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Review

Roles of motor proteins in building microtubule-based structures: a basic principle of cellular design

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Abstract

Eukaryotic cells must build a complex infrastructure of microtubules (MTs) and associated proteins to carry out a variety of functions. A growing body of evidence indicates that a major function of MT-associated motor proteins is to assemble and maintain this infrastructure. In this context, we examine the mechanisms utilized by motors to construct the arrays of MTs and associated proteins contained within the mitotic spindle, neuronal processes, and ciliary axonemes. We focus on the capacity of motors to drive the 'sliding filament mechanism' that is involved in the construction and maintenance of spindles, axons and dendrites, and on a type of particle transport called 'intraflagellar transport' which contributes to the assembly and maintenance of axonemes.

Keywords: Microtubule; Motor protein; Kinesin; Dynein; Polarity pattern; Axon; Dendrite; Spindle; Axoneme

1. Introduction

Microtubules (MTs) form a dynamic intracellular polymer system, or cytoskeleton, that plays an essential role in cellular function and development. In order to carry out a range of very different tasks, the MT cytoskeleton is designed to be extremely flexible, capable of rapidly assembling into a variety of distinct configurations during the life cycle of the cell. For example, during mitosis MTs are organized into the mitotic spindle, a bipolar machine upon which chromosomes are equally segregated from mother to daughter cells. Then, during interphase or after cells exit the cell cycle, cellular MT arrays are drastically reorganized to serve functions ranging from membrane traffic to cell motility and growth. The assembly of these MT arrays must occur quickly and accurately in order to drive proper cellular function and development. Thus, understanding the basic design principles that guide their construction is of fundamental importance to the fields of cellular and developmental biology.

In this review we focus on the roles of MT-based motor proteins in the construction of the arrays of MTs contained within mitotic spindles, neuronal processes, and axonemes (Fig. 1). These arrays differ greatly from one another with regard to their architecture and function; spindles drive chromosome segregation, axons and dendrites function in neuronal signaling and information processing, and axonemes function to move a cell relative to a fluid medium or, in a modified form, function as sensory receptors. Nevertheless, it is clear that motors play an integral role in the construction of each. In the sections that

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follow, we will outline the evidence suggesting that motors function in the formation of spindles and neuronal processes by using a 'sliding filament mechanism' to reorganize MT polymers, and in the formation of axonemes by transporting molecules required for the assembly of the MT array along the polymer lattice to active construction sites (Fig. 2).

2. A 'sliding filament mechanism' for mitotic spindle assembly

The segregation of chromosomes during mitosis depends upon the action of the mitotic spindle, a self-organizing protein machine that uses MTs to coordinate chromosome movements with cell division. Architecturally, the spindle consists of two partially overlapping radial arrays of MTs oriented with their minus ends focused at, and their plus ends radiating away from, duplicated centrosomes [1]. Thus, as shown in Fig. 1A, the mitotic spindle contains both antiparallel arrays of MT (where the half-spindles overlap) and parallel arrays of MTs (in non-overlapping regions). During the assembly and function of the spindle, it is thought that specific forces applied to the MTs within each of these regions ensure that the spindle poles are properly positioned in relation to one another and that the spindle itself is properly positioned within the cell. Multiple MT-motors are known to be involved in this activity, but their precise mechanisms of action remain controversial [2].

An appealing hypothesis for motor function during mitosis is the 'sliding filament mechanism' [3]. In this model, spindle movements are driven by motors that cross-link and slide adjacent MTs in relation to one another, a mechanism that is analogous to the interactions between actin and myosin that drive muscle contraction [4]. Although subsequent work has made it clear that 'sliding filaments' cannot account for all mitotic movements (such as chromosome movements on the spindle) [5] they may underlie many of the movements involved in positioning the spindle poles during spindle assembly and elongation [6]. In fact, studies conducted over the past decade have strongly suggested that at least three prominent mitotic motors function in this way. These are the bipolar kinesins, the C-terminal kinesins, and cytoplasmic dynein.

2.1. Bipolar kinesins cross-link and slide antiparallel MTs to push the spindle poles apart

The bipolar kinesin (bimC) motor family [7] was originally identified by screens for mitotic mutants in fungi [8–11] and, subsequently, closely related motors were identified in a variety of animal systems [12–14]. Because the inhibition of these motors uniformly results in the formation of mono-astral mitotic spindles with duplicated but closely spaced poles at their center it is widely accepted that bipolar kinesins function to establish or maintain the separation of spindle poles. Yet, precisely how and when these motors play a role in this process has been difficult to ascertain and the focus of a great deal of research and debate. Recently, studies from the budding yeast, *Saccharomyces cerevisiae*, and the fruitfly, *Drosophila melanogaster*, have provided insights into bipolar kinesin function suggesting that these motors separate the spindle poles by cross-linking and sliding antiparallel spindle MTs in relation to one another.

2.1.1. Cin8 and Kip1

The budding yeast, *S. cerevisiae*, is unique in that it expresses two clearly distinct, yet partially redundant bipolar kinesin family members, Cin8 and Kip1 [10,11]. Elegant genetic and structural analyses of Cin8 and Kip1 mutants, described below, have pro-

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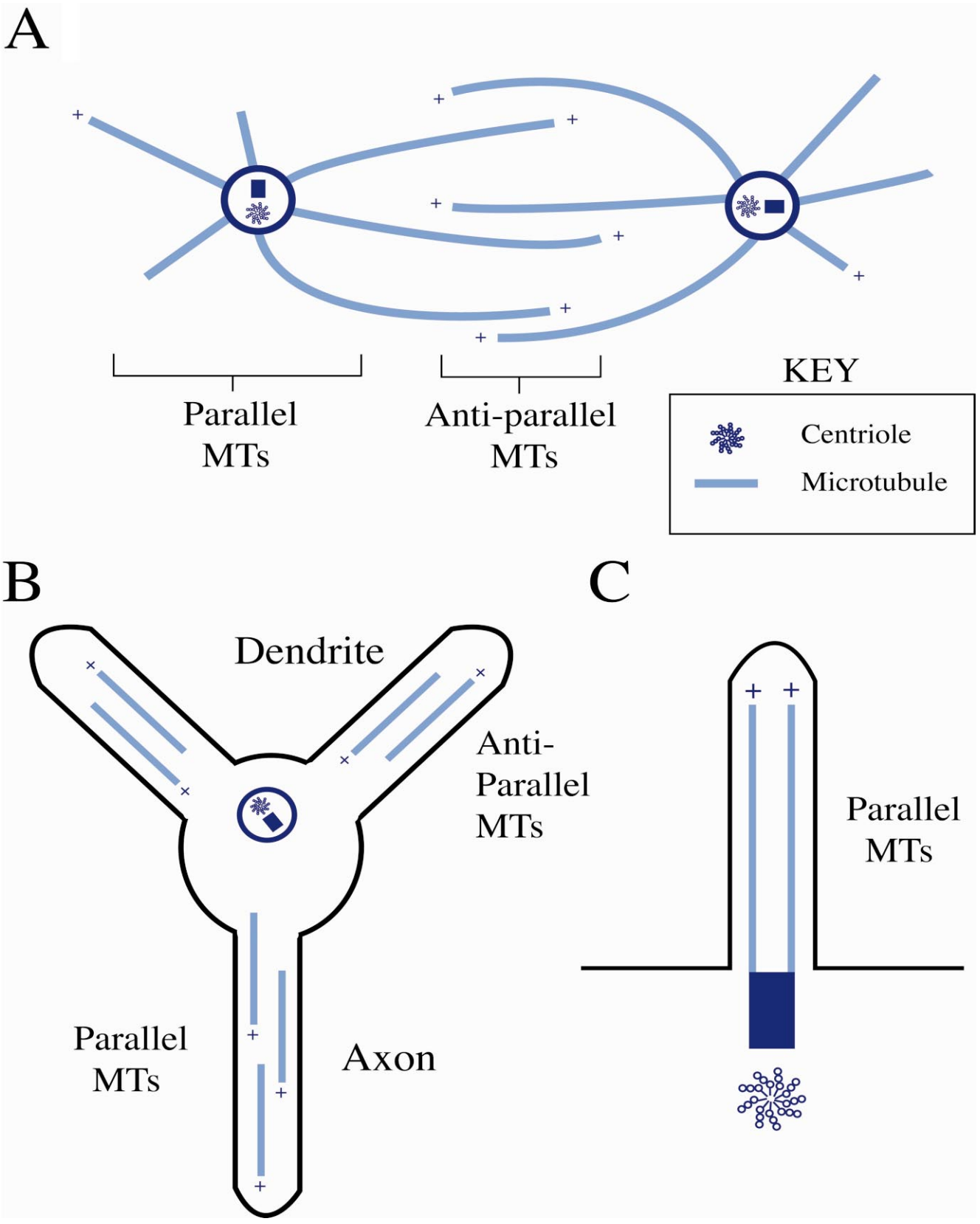
Fig. 1. Organization of the MT arrays contained within spindles, neuronal processes and axonemes. (A) The mitotic spindle. The spindle consists of two partially overlapping radial arrays of MTs. Because most spindle MTs are oriented with their minus ends focused at one of the two poles, the spindle contains antiparallel arrangements of MTs where the half-spindles overlap (the spindle midzone) and parallel arrangements of MTs near the poles and extending away from the central spindle toward the cell cortex (astral MTs). (B) Neuronal processes. Neuronal MTs do not maintain close contact with the centrosome but are organized into highly specific arrays within axons and dendrites nonetheless. Axons contain parallel arrangements of MTs (uniformly oriented with their plus ends distal to the cell body) while dendrites contain antiparallel arrangements of MTs. (C) Axonemes. Axonemal MTs remain attached to the basal body and are organized into parallel arrangements oriented with their plus ends distal to the cell body.


A
Parallel MTs
Anti-parallel MTs
KEY
Centriole
Microtubule
B
Dendrite
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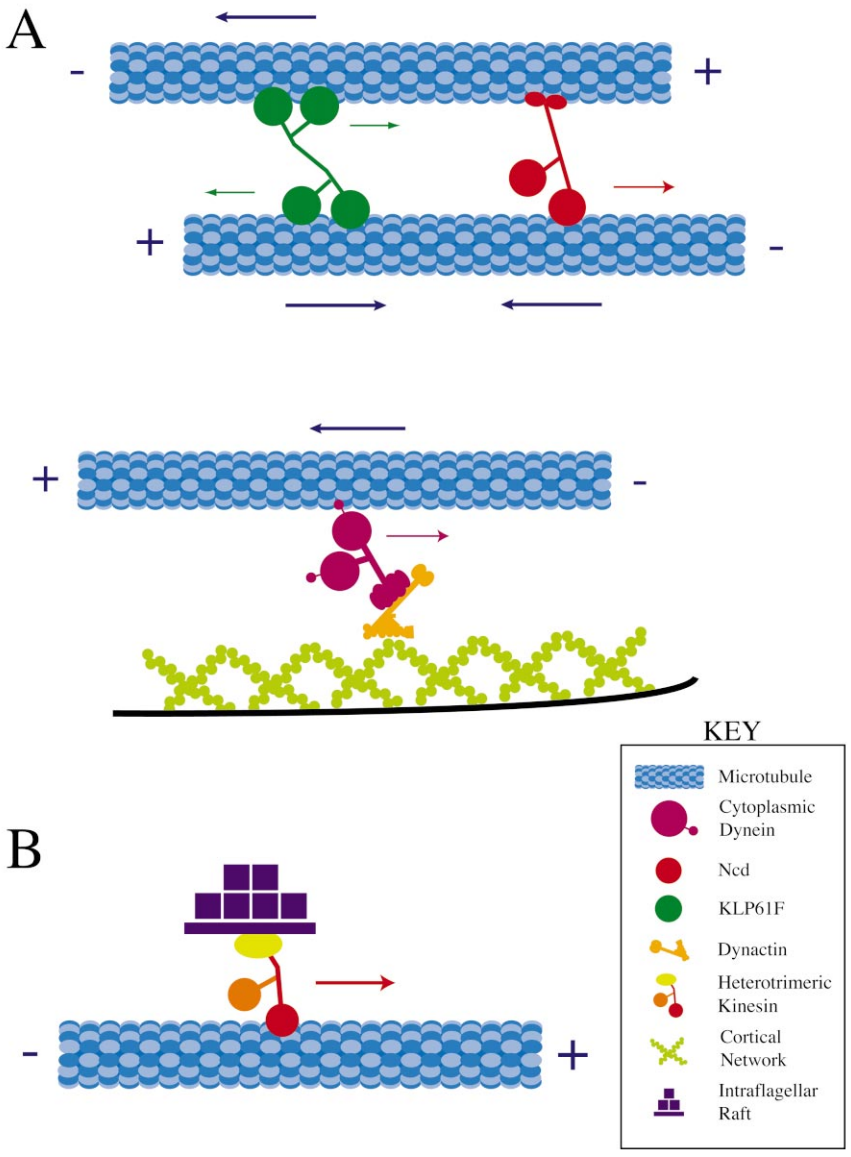

Fig. 2. The role of motors in sliding filament and particle transport mechanisms for the construction and maintenance of MT-based structures. (A) Sliding filament mechanism. Motors cross-link and slide MTs in relation to one another or other cytoskeletal elements to position spindle poles during mitosis or to move appropriately oriented MTs into neuronal processes. (B) Particle transport mechanism. Motors transport macromolecular assemblies of proteins and other components required for the assembly and maintenance of subcellular structures to their sites of incorporation.

vided extremely valuable insights into the mitotic function of these motors.

Although the simplest pathway for the formation of monopolar spindles is a failure in spindle pole separation, analyses of yeast spindles following the inactivation of Cin8 and Kip1 have revealed that these bipolar kinesins perform at least some of their functions during spindle maintenance and elongation. For example, when both Cin8 and Kip1 are inactivated in arrested pre-anaphase yeast cells that contain a bipolar spindle, the poles quickly slide back together to form monopolar structures [10,15]. Interestingly, this collapse is prevented by co-inhibiting the C-terminal kinesin Kar3 [16] (the C-terminal kinesins will be discussed in detail below). Alternatively, if yeast cells are allowed to proceed into ana-

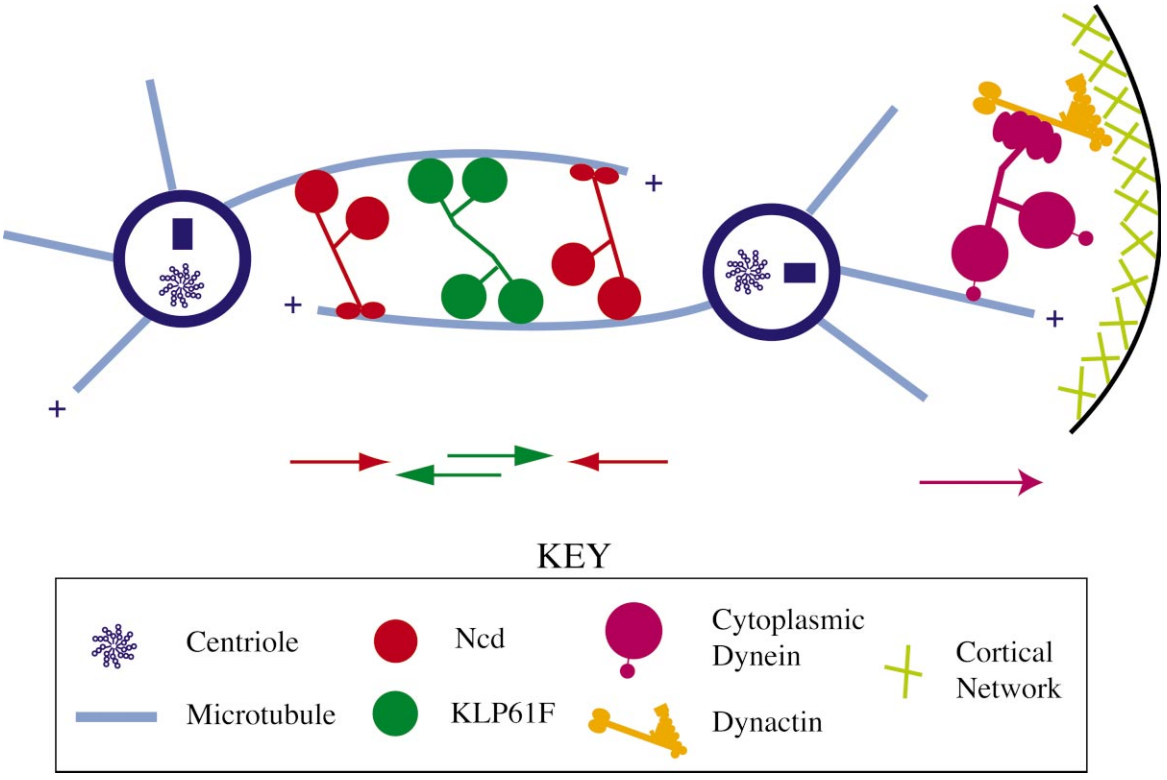

Fig. 3. A sliding filament mechanism for mitosis. Antiparallel MTs in the central spindle are cross-linked by bipolar motors such as Klp61F, which can slide microtubules with motor domains positioned at both ends of the molecule, and unipolar motors such as Ncd, which bind MTs in a nucleotide insensitive fashion with their tail domains and generate force against neighboring MTs with their motor domains. In this way, plus end-directed motors serve to push the poles apart while minus end-directed motors pull them together. Astral MTs are cross-linked to the cell cortex by the minus end-directed motor cytoplasmic dynein (which is anchored by dynactin) and transported with their plus ends leading to generate force that pulls the poles apart.

phase and then Cin8 and Kip1 are inactivated, spindles will not collapse but display severely hampered anaphase B spindle elongation [17,18]. Therefore, Cin8 and Kip1 act to maintain the bipolar morphology of the spindle through metaphase and elongate the spindle during anaphase. At present, the most straightforward explanation for these observations is that Cin8 and Kip1 act directly on MTs to generate forces that push the poles apart.

2.1.2. *Klp61F*

Studies of the *Drosophila* bipolar kinesin, Klp61F, have shown that this motor performs a similar function to its fungal counterparts and provide a specific structural model for how this function is carried out. Klp61F was first uncovered genetically [13] and, shortly thereafter, was purified in its native oligomeric state from *Drosophila* embryonic cytosol. Biochemical analyses of the purified Klp61F holoenzyme showed it to be a 'slow' plus end-directed MT-motor that forms a homotetrameric complex, in vivo [19], the first kinesin with such a subunit composition to be identified. Subsequent ultrastructural analyses using rotary shadowing EM showed that the four Klp61F motors within this complex are configured into a 'bipolar minifilament' with two MT-motor domains positioned at both ends of a central rod [20]. This striking architectural similarity to class II myosins [4] suggests that Klp61F motors, and potentially other bipolar kinesins, have the capacity to function by a 'sliding filament mechanism' in which they cross-link and slide apart antiparallel MTs to separate the poles [21,22] (see Fig. 3). This hypothesis has been strongly supported by both immuno-EM analyses in which Klp61F motors are shown to cross-link MTs within interpolar spindle MT bundles

[23] and also real-time functional analyses showing that, like Cin8 and Kip1, the inhibition of Klp61F causes bipolar spindles to collapse during prometaphase [24] or fail to elongate during anaphase B [25]. Interestingly, Klp61F does not appear to play a role in the initial separation of spindle poles during prophase because it is sequestered in the nucleus during this time [23], and its inhibition does not overtly affect this process [24].

Thus, members of both the fungal and animal bipolar kinesin families play integral roles in maintaining and elongating bipolar spindles. Some of these motors may also drive the initial separation of spindle poles but, at present, this has not been directly demonstrated. Together, the function, localization and biochemical characteristics of Klp61F are entirely consistent with a mechanism of action in which this bipolar kinesin cross-links and slides antiparallel MTs apart. Moreover, plus end-directed MT motility and MT bundling activity have recently been shown for Cin8 indicating that this motor, and presumably Kip1, also have the appropriate characteristics to drive MT-MT sliding [26]. Based on this, it is quite likely that other bipolar kinesins function by a similar 'sliding filament mechanism' as well.

2.2. The C-terminal kinesins cross-link and slide antiparallel MTs to pull the spindle poles together

Another major family of kinesin motors with members that are known to play a role in mitosis are the C-terminal kinesins. These motors are so named because their ATP-dependent motor domain is positioned at the carboxy-terminal end of the motor polypeptide, opposite to most other kinesins [7]. Moreover, C-terminal kinesins have also been shown to display the opposite transport properties to most other kinesins, moving toward the MT minus end [27–29]. As with the bipolar kinesins, work in *S. cerevisiae* and *D. melanogaster* has provided evidence that at least some C-terminal kinesins function to pull the poles together during mitosis by driving antiparallel MT-MT sliding.

2.2.1. Kar3

The founding member of the fungal C-terminal kinesin motor family, Kar3, was identified by a screen of budding yeast mutants showing defects in karyogamy [30,31]. A specific mitotic function for this motor was first revealed by the surprising finding that the co-inhibition of Kar3 with the bipolar kinesins Cin8 and Kip1 rescues the bipolar morphology of mitotic spindles [16]. This indicated that some sort of antagonistic interrelationship exists between fungal bipolar kinesins and C-terminal kinesins. The precise nature of this antagonism was further elaborated by studies showing that the overexpression of Kar3 causes abnormally short pre-anaphase spindles and, in the absence of bipolar kinesins, can completely inhibit anaphase B spindle elongation [15]. Thus, Kar3 serves to generate 'inward' forces that pull the spindle poles together, counterbalancing the 'outward' forces exerted by bipolar kinesins pushing the poles apart. Since Kar3 can bind MTs with its N-terminal tail (the opposite end of the molecule from the motor domain) [31] it is possible that the 'inward' force generated by Kar3 results from antiparallel MT bundling and sliding. However, there is also evidence that Kar3 concentrates at the spindle poles where it regulates MT assembly dynamics, suggesting an alternative mechanism of action for this motor [32].

2.2.2. Ncd

Like Kar3, the *Drosophila* C-terminal kinesin, Ncd, is a minus end-directed MT-motor [27,28] that influences the behavior of meiotic and mitotic spindles. Oocytes produced by Ncd null mutants display frayed spindle poles and are dynamically unstable [33] while Ncd null early embryos contain mitotic spindles that are abnormally spurred or branched and often become multipolar [34]. Several observations made during real-time assessments of spindle behavior in the absence of Ncd activity indicate that this C-terminal kinesin also functions by generating 'inward' forces on the poles. First, the co-inhibition of Ncd and the bipolar kinesin, Klp61F, prevents the spindle collapse resulting from the inhibition of Klp61F alone (see above) [24]. Thus, as in fungal systems, the *Drosophila* C-terminal and bipolar kinesin carry on an antagonistic interrelationship. Secondly, the rate of the initial separation of spindle poles in Ncd null embryos is significantly faster than it is in wild type embryos, suggesting that Ncd also antagonizes this process (which is not driven by Klp61F). Although Ncd is not a bipolar motor

like Klp61F, it does have characteristics suggesting that it can drive MT-MT sliding within the spindle. Because Ncd contains nucleotide insensitive MT-binding sites in its N-terminal tail [27,35,36] it can cross-link and slide MTs by anchoring its tail to one MT and generating force with its motor domain against adjacent MTs. Moreover, Ncd has been shown to concentrate on interpolar MT bundles during mitosis [36] positioning it appropriately to cross-link antiparallel MTs [34] and generate forces that pull the poles together (see Fig. 3). Prior to nuclear envelope breakdown, such an activity could prevent the premature or overseparation of spindle poles and, subsequently, could counterbalance Klp61F, constraining the morphology of the spindle and generating isometric tension. Interestingly, the absence of Ncd activity appears to have no effect on spindle morphology subsequent to metaphase [25], suggesting that the downregulation of this motor causes the spindle to elongate during anaphase B.

2.3. *Cytoplasmic dynein acts at the cell cortex to pull the spindle poles apart and position the spindle within the cell*

A modification of the 'sliding filament mechanism' in which MTs slide in relation to a fixed actin cortex has also been proposed to position spindle poles during mitosis [37] (Fig. 2A and 3). Indeed, interactions between astral MTs and actin at the cell cortex have been implicated in driving the movement of spindle poles in a variety of systems. For example, disruption of the actin cytoskeleton has been shown to inhibit the initial separation of centrosomes in tissue culture cells [38] and cause defects in the orientation and insertion of mitotic nuclei within the bud neck in budding yeast [39,40]. Moreover, association between astral MTs and the cell cortex has been shown to be sufficient to drive spindle elongation during anaphase B in mammalian cells [41] and to properly rotate nuclei in *Caenorhabditis elegans* embryos [42,43]. Given that astral MTs are oriented with their plus ends facing toward the cell periphery [1], these observations predict that minus end-directed motors can cross-link and slide astral MTs relative to the cortical actin network thereby generating pulling forces on the poles.

A prime candidate motor to mediate these interactions is cytoplasmic dynein. Cytoplasmic dynein is a large, minus end-directed motor complex involved in a variety of cellular activities and probably plays multiple functions during mitosis [44]. Several lines of evidence suggest that one of its major mitotic functions is to position spindle poles by generating actin-MT sliding. First, dynein has been shown to drive the separation of spindle poles in *Drosophila* early embryos, mammalian tissue culture cells, and budding yeast [17,25,45,46] and has also been implicated in orienting mitotic spindles in relation to specific actin-rich cortical sites in *C. elegans* and budding yeast [40,47–49]. Second, the immunolocalization of cytoplasmic dynein, in *Drosophila* early embryos, mammalian tissue culture cells, and budding yeast has shown that it concentrates on cortical sites during mitosis [25,40,50]. And third, biochemical analysis of the putative dynein 'receptor', dynactin, has revealed that one of its subunits, Arp1 or centractin, can associate with spectrin filaments [51] which are known to bind to the actin cytoskeleton in vivo. Therefore, cytoplasmic dynein may become anchored to the actin cortex via dynactin and spectrin allowing it to utilize its minus end-directed motility to generate pulling forces on the poles (Fig. 3).

2.4. *Cooperation between bipolar kinesins, C-terminal kinesins and cytoplasmic dynein*

Fig. 3 shows a schematic representation of how the bipolar kinesins, C-terminal kinesins, and cytoplasmic dynein may all utilize a slight modification of the same basic mechanistic theme to organize spindle MTs. All three generate forces on the spindle poles by sliding MTs. However, the bipolar kinesins and C-terminal kinesins cross-link antiparallel MTs together, sliding them in relation to one another in a way that causes expansion or contraction of the spindle, respectively. Cytoplasmic dynein, on the other hand, cross-links MTs to an immovable actin cortical network and generates forces that pull the poles to specific cortical sites. This, in turn, can drive the initial separation of the poles and position the spindle within the cell. In concert, these forces help ensure that the spindle forms in a controlled fashion, is maintained under tension, and is appropriately positioned to properly segregate sister chromatids into daughter nuclei.

3. A 'sliding filament mechanism' for organizing MTs in neuronal processes

Neurons are the principle cell type involved in information transfer and processing in the nervous system and thus must be capable of simultaneously sending, receiving, and processing numerous inputs. To carry this out, neurons generate two distinct types of cytoplasmic processes: axons, which are specialized to send information quickly over long distances, and dendrites, which receive and process information arriving from multiple sources. Many characteristic features of these processes allowing them to perform these distinct tasks probably arise from specific differences in the organization of MTs within them [52]. In particular, axons contain a parallel array of MTs oriented with their plus ends distal to the cell body [53], while dendrites contain an antiparallel array of MTs in which about half of the MTs are oriented with their minus ends distal to the cell body [54] (Fig. 1B). Given the role of MTs as both railways for the active transport of organelles and as structural support, this singular difference is likely to have a major impact on both the composition and morphology of these processes.

Although neurons are terminally postmitotic and no longer organize their MTs into bipolar spindles, there are important similarities between the neuronal and mitotic MT arrays. In particular, as mentioned above and shown in Fig. 1A,B, both neurons and mitotic spindles can be separated into regions containing either parallel or antiparallel arrangements of MTs. Moreover, because the active transport of neuronal MTs is involved in setting up these specific MT polarity patterns [55], neurons must employ a motor-driven 'sliding filament mechanism' to organize their MTs. Indeed, a series of recent studies have indicated that the specific mechanisms that organize the axonal and dendritic MT arrays are strikingly similar to those utilized to organize the spindle.

3.1. Cytoplasmic dynein transports plus end distal MTs into neuronal processes

All neuronal processes must acquire populations of MTs oriented with their plus ends distal to the cell body [56] and, thus, a major task throughout neuronal development is to move MTs into and down these processes with their plus ends leading. Recent studies suggest that cytoplasmic dynein, which has been proposed to move MTs with their plus ends leading to position spindle poles during mitosis (see above), works similarly to generate plus end distal MTs within neuronal processes.

Although cytoplasmic dynein's functions in neurons have been studied most extensively with regard to retrograde organelle transport, it has recently been shown that both dynein and its putative receptor dynactin move anterogradely down axons associated with the actin cytomatrix and not membranous organelles [57,58]. Thus, dynein is appropriately positioned to mediate actin-MT sliding. More recently, it has been shown that the inhibition of dynein by the disruption of dynactin in cultured neurons prohibits the outward movement of MTs from the centrosome into developing neurites [59], as well. Collectively, these findings along with the determined roles for dynein and dynactin during mitosis, strongly suggest that dynein functions by sliding MTs in relation to the actin cortex (Fig. 2A). This could serve to provide plus end distal MTs to immature neurites, axons (Fig. 4A), and dendrites (Fig. 4B).

3.2. CHO1/MKLP1 transports minus end distal MTs into dendrites

Unlike axons which acquire only plus end distal MTs during their development dendrites must also receive a population of minus end distal MTs [56]. Therefore, some MTs must be transported into these processes with their minus ends leading, perhaps towards the plus ends of other MTs. To date, only one motor, CHO1/MKLP1, has been shown to mediate this kind of antiparallel MT sliding, in vitro [60]. Moreover, in vivo functional analyses have suggested that the role of CHO1/MKLP1 during mitosis is to organize antiparallel MT arrays within the central spindle [61–64]. Recent studies of the mechanisms involved in dendritic development suggest that this 'mitotic' motor is also available to perform a similar function in neurons. For instance, consistent with a role in organizing dendritic MTs, CHO1/MKLP1 is expressed long after neurons exit the cell cycle where it localizes specifically to cell bodies and dendrites [65,66]. Functional analyses of CHO1/MKLP1 indicate that this motor is both necessary and sufficient

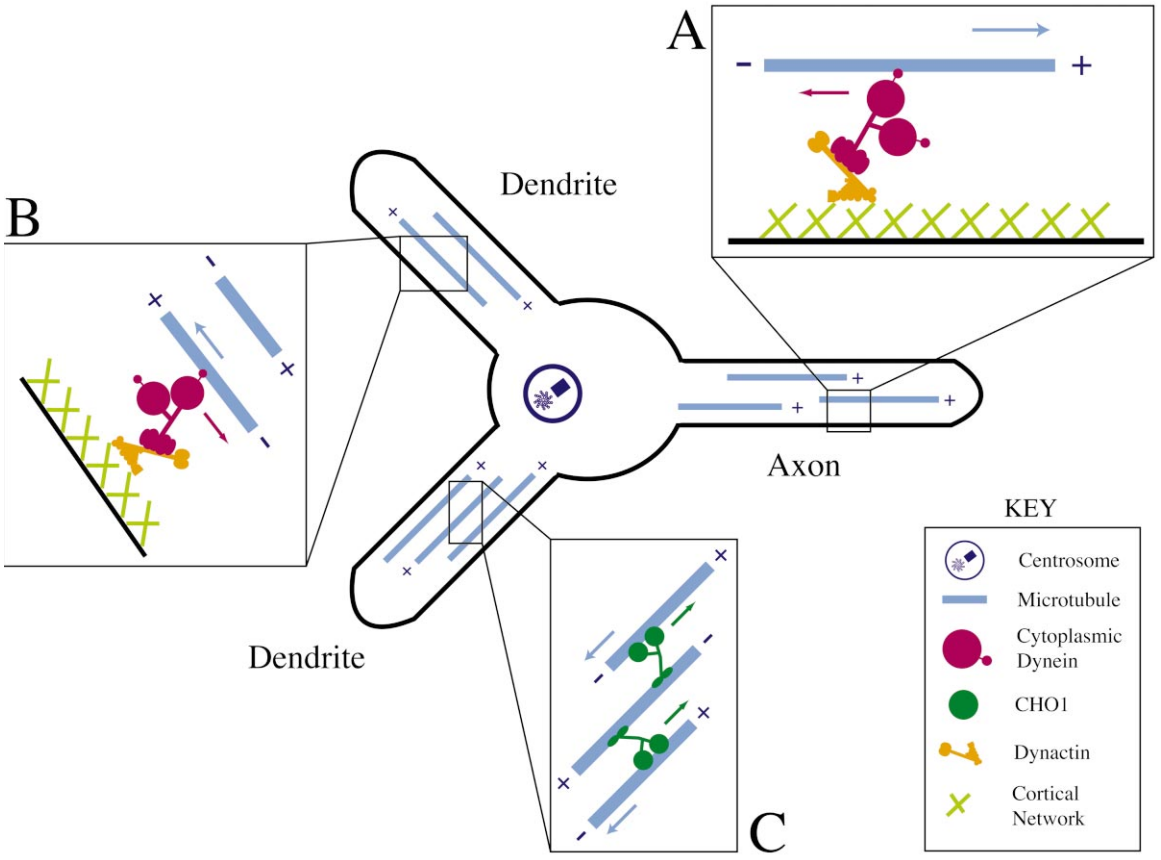

Fig. 4. A sliding filament mechanism for neuronal process formation. (A,B) Cytoplasmic dynein, tethered to the cell cortex by dynactin, slides MTs into both axons and dendrites with their plus ends leading. (C) The plus end-directed motor CHO1/MKLP1 cross-links antiparallel MTs using nucleotide insensitive binding sites in its tail and slides MTs into developing dendrites with their minus ends leading.

to drive the formation of processes with antiparallel MT arrays. Studies in neurons, as well as other cell types that are known to extend processes containing antiparallel MTs, have shown that the inhibition of CHO1/MKLP1 specifically prevents the acquisition of minus end distal MTs within these processes [65,67,68]. Moreover, the expression of a fragment of the motor in insect ovarian cells has been shown to cause the formation of dendrite-like processes containing non-uniform MT polarity patterns [66]. Interestingly, like Kar3 and Ncd discussed above, CHO1/MKLP1 contains nucleotide insensitive MT binding sites outside of the motor domain and has been shown to bundle MTs in vitro and in vivo [69]. It is, therefore, plausible that CHO1/MKLP1 acts by cross-linking antiparallel MTs within dendrites and generating forces that slide MTs with their minus ends leading toward the plus ends of MTs concomitantly being transported down developing dendrites by dynein (see Fig. 4C). How CHO1/MKLP1 is directed specifically into dendrites, however, is still a mystery.

3.2.1. *Conservation of the mitotic 'sliding filament mechanisms' in neurons*

It is quite striking and initially surprising that nearly identical 'sliding filament mechanisms' may be utilized to organize both mitotic and neuronal MTs. However, despite their functional differences, the organization of MTs within the mitotic spindle and neuronal processes, particularly with regard to specific polarity relationships, are quite similar. Perhaps, the use of the same motors and mechanisms to build structures with these fundamental similarities but with ultimately different functions may make sound evolutionary sense. For instance, it

may be inefficient and thus selectively disadvantageous for neurons, which were at one point in their life cycles mitotic cells, to abandon the basic mitotic mechanisms which, with a few twists, can continue to meet their specific needs during process development.

4. MT-motors involved in intraflagellar transport and axoneme assembly

Recent work has uncovered another way in which MT-based motor proteins contribute to the formation and maintenance of a subcellular structure, that is by driving a type of particle transport called 'intraflagellar transport' which underlies the assembly of ciliary axonemes (Fig. 2B) [70]. Motile and sensory cilia are MT-based organelles that have evolved to function in motility and sensory transduction, respectively. They share a common design, consisting of a membrane-bounded cylinder that surrounds nine doublet MTs (Fig. 1C and 5). In motile cilia (and flagella) accessory structures such as dynein arms, radial spokes and the central pair apparatus drive the coherent beating of the axoneme that moves a fluid over a cell surface or propels a cell through a fluid medium. Nodal cilia are modified motile cilia that appear to generate an unusual beat that is required for generating left-right asymmetry in mammalian embryos. Immotile sensory ciliary axonemes do not beat, and consequently they have a relatively simple structure, lacking the accessory structures required for motility.

These various types of ciliary axonemes are thought to assemble by the addition of subunits at the plus ends of the axonemal MTs located at the tip, distal to the cell body (Fig. 5). Therefore, as there is no protein synthesis in the axoneme itself, axonemal precursors and factors required for their incorporation must be synthesized in the cell body then translocated along the axoneme to the ciliary tip. The term intraflagellar transport refers to the process by which proteins that are required for the formation and maintenance of cilia (or flagella) are transported in the form of large protein complexes called IFT rafts between the cell body and the distal tip of the axoneme. The rafts move in both directions along the axoneme and it appears that both anterograde IFT (the movement of rafts towards the tip of the axoneme) and retrograde IFT (movement from the tip to the base of the axoneme) are required for the formation and maintenance of motile cilia and flagella [71,72], sensory cilia [73,74] and nodal cilia [75,76]. Anterograde IFT is driven by a subfamily of kinesins called the heteromeric kinesins, whereas retrograde IFT is driven by a form of cytoplasmic dynein, referred to as Dhc1b or che-3 dynein.

4.1. Heteromeric kinesins in anterograde intraflagellar transport

The prototypic heteromeric kinesin, heterotrimeric kinesin-II, was first purified from sea urchin eggs and embryos [77]. This motor protein is a 300 kDa complex of two heterodimerized motor subunits and a third accessory subunit (KAP), that moves to the plus ends of MT tracks at about 0.5 μm/s [77,78]. Similar heteromeric kinesins have now been identified in a broad range of eukaryotes where they are thought to participate in the intracellular transport of membrane-bounded organelles and macromolecular complexes [79].

Several heteromeric kinesins have been implicated in the assembly and maintenance of various types of ciliary or flagellar axonemes. For example, conditional loss-of-function mutations in the *Chlamydomonas fla-10* gene, which encodes a subunit of heterotrimeric kinesin-II [80], are characterized by defects in the assembly and maintenance of motile flagella at the restrictive temperature [71]. In echinoderm embryos, the microinjection of a monoclonal antibody to a subunit of kinesin-II leads to a striking and specific inhibition of ciliogenesis at the blastula stage, suggesting a role for kinesin-II in ciliary assembly in this system [72]. In mouse, there is evidence that kinesin-II is required for the normal assembly and function of nodal cilia, as mouse mutants lacking kif3 subunits of the heterotrimeric kinesin-II motor protein display defects in nodal ciliogenesis [75,76]. Finally, heteromeric kinesins have been implicated in the assembly and maintenance of sensory cilia as well. In the nematode *C. elegans*, sensory cilia are present on the dendritic endings of chemosensory neurons. These neurons contain two types of heteromeric kinesin, Osm-3-kinesin and heterotrimeric kinesin-II [74,81], both of which are concentrated in

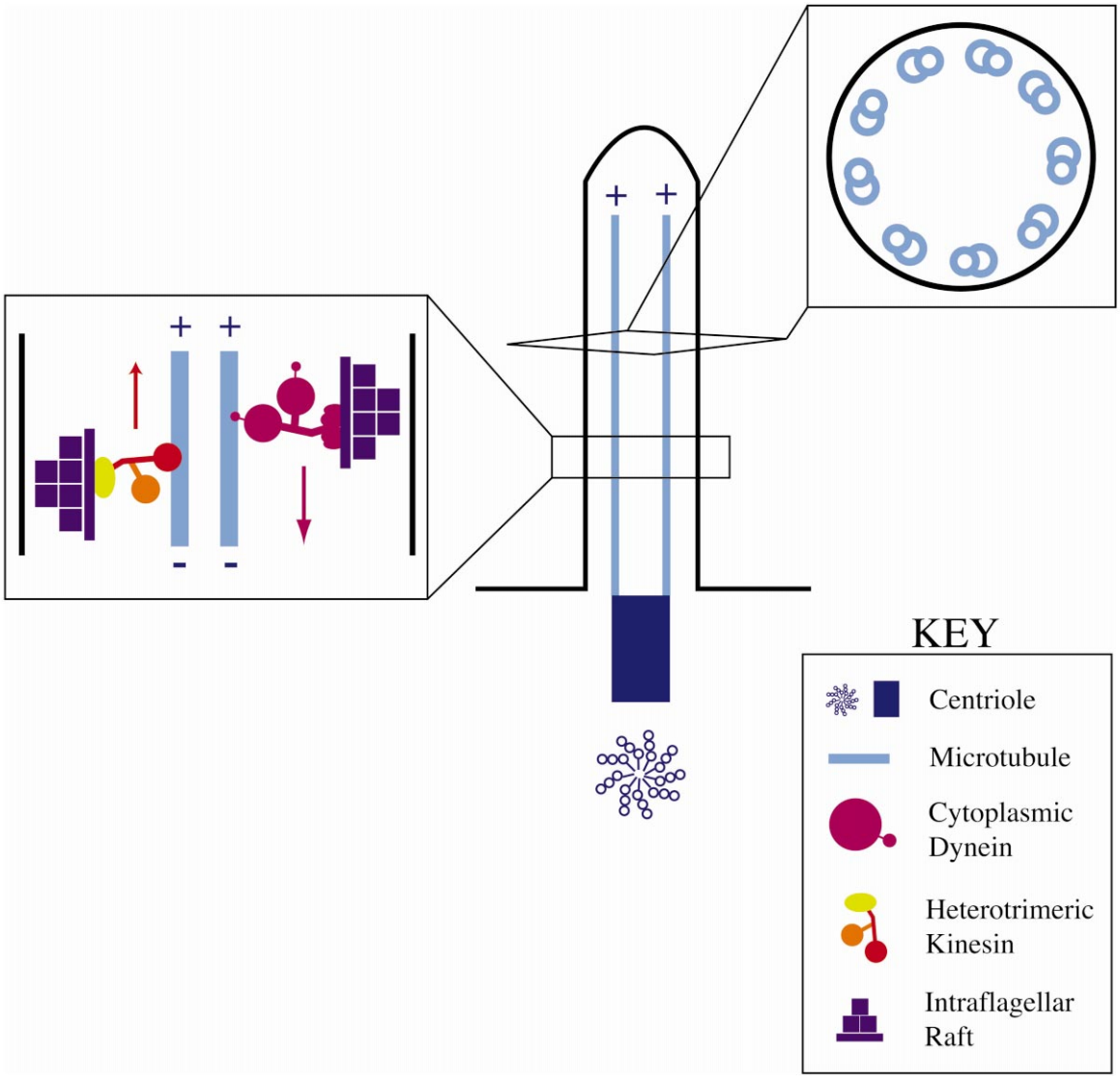

Fig. 5. Intraflagellar transport. The axoneme consists of nine MT doublets oriented with their plus ends facing away from the cell body. Macromolecular structures, known as raft particles, carrying proteins required for formation and maintenance of the axoneme are transported anterogradely to the tip of the axoneme by plus end-directed heteromeric kinesin motors and retrogradely by cytoplasmic dynein.

sensory cilia by immunofluorescence [81]. Mutations in one of these motors, Osm-3-kinesin, give rise to defects in the assembly and maintenance of sensory cilia characterized by a lack of the distal segments of the ciliary axonemes [73].

Although heteromeric kinesins are required for ciliogenesis in various systems, direct evidence that these motors drive anterograde transport along ciliary axonemes has only been obtained in *Chlamydomonas* and *C. elegans*. In the former case, intraflagellar transport can be visualized in the light microscope as the movement of 'bulges' in both directions along the flagellar membrane [71,82]. These bulges correspond to electron dense 'IFT rafts' made up from 16S subunits that contain multiple polypeptides including two, Osm-1 and Osm-6, that are essential for ciliary assembly [80]. In the aforementioned *fla-10* mutant, the transport of these bulges along the axoneme ceases at the restrictive temperature, consistent with the hypothesis that the plus end-directed motor, kinesin-II, drives the anterograde transport of the IFT rafts along the flagellar

axoneme [71,80,82]. In *C. elegans* it has been possible to directly visualize the movement of labeled IFT motor and cargo molecules along sensory ciliary axonemes [83]. This was done by producing transgenic lines of worms that express fusion proteins of heterotrimeric kinesin-II and its presumptive cargo molecules, Osm-1 and Osm-6, fused to green fluorescent protein. Using standard time-lapse fluorescence microscopy, it was then possible to directly observe these motor and cargo molecules moving along the sensory ciliary axoneme in the anterograde direction at about 0.7 μm/s [83]. Together, these studies provide compelling support for the hypothesis that heteromeric kinesins play important roles in ciliary assembly and stability by driving the anterograde component of intraflagellar transport.

4.2. *Cytoplasmic dynein in retrograde intraflagellar transport*

The DHC1b dynein isoform is a divergent cytoplasmic dynein that was first implicated in ciliary assembly and maintenance by the observation that its expression is upregulated during ciliogenesis in sea urchin embryos [84,85]. Support for the hypothesis that this form of dynein drives the retrograde transport of IFT raft particles was obtained in *Chlamydomonas*, where mutations in the DHC1b heavy chain, and an associated 8 kDa light chain both give rise to the accumulation of IFT raft particles around the flagellar axoneme and an associated truncation of the axoneme itself [86–88]. The accumulation of raft particles could result form the normal delivery of rafts in the anterograde direction, but the defective retrieval of the rafts. Direct evidence for a role of this dynein isoform in retrograde intraflagellar transport was obtained using the aforementioned time-lapse fluorescence IFT transport assay in sensory cilia of living *C. elegans* [83]. In this organism the *che-3* gene encodes a homolog of DHC1b dynein, and it was observed that in a *che-3* mutant background, there was a specific inhibition in the retrograde transport of IFT motors and raft particles, while anterograde IFT and bidirectional dendritic transport continued unabated [89]. Together, these studies provide compelling evidence for the hypothesis that ciliary assembly depends upon bidirectional intraflagellar transport, and that heteromeric kinesins, and DHC-1b (or che-3) dyneins are responsible for anterograde and retrograde IFT, respectively (Fig. 5).

5. Summary and concluding remarks

In this review, we have described two basic mechanisms by which motor proteins contribute to the assembly and maintenance of MT-based subcellular structures, one that involves a sliding filament mechanism (Fig. 2A) and a second one involving the MT-based transport of components that are required for the assembly of the structure to the site where assembly occurs (Fig. 2B). The former mechanism is utilized in the construction of mitotic spindles, axons and dendrites, where bipolar motors like Klp61F or unipolar motors like Ncd or CHO-1 drive MT-MT sliding to produce arrays of MTs of defined polarity patterns. In a modification of this mechanism, the motor protein cytoplasmic dynein slides MTs relative to a fixed network of cortical actin filaments during the assembly of mitotic spindles, and possibly also during the slow transport of MTs along axons. The latter mechanism utilizes heteromeric kinesin motors to drive the anterograde intraflagellar transport of components required for ciliary or flagellar assembly along axonemal MTs to the site of ciliary growth, while a cytoplasmic dynein isoform, Dhc1b or Che-3-dynein, drives transport in the opposite, retrograde direction.

Although we have focused on the deployment of the sliding filament mechanism in spindles, axons and dendrites, and on the role of intraflagellar particle transport in axonemes, it is clear that other factors are important in the assembly and maintenance of these structures, including MT nucleation by MT-organizing centers and MT dynamics. However, it is likely that the sliding filament and particle transport mechanisms discussed here illustrate how motors can be used in the assembly and maintenance of a broad range of subcellular structures.

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