depolymerize microtubules from the plus end. (3) Facilitation of slow microtubule growth by polymerases like CPAP and TOGARAM1 (green), which can bind soluble tubulin and promote its addition to microtubule plus ends with unfavorable configurations like blunt protofilaments or a small GTP cap (black arrow), or CLASP that facilitates growth by inhibiting catastrophes and promoting rescues.

β-tubulin site involved in the longitudinal interdimer interactions, demonstrated that a single drug molecule is sufficient to perturb polymerization (Doodhi et al., 2016).

In the centriolar cap complex, this function is mediated by the PN2-3 domain of CPAP. PN2-3 forms a 1:1 complex with free tubulin, independent of its nucleotide state (Cormier et al., 2009; Hsu et al., 2008). The overexpression of PN2-3 in cells results in cytoplasmic microtubule depolymerization, as PN2-3 sequesters soluble tubulin in a nonpolymerizable state. PN2-3 is structurally unique and distinct from other tubulin binders, like TOG domains or stathmin: it comprises an α-helical LID domain followed by an unstructured SAC domain. The SAC domain binds to the terminal α- and β-tubulin at the tip of the growing protofilament, while the LID domain associates with the solvent-exposed surface of that terminal β-tubulin; additional microtubule affinity is provided by the adjacent MBD (Campanacci et al., 2022; Sharma et al., 2016; Zheng et al., 2016) (Fig. 3). Together, these domains act like a "leaky lid" that transiently binds the plus end of a growing protofilament. When bound, the leaky lid hinders the longitudinal associations between the protofilament and soluble tubulin dimers, thereby inhibiting polymerization. Further, since PN2-3 was shown to inhibit nucleotide exchange and hydrolysis, CPAP-bound plus ends might retain a GTP cap in spite of slow growth or pausing (Cormier et al., 2009) (Fig. 3). As CPAP is a dimeric protein, each molecule can bind to two soluble tubulin dimers, and *in vitro* work showed that two CPAP dimers, which potentially can occlude four protofilaments, are sufficient to inhibit microtubule growth and suppress catastrophes (Sharma et al., 2016).

Within the ciliary tip module, CEP104 seems to play a role analogous to the CPAP PN2-3 domain, as CEP104 blocks microtubule growth *in vitro* through its TOG domain. The single TOG domain of CEP104 associates with soluble tubulin in a 1:1 complex, and as CEP104 dimerizes through its coiled-coil domain, each dimer can bind two tubulin dimers (Al-Jassar et al., 2017; Rezabkova et al., 2016; Yamazoe et al., 2020) (Fig. 3). This is similar to PN2-3, yet these domains have opposing effects on microtubule polymerization *in vitro*: while the PN2-3 domain inhibits polymerization by sequestering tubulin, the TOG domain of CEP104 promotes microtubule polymerization by lowering the critical concentration of tubulin, like other canonical TOG domains (Yamazoe et al., 2020). Crystal structure of the human CEP104 TOG domain reveals that it is similar to the canonical TOG domains, with a paddlelike shape composed of antiparallel α-helical pins termed HEAT repeats (Andrade et al., 2001). This paddle has conserved tubulin-interacting amino acids homologous to those in yeast Stu2, and mutating these residues decreases the *in vitro* polymerizing activity of the CEP104 TOG domain (Yamazoe et al., 2020). This is in sharp contrast with the activity of full-length CEP104, which strongly inhibits plus-end growth at the GMPCPP-stabilized seed and can also inhibit depolymerization of dynamic microtubule ends (Saunders et al., 2025). Two or three CEP104 dimers are sufficient for complete inhibition of growth, indicating that, similar to CPAP, CEP104 does not need to occupy all protofilaments to alter that dynamic state of the plus end.

CEP104 interacts with CSPP1 and CP110, both of which localize in the lumen of the microtubule (Iyer et al., 2025; van den Berg et al., 2023), as well as with EB3 (Jiang et al., 2012), which binds to the outer microtubule wall (Maurer et al., 2012) (Fig. 3). By interacting with proteins on the inner and outer surface of the microtubule, CEP104 may effectively span the terminal β-tubulin surface, creating a molecular cap that inhibits longitudinal interactions between the lattice and soluble tubulin dimers, thereby halting elongation.

Facilitation of slow tubulin incorporation

The third essential feature of slowly growing microtubules is controlled, attenuated incorporation of tubulin into the plus ends. Occlusion of protofilament ends and possibly also their blunt configuration, observed for slowly growing microtubule plus ends *in vitro* (Iyer et al., 2025; Saunders et al., 2025), may be unfavorable for tubulin binding. Furthermore, the reduction or loss of GTP cap associated with pausing ends can disfavor growth reinitiation (Wieczorek et al., 2015). These obstacles are likely to be overcome by specialized microtubule polymerases (Fig. 3).

In the centriolar cap complex, CPAP acts as a microtubule polymerase that can bind to soluble tubulin. The polymerase activity is attributed to the PN2-3 domain, followed by the positively charged unstructured MBD. The MBD is essential for centriolar biogenesis, as overexpression of mutant CPAP lacking the MBD impairs procentriole formation (Sharma et al., 2016). The combined action of MBD and PN2-3 positions CPAP at the microtubule plus end (Sharma et al., 2016), where, as discussed above, PN2-3 acts as a leaky lid that transiently associates with the solvent-exposed surface of the terminal β-tubulin. When bound, the leaky lid blocks longitudinal interactions; when released, it can capture a free tubulin dimer, thus bringing it closer to the protofilament tip and promoting polymerization. Thus, CPAP promotes slow yet processive growth and inhibits catastrophes *in vitro*.

Within the ciliary tip module, TOGARAM1 is the microtubule polymerase, which similar to CPAP overcomes growth inhibition imposed by other proteins (Saunders et al., 2025). It contains an array of TOG domains and linker regions that bind to both soluble tubulin and microtubules. The number of TOG domains in TOGARAM1 is species-specific, ranging from two in *Chlamydomonas* to four in mammals (Das et al., 2015). Structural analysis of the TOG2 domain of TOGARAM1 reveals it has a canonical TOG fold that binds soluble tubulin preferentially in a bent conformation (Das et al., 2015). Isolated TOG2 and TOG4 of TOGARAM promote tubulin polymerization into microtubules *in vitro* (Das et al., 2015). However, *in vitro* studies using full-length TOGARAM1 indicated that regulation of microtubule dynamics depends on TOG3 and TOG4, whereas TOG2 mediates binding to ciliary partners (Saunders et al., 2025) (Fig. 3).

In the centriolar cap complex, CPAP has a dual function, as the PN2-3 coordinates the slow incorporation of tubulin with the inhibition of longitudinal interactions. In the ciliary tip module, these two functions appear to be distributed between TOGARAM1 and CEP104, which can bind to each other. When combined *in vitro*, the two proteins can induce episodes of slow microtubule plus-end growth, suggesting that they can coordinate the inhibition of polymerization with tubulin incorporation (Saunders et al., 2025). Moreover, when TOGARAM1 and CEP104 are combined with CSPP1 and other ciliary module components, this results in uniform, processive slow microtubule growth and correlates with the formation of distinct electron-dense "corks" at microtubule plus ends (Saunders et al., 2025). In this reconstitution, TOGARAM1 is essential for polymerization, whereas leaving out one of the other components of the module has no major effect on the rate of slow growth, but makes this dynamic state less robust (Saunders et al., 2025). This might explain why, when the individual components of the ciliary tip module are mutated, cilia still form but have structural abnormalities, which in turn can cause defects in Hedgehog signaling and developmental aberrations.

Conclusions and outlook

Genetics, proteomics, and cell biological studies generated an inventory of proteins controlling microtubule growth in centrioles and cilia, and recent biochemical reconstitutions have provided the first glimpses of how these proteins work together. A common feature of microtubule regulation in all cell compartments seems to be the formation of complex and flexible protein interaction networks, which have some organelle-specific factors, such as CP110 or TOGARAM1, and some general components, including the EB proteins, that recruit different factors, and kinesin-13 depolymerases, which curb growth or trigger disassembly (Akhmanova and Steinmetz, 2008). Interestingly, proteins that regulate the dynamics of cytoplasmic or kinetochore microtubules—which typically undergo relatively rapid growth and shortening—predominantly bind to the outer microtubule surface. In contrast, centriolar and ciliary regulators span both surfaces and form luminal accumulations. Binding to the luminal side appears to be a general mechanism for generating stable microtubules, as seen in MAP6-dependent cold-stable microtubules in neurons (Cuveillier et al., 2020) and in axonemes of motile cilia (Leung et al., 2025; Ma et al., 2019). Because diffusion within the microtubule lumen is limited (Odde, 1998), luminal binding sites are likely used primarily in contexts where dynamics are slow, such as in cilia and centrioles.

The next challenge for the field is twofold: to increase the complexity of biochemical reconstitution assays—by, for example, combining stabilizing and destabilizing factors—and to perform quantitative measurements in cells to define the dynamics and copy numbers of regulatory proteins at individual microtubule tips. Multivalent protein interaction networks, including those formed by microtubule tip-binding proteins, are prone to condensate formation at elevated concentrations (reviewed in Volkov and Akhmanova [2024]), a property that may in some cases underlie their collective behavior. Yet, many centriolar and ciliary tip regulators are strikingly potent, with as few as two to three molecules sufficient to profoundly alter microtubule growth or shortening. Only precise quantitative measurements in cells will reveal which concentration regimes and mechanisms are physiologically relevant.

Reconstitution of ciliary microtubule regulators needs to take into consideration a potentially limiting supply of tubulin dimers, a topic extensively discussed in the context of ciliary length control (Craft Van De Weghe et al., 2020; Marshall, 2023), and the fact that microtubules elongate in a close proximity to a membrane barrier, which would exert a force on the microtubule plus end, potentially inhibit its growth, and affect its structure (Dogterom et al., 2005; Janson et al., 2003). Both factors can be taken into account in reconstitution assays, by varying tubulin concentration and by placing physical barriers in the path of microtubule growth (Janson et al., 2003). Another important challenge is to incorporate into *in vitro* reconstitutions microtubule substrates that are representative of centriolar and ciliary microtubules. A key limitation of the *in vitro* studies described above was that they were focused on singlet microtubules prepared from brain tubulin. However, in cells, centriole and ciliary proteins regulate growth and length of microtubule doublets and triplets built from specific tubulin isotypes and bearing specific posttranslational modifications. Recent advances have enabled the *in vitro* reconstitution of doublet-like microtubules using various approaches (Gopal et al., 2025; Khuntsariya et al., 2025, *Preprint*; Li et al., 2025a, *Preprint*; Li et al., 2025b; Schmidt-Cernohorska et al., 2019). These studies set the stage for mechanistic studies that would examine the effects of the centriolar cap and the ciliary tip proteins on more complex microtubule substrates. Ultimately, such complex reconstitutions will need to be combined with advanced structural analyses, simulations, and modeling. Together, these approaches will reveal how cooperative conformational transitions at microtubule tips integrate regulatory activities that govern microtubule growth dynamics to generate the unique architecture of centrioles and cilia.

Online supplemental material

Table S1 provides an overview of the proteins discussed in the review and shows their domain organization, interactions with microtubules, tubulin and other proteins, effects on microtubule dynamics in vitro, function during centriole and cilia biogenesis and disease relevance.

Acknowledgments

Author contributions: Saishree S. Iyer: conceptualization, visualization, and writing—original draft, review, and editing. Anna Akhmanova: conceptualization, supervision, and writing—original draft, review, and editing.

Disclosures: The authors declare no competing interests exist.

Submitted: 28 January 2026
Revised: 17 April 2026
Accepted: 17 April 2026

References

Abbühl, D., M. Pruzincova, L. Stepanek, E. Bouscasse, R. Azevedo, M. Matondo, V. Varga, S. Bonnefoy, and P. Bastin. 2025. A novel approach to tagging tubulin reveals microtubule assembly dynamics of the axoneme in Trypanosoma brucei. *J. Cell Sci.* 138:jcs264145. https://doi.org/10.1242/jcs.264145

Aher, A., M. Kok, A. Sharma, A. Rai, N. Olieric, R. Rodriguez-Garcia, E.A. Katrukha, T. Weinert, V. Olieric, L.C. Kapitein, et al. 2018. CLASP suppresses microtubule catastrophes through a single TOG domain. *Dev. Cell.* 46:40–58.e8. https://doi.org/10.1016/j.devcel.2018.05.032

Aher, A., D. Rai, L. Schaedel, J. Gaillard, K. John, Q. Liu, M. Altelaar, L. Blanchoin, M. Thery, and A. Akhmanova. 2020. CLASP mediates microtubule repair by restricting lattice damage and regulating tubulin incorporation. *Curr. Biol.* 30:2175–2183.e6. https://doi.org/10.1016/j.cub.2020.03.070

Ahmad, S., L. Pecqueur, B. Dreier, D. Hamdane, M. Aumont-Nicaise, A. Plückthun, M. Knossow, and B. Gigant. 2016. Destabilizing an interacting motif strengthens the association of a designed ankyrin repeat protein with tubulin. *Sci. Rep.* 6:28922. https://doi.org/10.1038/srep28922

Akhmanova, A., and M.O. Steinmetz. 2008. Tracking the ends: A dynamic protein network controls the fate of microtubule tips. *Nat. Rev. Mol. Cell Biol.* 9:309–322. https://doi.org/10.1038/nrm2369

Akizu, N., J.L. Silhavy, R.O. Rosti, E. Scott, A.G. Fenstermaker, J. Schroth, M.S. Zaki, H. Sanchez, N. Gupta, M. Kabra, et al. 2014. Mutations in CSPP1 lead to classical Joubert syndrome. *Am. J. Hum. Genet.* 94:80–86. https://doi.org/10.1016/j.ajhg.2013.11.015

Al-Dosari, M.S., R. Shaheen, D. Colak, and F.S. Alkuraya. 2010. Novel CENPJ mutation causes Seckel syndrome. *J. Med. Genet.* 47:411–414. https://doi.org/10.1136/jmg.2009.076646

Al-Jassar, C., A. Andreeva, D.D. Barnabas, S.H. McLaughlin, C.M. Johnson, M. Yu, and M. van Breugel. 2017. The ciliopathy-associated Cep104 protein interacts with tubulin and Nek1 kinase. *Structure.* 25:146–156. https://doi.org/10.1016/j.str.2016.11.014

Alsafh, R., A. Alhashem, A. Elsyed, Z. Yuksel, K. Graiess-Tlili, K. Hundallah, F. Thabet, and B. Tabarki. 2024. Multiplex consanguineous family highlights *CLASP1* as a candidate gene for lissencephaly. *Neurol. Genet.* 10:e200172. https://doi.org/10.1212/NXG.0000000000200172

Andrade, M.A., C. Petosa, S.I. O'Donoghue, C.W. Müller, and P. Bork. 2001. Comparison of ARM and HEAT protein repeats. *J. Mol. Biol.* 309:1–18. https://doi.org/10.1006/jmbi.2001.4624

Atherton, J., K. Jiang, M.M. Stangier, Y. Luo, S. Hua, K. Houben, J.J.E. van Hooff, A.P. Joseph, G. Scarabelli, B.J. Grant, et al. 2017. A structural model for microtubule minus-end recognition and protection by CAMSAP proteins. *Nat. Struct. Mol. Biol.* 24:931–943. https://doi.org/10.1038/nsmb.3483

Atherton, J., M. Stouffer, F. Francis, and C.A. Moores. 2018. Microtubule architecture in vitro and in cells revealed by cryo-electron tomography. *Acta Crystallogr. D Struct. Biol.* 74:572–584. https://doi.org/10.1107/S2059798318001948

Ayaz, P., S. Munyoki, E.A. Geyer, F.A. Piedra, E.S. Vu, R. Bromberg, Z. Otwinowski, N.V. Grishin, C.A. Brautigam, and L.M. Rice. 2014. A tethered delivery mechanism explains the catalytic action of a microtubule polymerase. *Elife*. 3:e03069. https://doi.org/10.7554/eLife.03069

Aydogan, M.G., A. Wainman, S. Saurya, T.L. Steinacker, A. Caballe, Z.A. Novak, J. Baumbach, N. Muschalik, and J.W. Raff. 2018. A homeostatic clock sets daughter centriole size in flies. *J. Cell Biol.* 217:1233–1248. https://doi.org/10.1083/jcb.201801014

Bacaj, T., Y. Lu, and S. Shaham. 2008. The conserved proteins CHE-12 and DYF-11 are required for sensory cilium function in Caenorhabditis elegans. *Genetics*. 178:989–1002. https://doi.org/10.1534/genetics.107.082453

Badano, J.L., N. Mitsuma, P.L. Beales, and N. Katsanis. 2006. The ciliopathies: An emerging class of human genetic disorders. *Annu. Rev. Genomics Hum. Genet.* 7:125–148. https://doi.org/10.1146/annurev.genom.7.080505.115610

Badv, R.S., M. Mahdiannasser, M. Rasoulinezhad, L. Habibi, and A. Rashidi-Nezhad. 2022. CEP104 gene may involve in the pathogenesis of a new developmental disorder other than Joubert syndrome. *Mol. Biol. Rep.* 49:7231–7237. https://doi.org/10.1007/s11033-022-07353-w

Banterle, N., and P. Gönczy. 2017. Centriole biogenesis: From identifying the characters to understanding the plot. *Annu. Rev. Cell Dev. Biol.* 33:23–49. https://doi.org/10.1146/annurev-cellbio-100616-060454

Barakeh, D., E. Faqeih, S. Anazi, M. S Al-Dosari, A. Softah, F. Albadr, H. Hassan, A.M. Alazami, and F.S. Alkuraya. 2015. The many faces of KIF7. *Hum. Genome*. 2:15006. https://doi.org/10.1038/hgv.2015.6

Basto, R., J. Lau, T. Vinogradova, A. Gardiol, C.G. Woods, A. Khodjakov, and J.W. Raff. 2006. Flies without centrioles. *Cell*. 125:1375–1386. https://doi.org/10.1016/j.cell.2006.05.025

Batman, U., J. Deretic, and E.N. Firat-Karalar. 2022. The ciliopathy protein CCDC66 controls mitotic progression and cytokinesis by promoting microtubule nucleation and organization. *PLoS Biol.* 20:e3001708. https://doi.org/10.1371/journal.pbio.3001708

Ben-Omran, T., R. Alsulaiman, H. Kamel, R. Shaheen, and F.S. Alkuraya. 2015. Intrafamilial clinical heterogeneity of CSPP1-related ciliopathy. *Am. J. Med. Genet. A*. 167A:2478–2480. https://doi.org/10.1002/ajmg.a.37175

Bie, L., G. Zhao, Y.-p. Wang, and B. Zhang. 2012. Kinesin family member 2C (KIF2C/MCAK) is a novel marker for prognosis in human gliomas. *Clin. Neurol. Neurosurg.* 114:356–360. https://doi.org/10.1016/j.clineuro.2011.11.005

Blaineau, C., M. Tessier, P. Dubessay, L. Tasse, L. Crobu, M. Pagès, and P. Bastien. 2007. A novel microtubule-depolymerizing kinesin involved in length control of a eukaryotic flagellum. *Curr. Biol.* 17:778–782. https://doi.org/10.1016/j.cub.2007.03.048

Blasius, T.L., Y. Yue, R. Prasad, X. Liu, A. Gennerich, and K.J. Verhey. 2021. Sequences in the stalk domain regulate auto-inhibition and ciliary tip localization of the immotile kinesin-4 KIF7. *J. Cell Sci.* 134:jcs258464. https://doi.org/10.1242/jcs.258464

Bond, J., E. Roberts, K. Springell, S.B. Lizarraga, S. Scott, J. Higgins, D.J. Hampshire, E.E. Morrison, G.F. Leal, E.O. Silva, et al. 2005. A centrosomal mechanism involving CDK5RAP2 and CENPJ controls brain size. *Nat. Genet.* 37:353–355. https://doi.org/10.1038/ng1539

Brouhard, G., and D. Sept. 2012. Microtubules: Sizing up the GTP cap. *Curr. Biol.* 22:R802–R803. https://doi.org/10.1016/j.cub.2012.07.050

Brouhard, G.J., and L.M. Rice. 2014. The contribution of alphabeta-tubulin curvature to microtubule dynamics. *J. Cell Biol.* 207:323–334. https://doi.org/10.1083/jcb.201407095

Brouhard, G.J., J.H. Stear, T.L. Noetzel, J. Al-Bassam, K. Kinoshita, S.C. Harrison, J. Howard, and A.A. Hyman. 2008. XMAP215 is a processive microtubule polymerase. *Cell*. 132:79–88. https://doi.org/10.1016/j.cell.2007.11.043

Campanacci, V., A. Urvoas, L. Ammar Khodja, M. Aumont-Nicaise, M. Noiray, S. Lachkar, P.A. Curmi, P. Minard, and B. Gigant. 2022. Structural convergence for tubulin binding of CPAP and vinca domain microtubule inhibitors. *Proc. Natl. Acad. Sci. USA*. 119:e2120098119. https://doi.org/10.1073/pnas.2120098119

Carlier, M.F., and D. Pantaloni. 1981. Kinetic analysis of guanosine 5′-triphosphate hydrolysis associated with tubulin polymerization. *Biochemistry*. 20:1918–1924. https://doi.org/10.1021/bi00510a030

Chainaphaphorn, P., C. Ngamphiw, S. Tongsima, K. Chintakanon, K. Kawasaki, T.C. Cox, A. Ohazama, J.R. Ketudat Cairns, and P.N. Kantaputra. 2025. Genetic variants in KIF7 may contribute to supernumerary tooth formation. *Int. Dent J.* 75:100928. https://doi.org/10.1016/j.identj.2025.100928

Chen, Z., V.B. Indjeian, M. McManus, L. Wang, and B.D. Dynlacht. 2002. CP110, a cell cycle-dependent CDK substrate, regulates centrosome duplication in human cells. *Dev. Cell*. 3:339–350. https://doi.org/10.1016/s1534-5807(02)00258-7

Chrétien, D., B. Buendia, S.D. Fuller, and E. Karsenti. 1997. Reconstruction of the centrosome cycle from cryoelectron micrographs. *J. Struct. Biol.* 120:117–133. https://doi.org/10.1006/jsbi.1997.3928

Chrétien, D., S.D. Fuller, and E. Karsenti. 1995. Structure of growing microtubule ends: Two-dimensional sheets close into tubes at variable rates. *J. Cell Biol.* 129:1311–1328. https://doi.org/10.1083/jcb.129.5.1311

Cleary, J.M., and W.O. Hancock. 2021. Molecular mechanisms underlying microtubule growth dynamics. *Curr. Biol.* 31:R560–R573. https://doi.org/10.1016/j.cub.2021.02.035

Comartin, D., G.D. Gupta, E. Fussner, E. Coyaud, M. Hasegan, M. Archinti, S.W. Cheung, D. Pinchev, S. Lawo, B. Raught, et al. 2013. CEP120 and SPICE1 cooperate with CPAP in centriole elongation. *Curr. Biol.* 23:1360–1366. https://doi.org/10.1016/j.cub.2013.06.002

Conduit, P.T., A. Wainman, Z.A. Novak, T.T. Weil, and J.W. Raff. 2015. Re-examining the role of Drosophila Sas-4 in centrosome assembly using two-colour-3D-SIM FRAP. *Elife*. 4:e08483. https://doi.org/10.7554/eLife.08483

Conkar, D., H. Bayraktar, and E.N. Firat-Karalar. 2019. Centrosomal and ciliary targeting of CCDC66 requires cooperative action of centriolar satellites, microtubules and molecular motors. *Sci. Rep.* 9:14250. https://doi.org/10.1038/s41598-019-50530-4

Conkar, D., E. Culfa, E. Odabasi, N. Rauniyar, J.R. Yates, and E.N. Firat-Karalar. 2017. The centriolar satellite protein CCDC66 interacts with CEP290 and functions in cilium formation and trafficking. *J. Cell Sci.* 130:1450–1462. https://doi.org/10.1242/jcs.196832

Cormier, A., M.J. Clement, M. Knossow, S. Lachkar, P. Savarin, F. Toma, A. Sobel, B. Gigant, and P.A. Curmi. 2009. The PN2-3 domain of centrosomal P4.1-associated protein implements a novel mechanism for tubulin sequestration. *J. Biol. Chem.* 284:6909–6917. https://doi.org/10.1074/jbc.M808249200

Craft Van De Weghe, J., J.A. Harris, T. Kubo, G.B. Witman, and K.F. Lechtreck. 2020. Diffusion rather than intraflagellar transport likely provides most of the tubulin required for axonemal assembly in Chlamydomonas. *J. Cell Sci.* 133:jcs249805. https://doi.org/10.1242/jcs.249805

Cuccurullo, C., G. Miele, G. Piccolo, L. Bilo, A. Accogli, A. D'Amico, M. Fratta, S. Guerrisi, M. Iacomino, V. Salpietro, et al. 2022. Hydranencephaly in CENPJ-related Seckel syndrome. *Eur. J. Med. Genet.* 65:104659. https://doi.org/10.1016/j.ejmg.2022.104659

Cutts, E.E., A. Inglis, P.J. Stansfeld, I. Vakonakis, and G.N. Hatzopoulos. 2015. The centriolar protein CPAP G-box: An amyloid fibril in a single domain. *Biochem. Soc. Trans.* 43:838–843. https://doi.org/10.1042/BST20150082

Cuveillier, C., J. Delaroche, M. Seggio, S. Gory-Faure, C. Bosc, E. Denarier, M. Bacia, G. Schoehn, H. Mohrbach, I. Kulić, et al. 2020. MAP6 is an intraluminal protein that induces neuronal microtubules to coil. *Sci. Adv.* 6:eaaz4344. https://doi.org/10.1126/sciadv.aaz4344

Dafinger, C., M.C. Liebau, S.M. Elsayed, Y. Hellenbroich, E. Boltshauser, G.C. Korenke, F. Fabretti, A.R. Janecke, I. Ebermann, G. Nürnberg, et al. 2011. Mutations in KIF7 link Joubert syndrome with Sonic Hedgehog signaling and microtubule dynamics. *J. Clin. Invest.* 121:2662–2667. https://doi.org/10.1172/JCI43639

Das, A., D.J. Dickinson, C.C. Wood, B. Goldstein, and K.C. Slep. 2015. Crescerin uses a TOG domain array to regulate microtubules in the primary cilium. *Mol. Biol. Cell*. 26:4248–4264. https://doi.org/10.1091/mbc.E15-08-0603

David-Pfeuty, T., H.P. Erickson, and D. Pantaloni. 1977. Guanosinetriphosphatase activity of tubulin associated with microtubule assembly. *Proc. Natl. Acad. Sci. USA*. 74:5372–5376. https://doi.org/10.1073/pnas.74.12.5372

Dekomien, G., C. Vollrath, E. Petrasch-Parwez, M.H. Boevé, D.A. Akkad, W.M. Gerding, and J.T. Epplen. 2010. Progressive retinal atrophy in Schapendoes dogs: Mutation of the newly identified CCDC66 gene. *Neurogenetics*. 11:163–174. https://doi.org/10.1007/s10048-009-0223-z

Delgehyr, N., H. Rangone, J. Fu, G. Mao, B. Tom, M.G. Riparbelli, G. Callaini, and D.M. Glover. 2012. Klp10A, a microtubule-depolymerizing kinesin-13, cooperates with CP110 to control Drosophila centriole length. *Curr. Biol.* 22:502–509. https://doi.org/10.1016/j.cub.2012.01.046

Dentler, W.L., and J.L. Rosenbaum. 1977. Flagellar elongation and shortening in Chlamydomonas. III. structures attached to the tips of flagellar microtubules and their relationship to the directionality of flagellar microtubule assembly. *J. Cell Biol.* 74:747–759. https://doi.org/10.1083/jcb.74.3.747

Deretic, J., S. Cengiz-Emek, E. Seyrek, and E.N. Firat-Karalar. 2025. CCDC66 regulation of cytoskeleton and cilia stability is important for signaling and epithelial organization. *PLoS Biol.* 23:e3003313. https://doi.org/10.1371/journal.pbio.3003313

Deretic, J., E. Odabasi, and E.N. Firat-Karalar. 2023. The multifaceted roles of microtubule-associated proteins in the primary cilium and ciliopathies. *J. Cell Sci.* 136:jcs261148. https://doi.org/10.1242/jcs.261148

Desai, A., and T.J. Mitchison. 1997. Microtubule polymerization dynamics. *Annu. Rev. Cell Dev. Biol.* 13:83–117. https://doi.org/10.1146/annurev.cellbio.13.1.83

Di Stazio, M., A. Morgan, M. Brumat, S. Bassani, D. Dell'Orco, V. Marino, P. Garagnani, C. Giuliani, P. Gasparini, and G. Girotto. 2020. New age-related hearing loss candidate genes in humans: An ongoing challenge. *Gene*. 742:144561. https://doi.org/10.1016/j.gene.2020.144561

Dobbelaere, J., M. Schmidt Cernohorska, M. Huranova, D. Slade, and A. Dammermann. 2020. Cep97 is required for centriole structural integrity and cilia formation in drosophila. *Curr. Biol.* 30:3045–3056.e7. https://doi.org/10.1016/j.cub.2020.05.078

Dogterom, M., J.W.J. Kerssemakers, G. Romet-Lemonne, and M.E. Janson. 2005. Force generation by dynamic microtubules. *Curr. Opin. Cell Biol.* 17:67–74. https://doi.org/10.1016/j.ceb.2004.12.011

Doodhi, H., A.E. Prota, R. Rodriguez-Garcia, H. Xiao, D.W. Custar, K. Bargsten, E.A. Katrukha, M. Hilbert, S. Hua, K. Jiang, et al. 2016. Termination of protofilament elongation by eribulin induces lattice defects that promote microtubule catastrophes. *Curr. Biol.* 26:1713–1721. https://doi.org/10.1016/j.cub.2016.04.053

Farmer, V., G. Arpağ, S.L. Hall, and M. Zanic. 2021. XMAP215 promotes microtubule catastrophe by disrupting the growing microtubule end. *J. Cell Biol.* 220:e202012144. https://doi.org/10.1083/jcb.202012144

Farmer, V.J., and M. Zanic. 2023. Beyond the GTP-cap: Elucidating the molecular mechanisms of microtubule catastrophe. *Bioessays*. 45:e2200081. https://doi.org/10.1002/bies.202200081

Foster, H.E., C. Ventura Santos, and A.P. Carter. 2022. A cryo-ET survey of microtubules and intracellular compartments in mammalian axons. *J. Cell Biol.* 221:e202103154. https://doi.org/10.1083/jcb.202103154

Franz, A., H. Roque, S. Saurya, J. Dobbelaere, and J.W. Raff. 2013. CP110 exhibits novel regulatory activities during centriole assembly in Drosophila. *J. Cell Biol.* 203:785–799. https://doi.org/10.1083/jcb.201305109

Friel, C.T., and J.P. Welburn. 2018. Parts list for a microtubule depolymerising kinesin. *Biochem. Soc. Trans.* 46:1665–1672. https://doi.org/10.1042/BST20180350

Frikstad, K.-M., E. Molinari, M. Thoresen, S.A. Ramsbottom, F. Hughes, S.J.F. Letteboer, S. Gilani, K.O. Schink, T. Stokke, S. Geimer, et al. 2019. A CEP104-CSPP1 complex is required for formation of primary cilia competent in hedgehog signaling. *Cell Rep.* 28:1907–1922. https://doi.org/10.1016/j.celrep.2019.07.025

Gerding, W.M., S. Schreiber, T. Schulte-Middelmann, A. de Castro Marques, J. Atorf, D.A. Akkad, G. Dekomien, J. Kremers, R. Dermietzel, A. Gal, et al. 2011. Ccdc66 null mutation causes retinal degeneration and dysfunction. *Hum. Mol. Genet.* 20:3620–3631. https://doi.org/10.1093/hmg/ddr282

Glass, I.A., J.C. Dempsey, M. Parisi, and D. Doherty. 1993. Joubert Syndrome. *In* GeneReviews((R)). M.P. Adam, S. Bick, G.M. Mirzaa, R.A. Pagon, S.E. Wallace, and A. Amemiya, editors. Seattle, University of Washington, Seattle.

Goodson, H.V., and E.M. Jonasson. 2018. Microtubules and microtubule-associated proteins. *Cold Spring Harb. Perspect. Biol.* 10:a022608. https://doi.org/10.1101/cshperspect.a022608

Gopal, D., J. Wu, J. Delaroche, C. Bosc, M. De Andrade, E. Denarier, G. Effantin, A. Andrieux, S. Gory-Fauré, L. Serre, and I. Arnal. 2025. The Mn-motif protein MAP6d1 assembles ciliary doublet microtubules. *Nat. Commun.* 16:6210. https://doi.org/10.1038/s41467-025-61679-0

Gopalakrishnan, J., V. Mennella, S. Blachon, B. Zhai, A.H. Smith, T.L. Megraw, D. Nicastro, S.P. Gygi, D.A. Agard, and T. Avidor-Reiss. 2011. Sas-4 provides a scaffold for cytoplasmic complexes and tethers them in a centrosome. *Nat. Commun.* 2:359. https://doi.org/10.1038/ncomms1367

Gudimchuk, N.B., and J.R. McIntosh. 2021. Regulation of microtubule dynamics, mechanics and function through the growing tip. *Nat. Rev. Mol. Cell Biol.* 22:777–795. https://doi.org/10.1038/s41580-021-00399-x

Gudimchuk, N.B., E.V. Ulyanov, E. O'Toole, C.L. Page, D.S. Vinogradov, G. Morgan, G. Li, J.K. Moore, E. Szczesna, A. Roll-Mecak, et al. 2020. Mechanisms of microtubule dynamics and force generation examined with computational modeling and electron cryotomography. *Nat. Commun.* 11:3765. https://doi.org/10.1038/s41467-020-17553-2

Guichard, P., D. Chrétien, S. Marco, and A.M. Tassin. 2010. Procentriole assembly revealed by cryo-electron tomography. *EMBO J.* 29:1565–1572. https://doi.org/10.1038/emboj.2010.45

Hatzopoulos, G.N., M.C. Erat, E. Cutts, K.B. Rogala, L.M. Slater, P.J. Stansfeld, and I. Vakonakis. 2013. Structural analysis of the G-box domain of the microcephaly protein CPAP suggests a role in centriole architecture. *Structure*. 21:2069–2077. https://doi.org/10.1016/j.str.2013.08.019

He, M., R. Subramanian, F. Bangs, T. Omelchenko, K.F. Liem Jr., T.M. Kapoor, and K.V. Anderson. 2014. The kinesin-4 protein Kif7 regulates mammalian Hedgehog signalling by organizing the cilium tip compartment. *Nat. Cell Biol.* 16:663–672. https://doi.org/10.1038/ncb2988

Helenius, J., G. Brouhard, Y. Kalaidzidis, S. Diez, and J. Howard. 2006. The depolymerizing kinesin MCAK uses lattice diffusion to rapidly target microtubule ends. *Nature*. 441:115–119. https://doi.org/10.1038/nature04736

Hildebrandt, F., T. Benzing, and N. Katsanis. 2011. Ciliopathies. *N. Engl. J. Med.* 364:1533–1543. https://doi.org/10.1056/NEJMra1010172

Hong, J., C. Lee, G. Madhu, O. Papoulas, E. Atayeter, G. Hoogerbrugge, J. Pan, M. Takagishi, N.I. Manzi, D.J. Dickinson, et al. 2025. A protein complex in the extreme distal tip of vertebrate motile cilia controls their organization, length, and function. *Nat. Commun.* 17:394. https://doi.org/10.1038/s41467-025-67086-9

Höög, J.L., S. Lacomble, E.T. O'Toole, A. Hoenger, J.R. McIntosh, and K. Gull. 2014. Modes of flagellar assembly in Chlamydomonas reinhardtii and Trypanosoma brucei. *Elife*. 3:e01479. https://doi.org/10.7554/eLife.01479

Hossein Garakani, M., K. Kakavand, M. Sabbaghian, A. Ghaheri, N.S. Masoudi, M. Shahhoseini, V. Hassanzadeh, M. Zamanian, A.M. Meybodi, and S.Z. Moradi. 2024. Comprehensive analysis of chromosomal breakpoints and candidate genes associated with male infertility: Insights from cytogenetic studies and expression analyses. *Mamm. Genome*. 35:764–783. https://doi.org/10.1007/s00335-024-10074-z

Hsu, W.B., L.Y. Hung, C.J. Tang, C.L. Su, Y. Chang, and T.K. Tang. 2008. Functional characterization of the microtubule-binding and -destabilizing domains of CPAP and d-SAS-4. *Exp. Cell Res.* 314:2591–2602. https://doi.org/10.1016/j.yexcr.2008.05.012

Hung, L.Y., H.L. Chen, C.W. Chang, B.R. Li, and T.K. Tang. 2004. Identification of a novel microtubule-destabilizing motif in CPAP that binds to tubulin heterodimers and inhibits microtubule assembly. *Mol. Biol. Cell*. 15:2697–2706. https://doi.org/10.1091/mbc.e04-02-0121

Hung, L.Y., C.J. Tang, and T.K. Tang. 2000. Protein 4.1 R-135 interacts with a novel centrosomal protein (CPAP) which is associated with the gamma-tubulin complex. *Mol. Cell Biol.* 20:7813–7825. https://doi.org/10.1128/MCB.20.20.7813-7825.2000

Iyer, S.S., F. Chen, F.E. Ogunmolu, S. Moradi, V.A. Volkov, E.J. van Grinsven, C. van Hoorn, J. Wu, N. Andrea, S. Hua, et al. 2025. Centriolar cap proteins CP110 and CPAP control slow elongation of microtubule plus ends. *J. Cell Biol.* 224:e202406061. https://doi.org/10.1083/jcb.202406061

Jaiswal, S., S. Sanghi, and P. Singh. 2023. Separation-of-function MCPH-associated mutations in CPAP affect centriole number and length. *J. Cell Sci.* 136:jcs261297. https://doi.org/10.1242/jcs.261297

Janson, M.E., M.E. de Dood, and M. Dogterom. 2003. Dynamic instability of microtubules is regulated by force. *J. Cell Biol.* 161:1029–1034. https://doi.org/10.1083/jcb.200301147

Jiang, K., G. Toedt, S. Montenegro Gouveia, N.E. Davey, S. Hua, B. van der Vaart, I. Grigoriev, J. Larsen, L.B. Pedersen, K. Bezstarosti, et al. 2012. A proteome-wide screen for mammalian SxIP motif-containing microtubule plus-end tracking proteins. *Curr. Biol.* 22:1800–1807. https://doi.org/10.1016/j.cub.2012.07.047

Jiang, S., N. Mani, E.M. Wilson-Kubalek, P.I. Ku, R.A. Milligan, and R. Subramanian. 2019. Interplay between the Kinesin and Tubulin mechanochemical cycles underlies microtubule tip tracking by the non-motile ciliary kinesin Kif7. *Dev. Cell*. 49:711–730. https://doi.org/10.1016/j.devcel.2019.04.001

Kalutskii, M., H. Grubmuller, V.A. Volkov, and M. Igaev. 2025. Microtubule dynamics are defined by conformations and stability of clustered protofilaments. *Proc. Natl. Acad. Sci. USA*. 122:e2424263122. https://doi.org/10.1073/pnas.2424263122

Kar, A., S.R. Phadke, A. Das Bhowmik, and A. Dalal. 2018. Whole exome sequencing reveals a mutation in ARMC9 as a cause of mental retardation, ptosis, and polydactyly. *Am. J. Med. Genet. A*. 176:34–40. https://doi.org/10.1002/ajmg.a.38537

Khoshbakht, S., M. Beheshtian, Z. Fattahi, N. Bazazzadegan, E. Parsimehr, M. Fadaee, R. Vazehan, M. Faraji Zonooz, A. Abolhassani, M. Makvand, et al. 2021. CEP104 and CEP290; genes with ciliary functions cause intellectual disability in multiple families. *Arch. Iran Med.* 24:364–373. https://doi.org/10.34172/aim.2021.53

Khuntsariya, D., U. Batman, A. Krishnan, D. Rozbesky, F. Lemaitre, C. Janke, V. Hamel, P. Guichard, M. Braun, and Z. Lansky. 2025. Cell-free reconstitution reveals synergistic stabilization of microtubule doublets by PACRG and FAP20. *bioRxiv*. https://doi.org/10.1101/2025.03.12.642377 (Preprint posted March 12, 2025).

Kiesel, P., G. Alvarez Viar, N. Tsoy, R. Maraspini, P. Gorilak, V. Varga, A. Honigmann, and G. Pigino. 2020. The molecular structure of mammalian primary cilia revealed by cryo-electron tomography. *Nat. Struct. Mol. Biol.* 27:1115–1124. https://doi.org/10.1038/s41594-020-0507-4

Klena, N., and G. Pigino. 2022. Structural biology of cilia and intraflagellar transport. *Annu. Rev. Cell Dev Biol.* 38:103–123. https://doi.org/10.1146/annurev-cellbio-120219-034238

Kleylein-Sohn, J., J. Westendorf, M. Le Clech, R. Habedanck, Y.D. Stierhof, and E.A. Nigg. 2007. Plk4-induced centriole biogenesis in human cells. *Dev. Cell*. 13:190–202. https://doi.org/10.1016/j.devcel.2007.07.002

Kobayashi, T., W.Y. Tsang, J. Li, W. Lane, and B.D. Dynlacht. 2011. Centriolar kinesin Kif24 interacts with CP110 to remodel microtubules and regulate ciliogenesis. *Cell*. 145:914–925. https://doi.org/10.1016/j.cell.2011.04.028

Kohlmaier, G., J. Loncarek, X. Meng, B.F. McEwen, M.M. Mogensen, A. Spektor, B.D. Dynlacht, A. Khodjakov, and P. Gönczy. 2009. Overly long centrioles and defective cell division upon excess of the SAS-4-related protein CPAP. *Curr. Biol.* 19:1012–1018. https://doi.org/10.1016/j.cub.2009.05.018

Kong, D., N. Sahabandu, C. Sullenberger, A. Vasquez-Limeta, D. Luvsanjav, K. Lukasik, and J. Loncarek. 2020. Prolonged mitosis results in structurally aberrant and over-elongated centrioles. *J. Cell Biol.* 219:e201910019. https://doi.org/10.1083/jcb.201910019

Kreis, N.N., H.H. Moon, L. Wordeman, F. Louwen, C. Solbach, J. Yuan, and A. Ritter. 2024. KIF2C/MCAK a prognostic biomarker and its oncogenic potential in malignant progression, and prognosis of cancer patients: A systematic review and meta-analysis as biomarker. *Crit. Rev. Clin. Lab Sci.* 61:404–434. https://doi.org/10.1080/10408363.2024.2309933

Kuriyama, R., and G.G. Borisy. 1981. Centriole cycle in Chinese hamster ovary cells as determined by whole-mount electron microscopy. *J. Cell Biol.* 91: 814–821. https://doi.org/10.1083/jcb.91.3.814

Lai, Y., B. Chen, J. Shi, J.N. Palmer, D.W. Kennedy, and N.A. Cohen. 2011. Inflammation-mediated upregulation of centrosomal protein 110, a negative modulator of ciliogenesis, in patients with chronic rhinosinusitis. *J. Allergy Clin. Immunol.* 128:1207–1215.e1. https://doi.org/10.1016/j.jaci.2011.09.001

Latour, B.L., J.C. Van De Weghe, T.D. Rusterholz, S.J. Letteboer, A. Gomez, R. Shaheen, M. Gesemann, A. Karamzade, M. Asadollahi, M. Barroso-Gil, et al. 2020. Dysfunction of the ciliary ARMC9/TOGARAM1 protein module causes Joubert syndrome. *J. Clin. Invest.* 130:4423–4439. https://doi.org/10.1172/JCI131656

Lawrence, E.J., M. Zanic, and L.M. Rice. 2020. CLASPs at a glance. *J. Cell Sci.* 133:jcs243097. https://doi.org/10.1242/jcs.243097

Legal, T., M. Parra, M. Tong, C.S. Black, E. Joachimiak, M. Valente-Paterno, K. Lechtreck, J. Gaertig, and K.H. Bui. 2023. CEP104/FAP256 and associated cap complex maintain stability of the ciliary tip. *J. Cell Biol.* 222: e202301129. https://doi.org/10.1083/jcb.202301129

Leidel, S., and P. Gönczy. 2003. SAS-4 is essential for centrosome duplication in C elegans and is recruited to daughter centrioles once per cell cycle. *Dev. Cell*. 4:431–439. https://doi.org/10.1016/s1534-5807(03)00062-5

Leung, M.R., M.C. Roelofs, R.T. Ravi, P. Maitan, H. Henning, M. Zhang, E.G. Bromfield, S.C. Howes, B.M. Gadella, H. Bloomfield-Gadelha, and T. Zeev-Ben-Mordehai. 2021. The multi-scale architecture of mammalian sperm flagella and implications for ciliary motility. *EMBO J.* 40:e107410. https://doi.org/10.15252/embj.2020107410

Leung, M.R., C. Sun, J. Zeng, J.R. Anderson, Q. Niu, W. Huang, W.E.M. Noteborn, A. Brown, T. Zeev-Ben-Mordehai, and R. Zhang. 2025. Structural diversity of axonemes across mammalian motile cilia. *Nature*. 637:1170–1177. https://doi.org/10.1038/s41586-024-08337-5

Li, M., G. Chen, Z. Chen, Z. Guo, Z. Wang, Y. Chai, W. Li, and G. Ou. 2025a. Physiological reconstitution of microtubule doublets. *bioRxiv*. https://doi.org/10.1101/2025.08.03.668368 (Preprint posted August 4, 2025).

Lin, Y.-C., C.-W. Chang, W.-B. Hsu, C.-J.C. Tang, Y.-N. Lin, E.-J. Chou, C.-T. Wu, and T.-K. Tang. 2013a. Human microcephaly protein CEP135 binds to hSAS-6 and CPAP, and is required for centriole assembly. *EMBO J.* 32: 1141–1154. https://doi.org/10.1038/emboj.2013.56

Li, M., Z. Chen, Z. Guo, Y. Wang, Y. Chai, W. Li, and G. Ou. 2025b. Alpha-tubulin tails regulate axoneme differentiation. *Proc. Natl. Acad. Sci. USA*. 122:e2414731122. https://doi.org/10.1073/pnas.2414731122

Lin, Y.-N., C.-T. Wu, Y.-C. Lin, W.-B. Hsu, C.-J.C. Tang, C.-W. Chang, and T.-K. Tang. 2013b. CEP120 interacts with CPAP and positively regulates centriole elongation. *J. Cell Biol.* 202:211–219. https://doi.org/10.1083/jcb.201212060

Loncarek, J., and M. Bettencourt-Dias. 2018. Building the right centriole for each cell type. *J. Cell Biol.* 217:823–835. https://doi.org/10.1083/jcb.201704093

Louka, P., K.K. Vasudevan, M. Guha, E. Joachimiak, D. Wloga, R.F.-X. Tomasi, C.N. Baroud, P. Dupuis-Williams, D.F. Galati, C.G. Pearson, et al. 2018. Proteins that control the geometry of microtubules at the ends of cilia. *J. Cell Biol.* 217:4298–4313. https://doi.org/10.1083/jcb.201804141

Lu, H., C. Wang, L. Xue, Q. Zhang, F. Luh, J. Wang, T.G. Lin, Y. Yen, and X. Liu. 2019. Human mitotic centromere-associated kinesin is targeted by MicroRNA 485-5p/181c and prognosticates poor survivability of breast cancer. *J. Oncol.* 2019:2316237. https://doi.org/10.1155/2019/2316237

Ma, M., M. Stoyanova, G. Rademacher, S.K. Dutcher, A. Brown, and R. Zhang. 2019. Structure of the decorated ciliary doublet microtubule. *Cell*. 179: 909–922. https://doi.org/10.1016/j.cell.2019.09.030

Maffini, S., A.R. Maia, A.L. Manning, Z. Maliga, A.L. Pereira, M. Junqueira, A. Shevchenko, A. Hyman, J.R. Yates 3rd, N. Galjart, et al. 2009. Motor-independent targeting of CLASPs to kinetochores by CENP-E promotes microtubule turnover and poleward flux. *Curr. Biol.* 19:1566–1572. https://doi.org/10.1016/j.cub.2009.07.059

Mandelkow, E.M., E. Mandelkow, and R.A. Milligan. 1991. Microtubule dynamics and microtubule caps: A time-resolved cryo-electron microscopy study. *J. Cell Biol.* 114:977–991. https://doi.org/10.1083/jcb.114.5.977

Mariappan, A., K. Soni, K. Schorpp, F. Zhao, A. Minakar, X. Zheng, S. Mandad, I. Macheleidt, A. Ramani, T. Kubelka, et al. 2019. Inhibition of CPAP-tubulin interaction prevents proliferation of centrosome-amplified cancer cells. *EMBO J.* 38:e99876. https://doi.org/10.15252/embj.201899876

Marshall, W.F. 2023. The flagellar length control system: Exploring the physical biology of organelle size. *Phys. Biol.* 20. https://doi.org/10.1088/1478-3975/acb1018d

Marshall, W.F., H. Qin, M. Rodrigo Brenni, and J.L. Rosenbaum. 2005. Flagellar length control system: Testing a simple model based on intraflagellar transport and turnover. *Mol. Biol. Cell*. 16:270–278. https://doi.org/10.1091/mbc.e04-07-0586

Maurer, S.P., N.I. Cade, G. Bohner, N. Gustafsson, E. Boutant, and T. Surrey. 2014. EB1 accelerates two conformational transitions important for microtubule maturation and dynamics. *Curr. Biol.* 24:372–384. https://doi.org/10.1016/j.cub.2013.12.042

Maurer, S.P., F.J. Fourniol, G. Bohner, C.A. Moores, and T. Surrey. 2012. EBs recognize a nucleotide-dependent structural cap at growing microtubule ends. *Cell*. 149:371–382. https://doi.org/10.1016/j.cell.2012.02.049

McIntosh, J.R., E. O'Toole, G. Morgan, J. Austin, E. Ulyanov, F. Ataullakhanov, and N. Gudimchuk. 2018. Microtubules grow by the addition of bent guanosine triphosphate tubulin to the tips of curved protofilaments. *J. Cell Biol.* 217:2691–2708. https://doi.org/10.1083/jcb.201802138

Mill, P., S.T. Christensen, and L.B. Pedersen. 2023. Primary cilia as dynamic and diverse signalling hubs in development and disease. *Nat. Rev. Genet.* 24:421–441. https://doi.org/10.1038/s41576-023-00587-9

Mitchison, T., and M. Kirschner. 1984. Dynamic instability of microtubule growth. *Nature*. 312:237–242. https://doi.org/10.1038/312237a0

Montenegro Gouveia, S., K. Leslie, L.C. Kapitein, R.M. Buey, I. Grigoriev, M. Wagenbach, I. Smal, E. Meijering, C.C. Hoogenraad, L. Wordeman, et al. 2010. In vitro reconstitution of the functional interplay between MCAK and EB3 at microtubule plus ends. *Curr. Biol.* 20:1717–1722. https://doi.org/10.1016/j.cub.2010.08.020

Morbidoni, V., E. Agolini, K.C. Slep, L. Pannone, D. Zuccarello, M. Cassina, E. Grosso, G. Gai, L. Salviati, B. Dallapiccola, et al. 2021. Biallelic mutations in the TOGARAM1 gene cause a novel primary ciliopathy. *J. Med. Genet.* 58:526–533. https://doi.org/10.1136/jmedgenet-2020-106833

Moriwaki, T., and G. Goshima. 2016. Five factors can reconstitute all three phases of microtubule polymerization dynamics. *J. Cell Biol.* 215: 357–368. https://doi.org/10.1083/jcb.201604118

Nachury, M.V., and D.U. Mick. 2019. Establishing and regulating the composition of cilia for signal transduction. *Nat. Rev. Mol. Cell Biol.* 20: 389–405. https://doi.org/10.1038/s41580-019-0116-4

Nigg, E.A., and T. Stearns. 2011. The centrosome cycle: Centriole biogenesis, duplication and inherent asymmetries. *Nat. Cell Biol.* 13:1154–1160. https://doi.org/10.1038/ncb2345

Niwa, S., K. Nakajima, H. Miki, Y. Minato, D. Wang, and N. Hirokawa. 2012. KIF19A is a microtubule-depolymerizing kinesin for ciliary length control. *Dev. Cell.* 23:1167–1175. https://doi.org/10.1016/j.devcel.2012.10.016

Odabasi, E., D. Conkar, J. Deretic, U. Batman, K.-M.M. Frikstad, S. Patzke, and E.N. Firat-Karalar. 2023. CCDC66 regulates primary cilium length and signaling via interactions with transition zone and axonemal proteins. *J. Cell Sci.* 136:jcs260327. https://doi.org/10.1242/jcs.260327

Odde, D. 1998. Diffusion inside microtubules. *Eur. Biophys. J.* 27:514–520. https://doi.org/10.1007/s002490050161

Patzke, S., H. Hauge, M. Sioud, E.F. Finne, E.A. Sivertsen, J. Delabie, T. Stokke, and H.C. Aasheim. 2005. Identification of a novel centrosome/microtubule-associated coiled-coil protein involved in cell-cycle progression and spindle organization. *Oncogene.* 24:1159–1173. https://doi.org/10.1038/sj.onc.1208267

Patzke, S., S. Redick, A. Warsame, C.A. Murga-Zamalloa, H. Khanna, S. Doxsey, and T. Stokke. 2010. CSPP is a ciliary protein interacting with nephrocystin 8 and required for cilia formation. *Mol. Biol. Cell.* 21: 2555–2567. https://doi.org/10.1091/mbc.e09-06-0503

Patzke, S., T. Stokke, and H.C. Aasheim. 2006. CSPP and CSPP-L associate with centrosomes and microtubules and differently affect microtubule organization. *J. Cell Physiol.* 209:199–210. https://doi.org/10.1002/jcp.20725

Pedersen, L.B., S. Geimer, R.D. Sloboda, and J.L. Rosenbaum. 2003. The microtubule plus end-tracking protein EB1 is localized to the flagellar tip and basal bodies in Chlamydomonas reinhardtii. *Curr. Biol.* 13: 1969–1974. https://doi.org/10.1016/j.cub.2003.10.058

Perlaza, K., M. Mirvis, H. Ishikawa, and W. Marshall. 2022. The short flagella 1 (SHF1) gene in Chlamydomonas encodes a crescerin TOG-domain protein required for late stages of flagellar growth. *Mol. Biol. Cell.* 33: ar12. https://doi.org/10.1091/mbc.E21-09-0472

Reilly, M.L., N.U. Ain, M. Muurinen, A. Tata, C. Huber, M. Simon, T. Ishaq, N. Shaw, S. Rusanen, M. Pekkinen, et al. 2022. Biallelic KIF24 variants are responsible for a spectrum of skeletal disorders ranging from lethal skeletal ciliopathy to severe acromesomelic dysplasia. *J. Bone Miner Res.* 37:1642–1652. https://doi.org/10.1002/jbmr.4639

Reynolds, M.J., T. Phetruen, R.L. Fisher, K. Chen, B.T. Pentecost, G. Gomez, P. Ounjai, and H. Sui. 2018. The developmental process of the growing motile ciliary tip region. *Sci. Rep.* 8:7977. https://doi.org/10.1038/s41598-018-26111-2

Rezabkova, L., S.H.W. Kraatz, A. Akhmanova, M.O. Steinmetz, and R.A. Kammerer. 2016. Biophysical and structural characterization of the centriolar protein Cep104 interaction network. *J. Biol. Chem.* 291: 18496–18504. https://doi.org/10.1074/jbc.M116.739771

Riparbelli, M.G., G. Callaini, and T.L. Megraw. 2012. Assembly and persistence of primary cilia in dividing Drosophila spermatocytes. *Dev. Cell.* 23:425–432. https://doi.org/10.1016/j.devcel.2012.05.024

Roostalu, J., C. Thomas, N.I. Cade, S. Kunzelmann, I.A. Taylor, and T. Surrey. 2020. The speed of GTP hydrolysis determines GTP cap size and controls microtubule stability. *Elife.* 9:e51992. https://doi.org/10.7554/eLife.51992

Rosenbaum, J.L., and F.M. Child. 1967. Flagellar regeneration in protozoan flagellates. *J. Cell Biol.* 34:345–364. https://doi.org/10.1083/jcb.34.1.345

Rosenbaum, J.L., J.E. Moulder, and D.L. Ringo. 1969. Flagellar elongation and shortening in chlamydomonas. The use of cycloheximide and colchicine to study the synthesis and assembly of flagellar proteins. *J. Cell Biol.* 41: 600–619. https://doi.org/10.1083/jcb.41.2.600

Ryniawec, J.M., M.R. Hannaford, M.E. Zibrat, C.J. Fagerstrom, B.J. Galletta, S.E. Aguirre, B.A. Guice, S.M. Dean, N.M. Rusan, and G.C. Rogers. 2023. Cep104 is a component of the centriole distal tip complex that regulates centriole growth and contributes to drosophila spermiogenesis. *Curr. Biol.* 33:4202–4216.e9. https://doi.org/10.1016/j.cub.2023.08.075

Satish Tammana, T.V., D. Tammana, D.R. Diener, and J. Rosenbaum. 2013. Centrosomal protein CEP104 (Chlamydomonas FAP256) moves to the ciliary tip during ciliary assembly. *J. Cell Sci.* 126:5018–5029. https://doi.org/10.1242/jcs.133439

Saunders, H.A.J., C.M. van den Berg, R.A. Hoogebeen, D. Schweizer, K.E. Stecker, R. Roepman, S.C. Howes, and A. Akhmanova. 2025. A network of interacting ciliary tip proteins with opposing activities imparts slow and processive microtubule growth. *Nat. Struct. Mol. Biol.* 32:979–994. https://doi.org/10.1038/s41594-025-01483-y

Schmidt-Cernohorska, M., I. Zhernov, E. Steib, M. Le Guennec, R. Achek, S. Borgers, D. Demurtas, L. Mouawad, Z. Lansky, V. Hamel, and P. Guichard. 2019. Flagellar microtubule doublet assembly in vitro reveals a regulatory role of tubulin C-terminal tails. *Science.* 363:285–288. https://doi.org/10.1126/science.aav2567

Schmidt, T.I., J. Kleylein-Sohn, J. Westendorf, M. Le Clech, S.B. Lavoie, Y.D. Stierhof, and E.A. Nigg. 2009. Control of centriole length by CPAP and CP110. *Curr. Biol.* 19:1005–1011. https://doi.org/10.1016/j.cub.2009.05.016

Schreiber, S., E. Petrasch-Parwez, E. Porrmann-Kelterbaum, E. Forster, J.T. Epplen, and W.M. Gerding. 2018. Neurodegeneration in the olfactory bulb and olfactory deficits in the Ccdc66 -/- mouse model for retinal degeneration. *IBRO Rep.* 5:43–53. https://doi.org/10.1016/j.ibror.2018.08.004

Schrøder, J.M., J. Larsen, Y. Komarova, A. Akhmanova, R.I. Thorsteinsson, I. Grigoriev, R. Manguso, S.T. Christensen, S.F. Pedersen, S. Geimer, and L.B. Pedersen. 2011. EB1 and EB3 promote cilia biogenesis by several centrosome-related mechanisms. *J. Cell Sci.* 124:2539–2551. https://doi.org/10.1242/jcs.085852

Seetapun, D., B.T. Castle, A.J. McIntyre, P.T. Tran, and D.J. Odde. 2012. Estimating the microtubule GTP cap size in vivo. *Curr. Biol.* 22:1681–1687. https://doi.org/10.1016/j.cub.2012.06.068

Shaheen, R., H.E. Shamseldin, C.M. Loucks, M.Z. Seidahmed, S. Ansari, M. Ibrahim Khalil, N. Al-Yacoub, E.E. Davis, N.A. Mola, K. Szymanska, et al. 2014. Mutations in CSPP1, encoding a core centrosomal protein, cause a range of ciliopathy phenotypes in humans. *Am. J. Hum. Genet.* 94:73–79. https://doi.org/10.1016/j.ajhg.2013.11.010

Sharma, A., A. Aher, N.J. Dynes, D. Frey, E.A. Katrukha, R. Jaussi, I. Grigoriev, M. Croisier, R.A. Kammerer, A. Akhmanova, et al. 2016. Centriolar CPAP/SAS-4 imparts slow processive microtubule growth. *Dev. Cell.* 37: 362–376. https://doi.org/10.1016/j.devcel.2016.04.024

Shoda, T., K. Yamazoe, Y. Tanaka, Y. Asano, and Y.H. Inoue. 2021. Orbit/CLASP determines centriole length by antagonising Klp10A in Drosophila spermatocytes. *J. Cell Sci.* 134:jcs251231. https://doi.org/10.1242/jcs.251231

Simon, J.R., and E.D. Salmon. 1990. The structure of microtubule ends during the elongation and shortening phases of dynamic instability examined by negative-stain electron microscopy. *J. Cell Sci.* 96:571–582. https://doi.org/10.1242/jcs.96.4.571

Spektor, A., W.Y. Tsang, D. Khoo, and B.D. Dynlacht. 2007. Cep97 and CP110 suppress a cilia assembly program. *Cell.* 130:678–690. https://doi.org/10.1016/j.cell.2007.06.027

Srour, M., F.F. Hamdan, D. McKnight, E. Davis, H. Mandel, J. Schwartzentruber, B. Martin, L. Patry, C. Nassif, A. Dionne-Laporte, et al. 2015. Joubert syndrome in French Canadians and identification of mutations in CEP104. *Am. J. Hum. Genet.* 97:744–753. https://doi.org/10.1016/j.ajhg.2015.09.009

Suzuki, H., Y. Muramatsu, F. Miya, H. Asada, M. Yamada, G. Nishimura, K. Kosaki, and T. Takenouchi. 2024. Biallelic loss-of-function variants in the centriolar protein CCP110 leads to a ciliopathy-like phenotype. *Eur. J. Med. Genet.* 70:104955. https://doi.org/10.1016/j.ejmg.2024.104955

Tang, C.J., R.H. Fu, K.S. Wu, W.B. Hsu, and T.K. Tang. 2009. CPAP is a cell-cycle regulated protein that controls centriole length. *Nat. Cell Biol.* 11: 825–831. https://doi.org/10.1038/ncb1889

Tuz, K., R. Bachmann-Gagescu, D.R. O'Day, K. Hua, C.R. Isabella, I.G. Phelps, A.E. Stolarski, B.J. O'Roak, J.C. Dempsey, C. Lourenco, et al. 2014. Mutations in CSPP1 cause primary cilia abnormalities and Joubert syndrome with or without Jeune asphyxiating thoracic dystrophy. *Nat. Cell Biol.* 94:62–72. https://doi.org/10.1016/j.ajhg.2014.01.003

Van De Weghe, J.C., T.D.S. Rusterholz, B. Latour, M.E. Grout, K.A. Aldinger, R. Shaheen, J.C. Dempsey, S. Maddirevula, Y.-H.H. Cheng, I.G. Phelps, et al. 2017. Mutations in ARMC9, which encodes a basal body protein, cause Joubert syndrome in humans and ciliopathy phenotypes in zebrafish. *Nat. Cell Biol.* 101:23–36. https://doi.org/10.1016/j.ajhg.2017.05.010

van den Berg, C.M., V.A. Volkov, S. Schnorrenberg, Z. Huang, K.E. Stecker, I. Grigoriev, S. Gilani, K.-A.M. Frikstad, S. Patzke, T. Zimmermann, et al. 2023. CSPP1 stabilizes growing microtubule ends and damaged lattices from the luminal side. *J. Cell Biol.* 222:e202208062. https://doi.org/10.1083/jcb.202208062

Vásquez-Limeta, A., K. Lukasik, D. Kong, C. Sullenberger, D. Luvsanjav, N. Sahabandu, R. Chari, and J. Loncarek. 2022. CPAP insufficiency leads to incomplete centrioles that duplicate but fragment. *J. Cell Biol.* 221: e202108018. https://doi.org/10.1083/jcb.202108018

Volkov, V.A., and A. Akhmanova. 2024. Phase separation on microtubules: From droplet formation to cellular function? *Trends Cell Biol.* 34:18–30. https://doi.org/10.1016/j.tcb.2023.06.004

Wang, Y., N. Kraemer, J. Schneider, O. Ninnemann, K. Weng, M. Hildebrand, J. Reid, N. Li, H. Hu, S. Mani, and A.M. Kaindl. 2025. Togaram1 is expressed in the neural tube and its absence causes neural tube closure defects. *HGG Adv.* 6:100363. https://doi.org/10.1016/j.xhgg.2024.100363

Wang, Z., W. Wang, S. Liu, F. Yang, X. Liu, S. Hua, L. Zhu, A. Xu, D.L. Hill, D. Wang, et al. 2024. CSPP1 stabilizes microtubules by capping both plus and minus ends. *J. Mol. Cell Biol.* 22:mjae007. https://doi.org/10.1093/jmcb/mjae007

Wieczorek, M., S. Bechstedt, S. Chaaban, and G.J. Brouhard. 2015. Microtubule-associated proteins control the kinetics of microtubule nucleation. *Nat. Cell Biol.* 17:907–916. https://doi.org/10.1038/ncb3188

Xie, S., N. Naslavsky, and S. Caplan. 2024. Emerging insights into CP110 removal during early steps of ciliogenesis. *J. Cell Sci.* 137:jcs261579. https://doi.org/10.1242/jcs.261579

Yamazoe, T., T. Nagai, S. Umeda, Y. Sugaya, and K. Mizuno. 2020. Roles of TOG and jelly-roll domains of centrosomal protein CEP104 in its functions in cilium elongation and Hedgehog signaling. *J. Biol. Chem.* 295: 14723–14736. https://doi.org/10.1074/jbc.RA120.013334

Yue, Y., T.L. Blasius, S. Zhang, S. Jariwala, B. Walker, B.J. Grant, J.C. Cochran, and K.J. Verhey. 2018. Altered chemomechanical coupling causes impaired motility of the kinesin-4 motors KIF27 and KIF7. *J. Cell Biol.* 217: 1319–1334. https://doi.org/10.1083/jcb.201708179

Zabeo, D., J.M. Heumann, C.L. Schwartz, A. Suzuki-Shinjo, G. Morgan, P.O. Widlund, and J.L. Höög. 2018. A lumenal interrupted helix in human sperm tail microtubules. *Sci. Rep.* 8:2727. https://doi.org/10.1038/s41598-018-21165-8

Zhang, R., G.M. Alushin, A. Brown, and E. Nogales. 2015. Mechanistic origin of microtubule dynamic instability and its modulation by EB proteins. *Cell.* 162:849–859. https://doi.org/10.1016/j.cell.2015.07.012

Zhao, L., C. Jin, Y. Chu, C. Varghese, S. Hua, F. Yan, Y. Miao, J. Liu, D. Mann, X. Ding, et al. 2010. Dimerization of CPAP orchestrates centrosome cohesion plasticity. *J. Biol. Chem.* 285:2488–2497. https://doi.org/10.1074/jbc.M109.042614

Zheng, X., A. Ramani, K. Soni, M. Gottardo, S. Zheng, L. Ming Gooi, W. Li, S. Feng, A. Mariappan, A. Wason, et al. 2016. Molecular basis for CPAP-tubulin interaction in controlling centriolar and ciliary length. *Nat. Commun.* 7:11874. https://doi.org/10.1038/ncomms11874

Zhu, X. 2025. Mammalian motile cilia: Structure, formation, organization, and function. *Semin. Cell Dev Biol.* 175:103651. https://doi.org/10.1016/j.semcdb.2025.103651

Supplemental material

Provided online is Table S1. Table S1 provides an overview of the proteins discussed in the review and shows their domain organization, interactions with microtubules, tubulin and other proteins, effects on microtubule dynamics *in vitro*, function during centriole and cilia biogenesis and disease relevance.