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Unconventional roles of cytoskeletal mitotic machinery in neurodevelopment

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Abstract

At first look, cell division and neurite formation seem to be two very different, essential biological processes. However, both processes require extensive reorganization of the cytoskeleton, and especially microtubules. Remarkably, in recent years, independent work from several groups has shown that multiple cytoskeletal components previously considered specific for the mitotic machinery play important roles in neurite initiation and extension. In this review, we describe how several cytoplasmic and “mitotic” microtubule motors, components of mitotic kinetochores and cortical actin participate in reorganization of the microtubule network required to form and maintain axons and dendrites. The emerging similarities between these two biological processes will certainly generate new insights into the mechanisms generating the unique morphology of neurons.

Keywords

neuron; neurites; cytoskeleton; cell division; microtubule; motor; sliding

Introduction: Microtubules are key players in neurite formation

Each individual neuron is developed from a precursor that has to extend neurites, called axons and dendrites, to form connections for communication. Axons are responsible for sending signals from cell bodies to other neurons or non-neuronal cells. In multipolar neurons, generally the rest of the neurites are dendrites, responsible for receiving and integrating signals. Both axons and dendrites are filled with microtubules, actin microfilaments and, in the case of vertebrate neurons, neurofilaments [reviewed in [1]]. Forces generated by cytoskeletal components are responsible for neurite formation, mainly through the dynamics of microtubule and actin networks. Experiments in primary neuronal cultures show that the microtubule network plays a major role in neurite formation (see Box 1 for strategies of imaging microtubule behavior in neurites). In the search for mechanisms responsible for microtubule reorganization in neurons, initial studies in the early 90s

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demonstrated that several “mitotic” motors, well-known for their role in mitotic spindle formation, surprisingly control neurite length of post-mitotic neurons [reviewed in [2]]. In agreement with these early observations, there has been an explosion of recent data showing that not only mitotic motors, but other important mitotic components are involved in generating the mechanical forces required for proper neurite outgrowth and maintenance. In this review, we discuss how “mitotic” machinery is repurposed in neurodevelopment.

Conserved mechanisms of microtubule reorganization in cell division and neuronal development

Despite obvious morphological differences between these two processes, the forces that act on microtubules in the spindle are equally important in neurodevelopment. Indeed, microtubules, regardless of the motors involved, can undergo sliding against each other and this sliding is required both for cell division and neurite extension. Furthermore, in both cases cytoplasmic dynein, anchored at the actin cortex, pulls on microtubules through similar mechanisms. Here we describe how microtubule rearrangements can be mechanistically similar while performing different functions in mitotic and postmitotic cells.

Sliding of microtubules against each other drives both formation of bipolar spindle and neurite outgrowth

Microtubule-microtubule sliding is the ability of microtubule motors to move microtubule against one another. A common feature of microtubule sliding is that, independently of the identity of the microtubule motor, a microtubule can function as both the cargo and track of the microtubule motor. Motors moving microtubules generate forces that are used either to reorganize microtubule network, i.e. mitotic spindle formation, or to change cell shape, i.e. neurite formation and outgrowth.

During mitosis, chromosome segregation requires a massive reorganization of the microtubule network from radial to bipolar array. Although bipolar spindle formation requires the cooperation of several mitotic motors, it is microtubule-microtubule sliding by the kinesin-5 motor that provides the main force that establishes spindle bipolarity and drives spindle elongation [3]. Kinesin-5 forms a homotetramer complex with pairs of motors at opposite ends (see Fig.1, left panel). As a result, kinesin-5 can crosslink two antiparallel microtubules and move them against each other [4]. Inactivation of kinesin-5 leads collapse of the bipolar spindle to monopolar asters and chromosome segregation failure [3]. Like mitosis, neurite initiation requires the generation of cytoplasmic forces that drive changes in cell shape. The microtubule network in cultured *Drosophila* neurons is continuously reorganized on a time scale of seconds. Rapidly moving microtubules push the plasma membrane at the tips of growing neurites [5]. Similar movement is also seen in developing mammalian neurons [6, 7]. Based on its role in mitosis, kinesin-5 seemed like a good candidate motor that drives microtubule sliding in neurons. However, depletion of kinesin-5 in neurons promotes rather than inhibits neurite outgrowth [8]. Additionally, the reported speed for kinesin-5 is too slow for the microtubule transport rates observed in primary cultures [9]. Instead microtubule movement in the cytoplasm is mainly driven by kinesin-1. Kinesin-1 knockdown impaired microtubule sliding and neurite outgrowth in cultured

Drosophila neurons [5]. Kinesin heavy chain contains two microtubules microtubule-binding domains (MBDs); the N-terminal motor domain and C-terminal ATP-independent MBD [10–12]. As a result, a microtubule that binds to the C-terminus of the kinesin heavy chain becomes a "cargo" that is moved by the motor along another "track" microtubule (Fig.1, right panel). Because kinesin-1 is a plus-end directed motor, the only possible symmetrical model of microtubule-microtubule sliding is the movement of microtubules against each other with minus-ends leading and plus-ends trailing (Fig. 1, right panel). Mutations in the C-terminal ATP-independent MBD create a motor with impaired sliding but intact ability to move other cargoes. Replacement of the wild-type kinesin with a sliding-deficient mutant in *Drosophila* causes severe neurological defects. Detailed examination of the nervous system in these animals reveals that neurons develop very short axons (e.g. in optic lobes) and dendrites (e.g. in sensory neurons) [13].

Moreover, microtubule sliding is stimulated during axon regeneration. Axonal injury causes a fast spike of calcium influx that destabilizes microtubules near the axotomy site and creates new microtubule arrays with mixed orientation. This microtubule configuration leads to reactivation of kinesin-1 driven microtubule sliding and subsequently axon regeneration [14].

Role of cortical dynein in neuronal development

Cytoplasmic dynein, together with the dynactin complex, is the major minus-end directed motor involved in many interphase processes such as organelle positioning and cargo trafficking. During cell division, dynein activity is responsible for centrosome separation and chromosome segregation by pulling kinetochores, but it is also involved in proper mitotic spindle positioning and orientation by pulling on astral microtubules. The latter activity requires enrichment of dynein/dynactin complex at the cortex distal to the spindle poles [15]. For example, this cortical association in asymmetrical cell division is mediated by Gαi/LGN/NuMA-dependent pathway [reviewed in [16]] (Fig 2, top panel).

Dynein has been shown to have important roles on microtubule reorganization in neurites.

Photobleaching of GFP-tagged microtubules showed that short fragments of microtubules are moving in the axons of developed cultured neurons [6]. The motion of these microtubules is rapid, highly asynchronous and bidirectional. The transport of short microtubules (both anterograde and retrograde) is diminished after inactivation of cytoplasmic dynein [7]. Recent work has shown that dynein is responsible not only for transport of short fragments of microtubules, but also the bulk cytoskeletal network that powers axonal elongation [17].

At earlier developmental stages, neurons develop neurites that contain microtubules with mixed orientation [18, 19]. Later in development, only one of these neurites will become an axon and its microtubule orientation will gradually switch to uniformly, plus-end out [19] [20]. The uniform orientation of axonal microtubules in *Drosophila* neurons requires the activity of cytoplasmic dynein [21]. In agreement with these data, microtubule polarity defects were found in axons of cultured *Drosophila* and mammalian neurons after knocking down dynein heavy chain or dynactin components [19] or pharmacological inhibition of

dynein [22]. Together, these data suggest that the dynein/dynactin complex is universally required for sorting microtubules in axons. Cytoplasmic dynein can cross-link and slide microtubules *in vitro*. It was suggested that the two motor domains of dynein can bind and move along two separate microtubules [23]. An alternative possibility, based on imaging fluorescently labeled components of the dynein/dynactin complex, is that dynein, immobilized at the actin cell cortex, captures and pulls microtubules [24] similar to cortical dynein pulling on astral microtubules during cell division [25]. In agreement with the cortical recruitment model, treatment of *Drosophila* cultured neurons with actin-depolymerizing drugs prevents dynein-driven microtubule sorting in the axons [19] (Fig.2 bottom panel). Furthermore, direct artificial recruitment of dynein to the plasma membrane bypassed the actin requirement for dynein-driven sorting of microtubules [19] confirming the role of the actin cortex in anchoring dynein pulling on axonal microtubules.

As dynein inactivation only affects microtubule polarity in axons but not in dendrites [21] it is likely that the mechanism of dynein recruitment to the cortex is axon-specific. There is some evidence that NDEL1 (a dynein cofactor) is enriched to the axonal initial segment via Ankyrin-G in mouse hippocampal neurons. Cultured neurons isolated from NDEL1 conditional knockout animals displayed defects in axon morphology as well as cargo sorting defects suggesting that recruitment of NDEL1 to the axon initial segment locally activates dynein [18]. We favor a scenario where dynein/dynactin cofactors are anchored at the cortical actin specifically in the axons but not in dendrites. It would be very interesting to identify the cortical recruitment mechanism of dynein in axons required for its microtubule sorting activity.

Role of spindle-reorganizing kinesins in neurite outgrowth

Kinesin-5 is the main motor responsible for sliding the overlapping sets of antiparallel microtubules that is required for bipolar spindle formation. Its microtubule sliding forces are counter-acted by other sets of microtubule motors such as kinesin-6 and kinesin-12, and the minus-end directed motor kinesin-14. Kinesin-6, kinesin-12 and kinesin-14 bind to mitotic microtubules in the metaphase spindle and act as an antagonist to kinesin-5-driven microtubule sliding [reviewed in [26, 27]]. These “mitotic” motors responsible for building the mitotic spindle are expressed in developing neurons, and post-mitotic depletion of these motors have implications in neurite outgrowth (Table 1). Here, we discuss the post-mitotic role of four mitotic kinesins, kinesin-5, kinesin-6, kinesin-12 and kinesin-14 in neurodevelopment.

Kinesin-5

Mammalian Eg5 (a kinesin-5 family member) is highly expressed in developing neurons that have already lost their ability to divide [28]. In post-mitotic neurons, a fraction of cytoplasmic Eg5 can be found in axons, especially in growth cones [29]. Knockdown of Eg5, or its chemical inhibition, accelerates the growth rate of axons of cultured sympathetic neurons and prevents proper microtubule polarization within growth cones, suggesting that Eg5 generates forces that oppose axonal growth, most likely suppressing retrograde

transport of microtubules [8, 30]. Parallels can also be drawn between regulation of kinesin-5 itself in mitosis and neuronal development.

Kinesin-5 activity during cell division is regulated by several proteins such as NEK7 and TPX2. NEK7 is a mitotic kinase that is required for recruitment of kinesin-5 around centrosomes to promote spindle assembly and mitotic progression [31]. TPX2 is a Ran-regulated microtubule associated protein (MAP) that plays several roles in spindle assembly [32]. TPX2 can simultaneously bind to spindle microtubules and to kinesin-5. It is believed that TPX2 works as a mechanical brake that slows down microtubule sliding driven by kinesin-5 [33]. NEK7 and TPX2 also affect neurite outgrowth. NEK7 kinase activity regulates dendrite growth and branching, as well as formation of dendritic spines [34]. Depletion of TPX2 in neurons resulted in faster neurite outgrowth with rates similar to those observed after inactivation of Kinesin-5. Normal neurite extension rates can be rescued with the full-length TPX2 but not with the variant missing its kinesin-5 interacting domain [35]. It would be of interest to further examine the roles of other kinesin adaptor/modifier proteins between mitosis and neurodevelopment.

Kinesin-6

Kinesin-6 is a major constituent of the Centralspindlin complex. Centralspindlin accumulates at the spindle midzone in anaphase and initiates cleavage furrow formation via Rho signaling [36]. Here, kinesin-6 stabilizes the mitotic spindle by bundling antiparallel microtubules (Fig.3, left panel). Kinesin-6 depletion induces defects in morphology of the mitotic spindle and failure to recruit contractile ring components [37].

The kinesin-6 proteins continue to be expressed in post-mitotic neurons [38]. Mammals express three different kinesin-6 family members: Kif23 (MKLP1), Kif20a (MKLP2), and Kif20b. MKLP1 was the first kinesin-6 reported to regulate microtubule polarity in mammalian dendrites [39]. In addition, Kif20b plays an important role in neuronal development during corticogenesis. Loss of function mutations of Kif20b in cortical mouse neurons disrupts polarization, neurite outgrowth and branching [40]. In a screen looking for microtubule-microtubule sliding regulators, it was found that depletion of Pav-KLP (Pavarotti, the *Drosophila* ortholog of MKLP1) induced a dramatic increase in microtubule sliding and neurite length in *Drosophila* neurons [41]. Inversely, ectopic expression of Pav-KLP variants that cross-link, or bundle, microtubules, block microtubule sliding and neurite outgrowth, confirming that Pav-KLP is a negative regulator of microtubule sliding driven by other motors (kinesin-1 and cortical dynein) (Fig 3, right panel). This is consistent with the role of Pav-KLP as a microtubule bundler in mitosis [36]. Pav-KLP expression levels are transcriptionally controlled via Toll-6-FoxO signaling, and that regulates microtubule rearrangements required during remodeling of synaptic terminals [42]. Taken together, these works show the important role of kinesin-6 family members in neuronal polarization and neurite outgrowth through microtubule rearrangement. This activity requires a tight spatial and temporal regulation, most likely through controlling microtubule bundling properties of kinesin-6, and so contributes to the developmental downregulation observed for microtubule sliding in neurons.

Kinesin-14

The minus-end directed KIFC1 (kinesin-14 family member) is another “mitotic” motor expressed in mature neurons [43]. During cell division, Kinesin-14 regulates spindle organization and assembly through its microtubule crosslinking and sliding activity [44, 45]. Post-mitotic inhibition of KIFC1 activity resulted in cultured neurons that displayed shorter axons and lower number of axonal branches, as well as microtubule polarity defects [46]. Rescue assays with KIFC1 mutants that can slide but not cross-link, and vice versa, showed that microtubule sliding activity is important for axon growth, microtubule orientation and normal spacing between neighboring microtubules. In contrast, its cross-linking activity seems to be important for opposing normal microtubule sliding driven by other motors and for preventing axonal retraction. [46].

Kinesin-12

Kinesin-12 (KIF15) concentrates in the midzone of the mitotic spindle where microtubules from opposite poles overlap. It is thought that kinesin-12 microtubule crosslinking activity is important for maintaining the mitotic spindle [47]. KIF15 is expressed in developing vertebrate neurons; its expression is downregulated at later stages. Depletion of KIF15 in developing neurons enhances axonal extension and bidirectional microtubule transport in the axons [48]. Inversely, its overexpression results in shorter axons [49]. Growth cones in KIF15-depleted cultured neurons display increased invasion of filopodia by microtubules resulting in inhibition of proper growth cone turning and guidance. The ability of KIF15 to bind to actin, a major component of growth cones, might be responsible for the phenotypes observed in these structures [48]. More recently, it has been shown that depletion of KIF15 increases the regeneration velocities of injured axons in zebrafish neurons [50].

Role of microtubule dynamic instability in neurite outgrowth

Simultaneously to microtubule transport, microtubule reorganization is influenced by polymerization/depolymerization events. Microtubules are dynamic polymers that undergo states of growth, catastrophe, shortening and rescue, together termed microtubule dynamic instability [51]. Dynamic instability is regulated by local concentration of tubulin dimers, microtubule-associated proteins, and several kinesins. Microtubule dynamic instability is essential in mitosis and for process extension [52]. During mitosis, dynamic instability allows spatial exploration for spindle microtubules in their search for kinetochores. This activity prevents spontaneous microtubule assembly, controls proper spindle assembly and kinetochore-microtubule attachments. Kinetochore-microtubule attachments are regulated by the microtubule-depolymerization activity of several kinesins, including kinesin-13 [reviewed in [53]] and kinesin-8 [54]. Several mitotic kinesins (kinesin-13 and kinesin-8) [55, 56] that control dynamics of spindle microtubules also regulate neurite outgrowth.

The kinesin-13 family is a group of four subfamilies (KIF2A, KIF2B, KIF2C and KIF24) that catalyze microtubule depolymerization. KIF2A is enriched in developing mouse neurons and its inhibition induces formation of neurons with extended branches [56]. A Kif2a conditional knockout mouse develops hippocampal neurons with multiple axons [57]. KIF2A microtubule depolymerization activity is regulated by several kinases.

Phosphorylation of KIF2A by CDK5 and PAK1 kinases decrease its depolymerizing activity and stimulate neurite outgrowth. Inversely, upregulation of KIF2A activity by ROCK2 inhibits outgrowth [58]. Kinesin-13 activity is locally downregulated after axonal injury, leading to upregulation of growing microtubules required for axon regeneration [59].

The role of kinetochore proteins in neurodevelopment

In addition to “mitotic” molecular motors, multiple components of the kinetochore-microtubule attachment machinery (termed KMN network for constituent proteins of the three subcomplexes) are required for nervous system development.

The KMN complex is assembled on inner components in contact with the centromere, e.g. CENP-A [60]. Intermediate complexes Mis12 and KNL-1 act as platforms for signaling and kinetochore assembly. The Ndc80 complex is the major site of microtubule attachment [61]. The KMN complex is necessary for proper alignment and subsequent segregation of chromosomes [61]. To ensure correct segregation, the KMN network must sense tension between sister chromatids and kinetochore microtubules. In order for progression into anaphase, the kinetochore must inactivate the spindle assembly checkpoint. This is achieved via a concerted action of kinases including Plk1 and Aurora B, and subsequent dephosphorylation [60].

In neurons, decreased neuronal expression of proteins from different KMN subcomplexes, (Mis12 and Ndc80) results in defects in NMJ formation and central nervous system (CNS) development in *Drosophila* [62]. Knl1 and Ndc80 are expressed in post-mitotic neurons in *C. elegans* and their depletion led to sensory neuron disorganization in *C. elegans* [63]. Nuf2, a component of the Ndc80 subcomplex is concentrated at microtubule rich dendrites, supportive of microtubule binding roles. Importantly, via sensory neuron-specific degradation, the authors were able to eliminate expression of endogenously Knl1 and Ndc80 in these cells. Under such conditions, a decrease in dendrite extension rate was noted, as was abnormal positioning of cell bodies of these head sensory neurons [63]. The disorganization/elongation of these neurons mirrors a phenotype observed in the *Drosophila* CNS in a Mis12 mutant, where the ventral nerve cord was narrower and longer than that of wild type embryos. Finer analysis of defects induced by loss of Mis12 revealed hyperextension of neurites at the NMJ and failure to form a normal pre-synapse, although neuronal differentiation itself was unaffected [62]. Notably, this hyperextension may be due to a similar mechanism by which kinesin-6 depletion leads to axon overextension in *Drosophila* [41]. Furthermore, cultured rat hippocampal neurons show an increase in filopodia-like dendritic extensions and expression of Mis12 at the protein level was confirmed in human iPSC derived cortical and motor neurons [62]. These findings suggest that repurposing of kinetochore machinery in cytoskeletal organization may be conserved from flies and worms to human.

Importantly, the Mis12 hyperextension phenotype was observed with multiple components of the KMN network in *Drosophila*, including each of the Ndc80 and Knl1 subcomplexes, as well as other Mis12 subunits [63]. These findings suggest that the KMN network functions as a comparable system in mitosis and in neurons. In contrast, both Ndc80 MBDs are

required in nervous system development, while only one of these two MBDs is needed for correct chromosome segregation [63]. Therefore, divergence between mitosis and neuronal development, and the precise mechanisms by which KMN proteins contribute to neuronal and synaptic maturation require further investigation.

Concluding Remarks

In recent years, an explosion of experimental data has shown that neurons repurpose the machinery involved in bipolar spindle formation to generate microtubule-based neurites.

Here we have discussed the conserved and contrasting roles of the cytoskeleton in cell cycle progression and neuronal development. Multiple studies have shed light on expression of many “mitotic” proteins in non-dividing neurons; and the functions of these proteins have been tailored to meet the demands of the morphologically complex cytoskeletal rearrangements in neurons. Common themes emerging from these studies include the remodeling of the cytoskeleton by microtubule sliding and polymerization/depolymerization events. These parallels are extended when we note the factors involved in kinesin-5 regulation are common between mitosis and neurodevelopment. In contrast, it is interesting to note that, in the case of microtubule sliding, the motors responsible are different. In the case of dynein mediated microtubule reorganization, both cell division and microtubule sorting in axons require cortical recruitment of dynein, but the recruiting cortical anchors likely differ (see Outstanding Questions).

This review was written from a microtubule-centric point of view. Obviously, there are other mitotic components, such as the anaphase promoting complex that also regulates synapse formation through transcription regulation and protein turnover. Based on the similarities found between mitosis and neurodevelopment, it would be interesting to investigate if signaling that controls the function of “mitotic” motors in mitosis is also conserved in neurodevelopment. We expect that additional mitotic proteins involved in neuronal development will be identified soon, followed by a more general picture that integrates these numerous components in a logical pathway.

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BOX 1

Strategies for imaging and quantify microtubule transport and dynamics in neurons.

In the search for mechanisms responsible for microtubule reorganization in neurons, several labs have taken advantage of different microscopy approaches that allow visualization and quantification of microtubule motility (Fig.I). Three common strategies to image and quantify microtubule motility in neurites include: i. Photobleaching of GFP-tubulin (FRAP) to visualize movement of non-photobleached microtubules through the photobleached region. This technique also enables scoring of frequencies for anterograde and retrograde direction of microtubule movement [6, 7] (Fig.I, left panel); ii.

Fusion of photoconvertible proteins to tubulin, e.g. EOS-tub provides specific marks on microtubules using UV light so the motility of photoconverted microtubules can be easily tracked and quantified [14, 64] (Fig.I, middle panel); and iii. Indirect measurement of microtubule motility by tracking the movement of mitochondria docked on motile microtubules [17] (Fig.I, right panel).

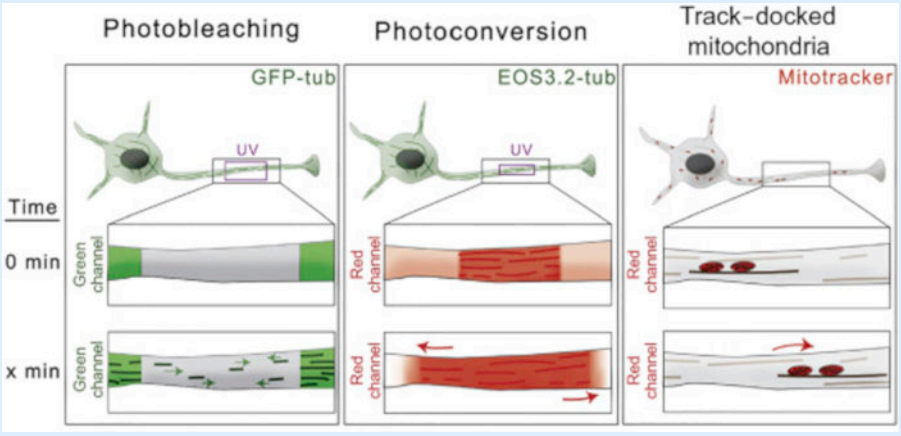

Figure I for Text Box 1. Strategies for imaging and quantify microtubule transport and dynamics in neurons.

Left Panel. Neurons expressing GFP-tubulin. Photobleaching of a small region in the axons allows to visualize movement of non-bleached microtubules through the photobleached region.

Middle Panel. Neurons expressing tubulin fused to a photoconvertible protein EOS3.2 display microtubules in green. Photoconversion of a small region of EOS-labeled microtubules with UV light creates red marks on microtubules. Photoconverted microtubules can be easily tracked and quantified in the red channel.

Right Panel. Neurons are labeled with a mitochondria-specific dye. Movement of microtubules can be tracked indirectly by observing the position of mitochondria docked on microtubules.

Outstanding Questions Box

How do the “mitotic” kinesins regulate sliding during neurite development? Do signaling pathways identified in cell division play a role in neurodevelopment?

Why do microtubules get sorted in axons but not in dendrites?

What are the components that recruit dynein to the cortical actin in axons?

What is the mechanism of action of “kinetochore” proteins in nervous system development?

Highlights

- Many components of the cell division machinery are repurposed in post-mitotic neurons to control neurite outgrowth.
- The mechanical forces generated by motor-driven reorganization of the microtubule network play a major role in neurite outgrowth
- Microtubule transport driven by kinesin-1 and cortical dynein generates forces responsible for neurite formation
- Several mitotic kinesins regulate neurite outgrowth by counteracting microtubule transport driven by kinesin-1 and dynein
- Cortical dynein is responsible for sorting of microtubules in axons
- Kinetochore proteins are required for nervous system development

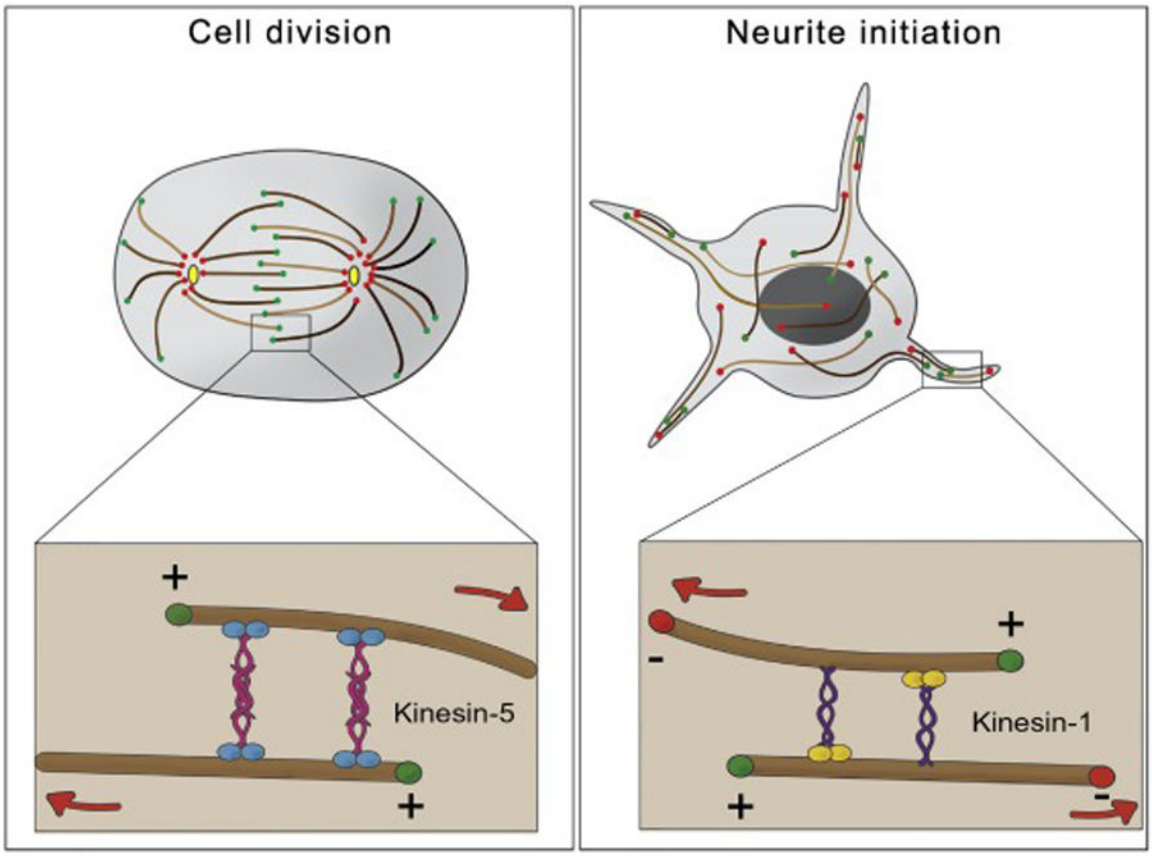

Figure 1. Roles of microtubule-microtubule sliding in neurodevelopment and cell division

Left Panel. In cell division, microtubule-microtubule sliding, driven by the mitotic kinesin-5 family motors, is responsible for pole segregation and maintaining spindle bipolarity. Kinesin-5 motors form homotetramers with two pairs of plus-end directed motor heads at opposite ends that cross-link and slide antiparallel microtubules.

Right Panel. Neurite initiation requires the forces generated by microtubule-microtubule sliding of antiparallel microtubules by kinesin-1. Kinesin-1 links two microtubules through the kinesin heavy chain and slides microtubules with their minus-ends leading and plus-ends trailing.

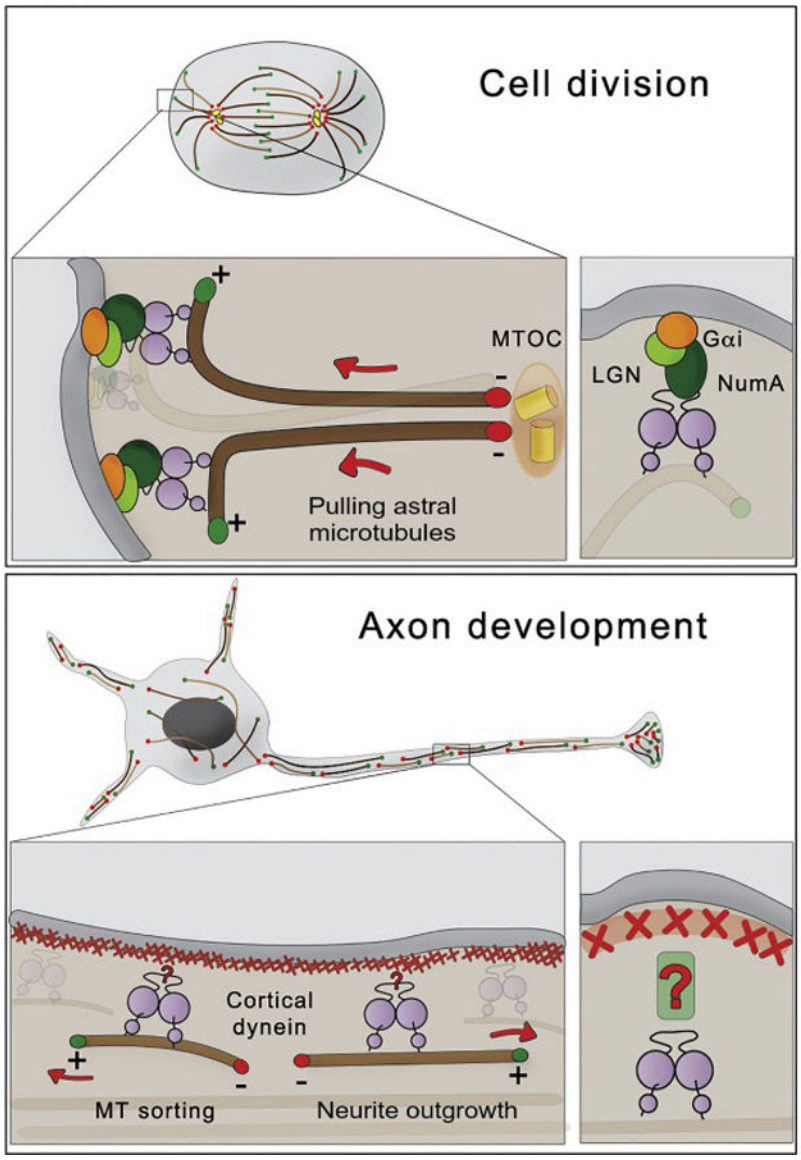

Figure 2. Roles of cortical dynein in neurodevelopment and cell division

Top Panel. During cell division, dynein is enriched at the cortex distal to the spindle poles. Cortical dynein generates pulling forces on astral microtubules required for centrosome separation, positioning and orientation of the mitotic spindle. The recruitment of dynein to the cortex in mitotic cells is mediated by Gαi/LGN/NuMA.

Bottom Panel. Cytoplasmic dynein is recruited to the cortical actin in axons. This recruitment is required for removing microtubules with “wrong” (minus-ends out) orientation from axons. On the other hand, microtubules with “right” (plus-ends out) orientation are transported towards the tip of the axons, inducing neurite outgrowth. Currently, it is unknown how dynein is targeted to the cortical actin in the axons.

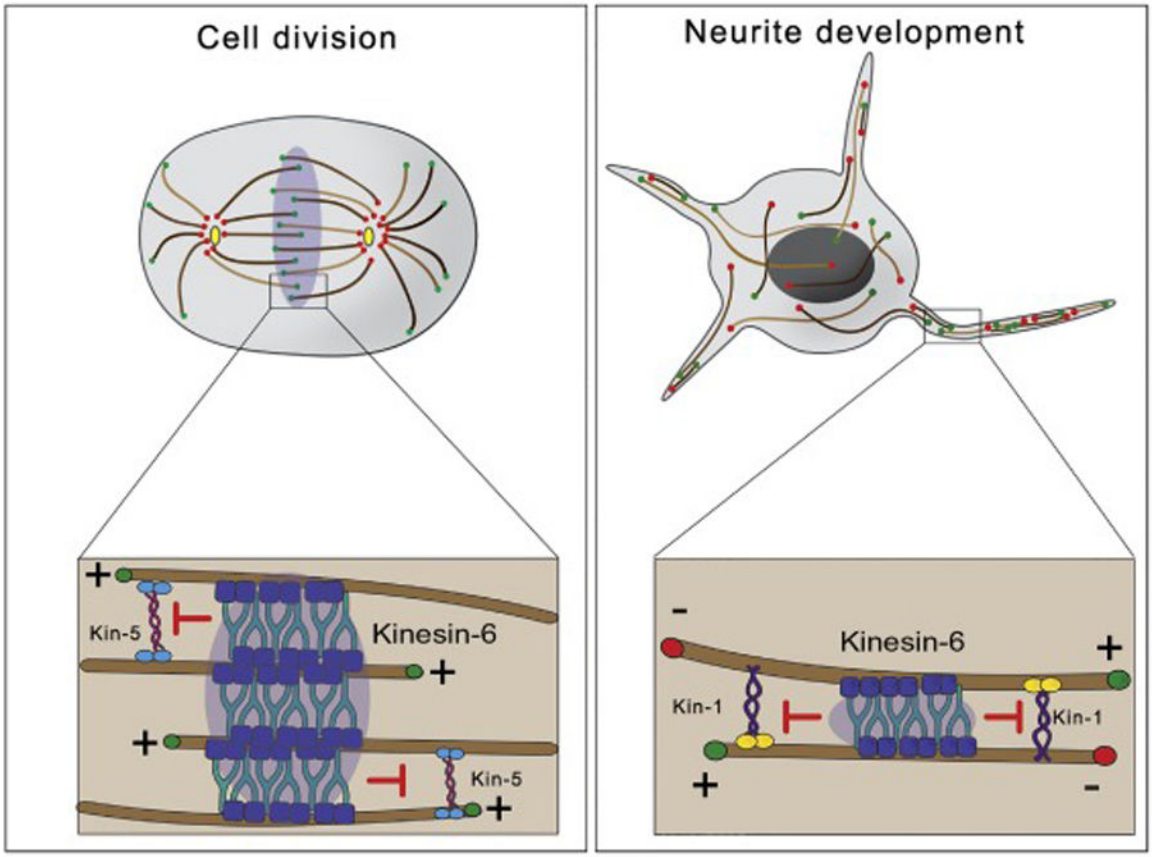

Figure 3. Roles of kinesin-6 in neurodevelopment and cell division.

Left panel. In anaphase B, kinesin-6 motors concentrate at the midzone of the mitotic spindle. There, microtubule bundling activity of kinesin-6 counteracts microtubule sliding forces driven by kinesin-5 and stabilizes the mitotic spindle.

Right panel. In developing neurons, neurite outgrowth is mainly driven by kinesin-1-powered microtubule sliding. Sliding and neurite outgrowth are downregulated in mature neurons by the microtubule bundling activity of kinesin-6.

Table 1.

Cytoskeletal proteins in neurite outgrowth

Protein	Role in mitosis	Experimental approaches	Role in neuronal development	Reference
Kinesin-5	Bipolar spindle formation	siRNA in cultured rat neurons. Chemical inhibition in cultured rat neurons.	Axon outgrowth, Brake on microtubule transport, Growth cone turning	[8, 29, 30]
Kinesin-1		RNAi and mutation in cultured Drosophila neurons. Mutation in Drosophila *in vivo*	Neurite outgrowth. Microtubule sliding	[5, 13]
Dynein	Centrosome separation, mitotic spindle positioning	Dynein heavy chain RNAi in cultured Drosophila neurons Dynein complex component mutation in Drosophila *in vivo* siRNA/Chemical inhibition in rat cultured neurons	Microtubule polarity Microtubule transport	[7, 19, 21, 22]
Kinesin-6	Bundle spindle microtubules	RNAi in Drosophila *in vitro* and *in vivo* RNAi in mouse hippocampal cultures	Brake on sliding Brake on neurite overextension Microtubule polarity	[39, 41]
Kinesin-12	Microtubule crosslinker. Maintain spindle.	siRNA in rat hippocampal cultures Knock out in zebrafish *in vivo*	Axon outgrowth Bidirectional microtubule transport Regulate microtubule invasion into growth cones Axon outgrowth Axon regeneration	[48–50]
Kinesin-14	Spindle organization	siRNA and chemical inhibition in rat hippocampal or sympathyetic cultures	Axon outgrowth Axon branching Microtubule polarity	[46]
Kinesin-13	Microtubule depolymerizer	Inhibition and Conditional knockout in mouse culture. Mutant Kinesin-13 in *C. elegans in vivo*	Correct branching. Axon specification	[57–59]
Mis-12	Kinetochore component	Drosophila RNAi and mutant *in vivo*	Neurite extension. NMJ formation.	[62]
Ndc80	Kinetochore-microtubule attachment	Drosophila RNAi *in vivo C. elegans* depletion and mutation *in vivo*	Neurite extension. NMJ formation.	[62, 63]