HHS Public Access

Author manuscript

Curr Opin Neurobiol. Author manuscript; available in PMC 2019 August 01.

Published in final edited form as:

Curr Opin Neurobiol. 2019 August ; 57: 39–45. doi:10.1016/j.conb.2019.01.003.

Microtubule control of functional architecture in neurons

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Abstract

Neurons are exquisitely polarized cells whose structure and function relies on microtubules. Microtubules in signal-receiving dendrites and signal-sending axons differ in their organization and microtubule-associated proteins. These differences, coupled with microtubule post-translational modifications, combine to locally regulate intracellular transport, morphology, and function. Recent discoveries provide new insight into the regulation of non-centrosomal microtubule arrays in neurons, the relationship between microtubule acetylation and mechanosensation, and the spatial patterning of microtubules that regulates motor activity and cargo delivery in axons and dendrites. Together, these new studies bring us closer to understanding how microtubule function is locally tuned to match the specialized tasks associated with signal reception and transmission.

Introduction

Cell shape and polarity are integral to cell function. Neurons display a variety of morphologies to match the range of functions that they carry out. Simple or complex in shape, all neurons are highly compartmentalized with distinct signal-receiving dendrites and signal-sending axons. A neuron's morphology and function depend on the underlying microtubule cytoskeleton, which both sculpts neuronal structure and mediates the transport of RNAs, proteins, vesicles, and organelles that sustain neuronal activity. Given the distinct compartmentalization of neuronal activity, it is not surprising that microtubule organization and function differ between axons and dendrites and also vary within these compartments. A major challenge is to determine how microtubules are locally regulated to match specific neuronal activities. Here we highlight recent studies on the patterning of microtubules that diversify microtubule function regionally and regulate transport by molecular motors.

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Conflict of interest statement
Nothing declared.

Microtubule organization in neurons

The neuronal microtubule cytoskeleton shapes and supports the axonal and dendritic projections that carry out the distinct functions of signal reception and transmission. Microtubules in axons and dendrites are distinguished by their polarity, organization, post-translational modifications, and microtubule-binding proteins (Figure 1). Microtubules have an intrinsic polarity due to the head-to-tail assembly of α- and β-tubulin dimers (the β- and α-tubulin ends are referred to as the plus- and minus-ends, respectively). Molecular motors and other proteins read-out this polarity, which is thought to be a central underpinning of neuronal polarity. In axons, microtubules are uniformly arrayed with their plus-end positioned distal towards the axon terminal, whereas in dendrites, microtubule polarity is mixed to varying degrees depending on position within the dendritic arbor, neuronal type, and organism (Figure 1A). Microtubule orientation is regulated in part by the molecular motors dynein and kinesin, which play key roles in constructing the tracks that they use for transport [1–5]. Several additional proteins also regulate microtubule orientation, including the mammalian tripartite motif-containing (TRIM) protein TRIM46, whose exact molecular activity remains mysterious [6]. Loss of TRIM46 disrupts the uniform orientation of axonal microtubules. However, the localization of dendritic cargos, such as AMPA receptors, are unaffected. This suggests that disrupting microtubule polarity alone may not be sufficient to alter the functional polarity of neurons. Given the additional ways in which microtubules are patterned and microtubule-based transport is regulated, microtubule polarity may be just one part of creating distinct axonal and dendritic compartments.

Microtubules in neurons, unlike in other cell types, are not typically anchored at a defined microtubule organizing center (MTOC). In some organisms, such as fruit flies, neurons lack a centrosome entirely, whereas in mammals, the post-mitotic neuronal centrosome mainly supports ciliary function [7–9]. In the absence of a central MTOC, microtubule nucleation, growth, and organization are locally regulated. Recent work has shed light on the regulation of gamma-tubulin-mediated nucleation by the augmin complex, the CAMSAP microtubule minus-end-binding proteins, and the microtubule-binding proteins TPX2 and SSNA1 [9–19]. Augmin, which recruits gamma-tubulin to microtubules to amplify existing arrays, increases microtubule density to enable efficient transport in both axons and dendrites [9,10]. In mammals, loss of augmin function perturbs axonal microtubule polarity, possibly by de-coupling gamma-tubulin from existing microtubules, which then allows for the formation of misoriented microtubules. In dendrites,however,loss of augmin alone does not affect microtubule polarity, and studies in flies suggest that augmin activity is restricted by centrosomin/CDK5RAP2 [10,11]. Centrosomin/CDK5RAP2, along with GM130 and AKAP450, are proposed to couple gammatubulin-mediated microtubule nucleation to dendritic Golgi outposts, which serve as local MTOCs in developing neurons [11,20,21]. In addition to shaping microtubule networks through microtubule nucleation, a recent study in worms has shown that the growth of existing microtubules in dendrites is locally controlled by dynein, which tethers microtubule plus-ends to restrain their growth [22]. Thus, neurons use a combination of mechanisms to spatially and temporally tune the microtubule cytoskeleton. Such local regulation of nucleation, growth, and organization gives

microtubules the flexibility to mediate distinct activities and to respond dynamically to changes in neuronal function.

Diversifying microtubule function through post-translational modifications: focus on acetylation

Microtubules are patterned by a variety of post-translational modifications (PTMs), such as acetylation, polyglutamylation, phosphorylation, and detyrosination. An appealing model is that microtubule function, and thus its role in neuronal activity, is locally controlled by different PTM patterns that could affect microtubule stability and growth as well as microtubule-associated proteins (MAPs), including molecular motors. One of the best-studied microtubule modifications is the acetylation of α-tubulin lysine 40 (K40), a residue in the microtubule lumen. This modification has intrigued researchers since its discovery over 30 years ago in large part because it is highly conserved and correlates with stable long-lived microtubules in virtually all cells, including neurons [23,24]. Acetylation itself, however, does not confer stability to microtubules; rather, recent work suggests that acetylation protects microtubules against mechanical stresses by strengthening lateral interactions between protofilaments [25]. By making microtubules resilient to mechanical stress, acetylation of α-tubulin K40 may enable microtubules to persist in cells and/or may reinforce the stability of long-lived microtubules [26]. Neurons, which survive throughout an organism's lifetime, may be particularly reliant on α-tubulin K40 acetylation to preserve stable connections. Indeed, loss of α-tubulin K40 acetylation has been associated with neuronal degeneration [27].

Microtubules in mechanosensory neurons, which experience repeated mechanical stresses, are typically highly acetylated [24]. Eliminating microtubule acetylation by either mutating α-tubulin K40 or deleting the modifying enzyme α-tubulin acetyltransferase 1 (αTAT1) does not cause lethality but does impair touch sensitivity [24]. Two recent studies point to the mechanistic roles that acetylated microtubules may play in mechanosensation (Figure 1B). Touch and other mechanical stimuli trigger ion channel opening when force is exerted on a neuronal membrane. Some mechanosensory ion channels, including the TRP channel NOMP, directly contact microtubules [28]. New data from mice suggest that acetylated microtubules beneath the cell membrane increase cell stiffness to a range that allows for optimal gating of mechanosensory ion channels [29]. Work from flies has revealed that acetylation preserves the microtubule scaffold that NOMPC relies on for its activity [30]. Thus, acetylated microtubules likely serve a dual function in mechanosensation by tuning cell stiffness and serving as a stable scaffold for mechanosensory ion channels such as NOMPC.

In addition to their role in mechanosensation, acetylated microtubules and αTAT1 can shape neuronal morphology. In developing neurons, acetylated microtubules and αTAT1 generally, though not always, act to restrict neurite growth. For example, mutating α-tubulin K40 in flies disrupts the refinement of sensory dendrites leading to excess branching [31]. Conversely, mimicking the acetylation of α-tubulin K40 suppresses axon terminal overgrowth induced by over –expressed mutant human Tau [32]. The loss of αTAT1 in mice

similarly results in increased axon growth and branching in the developing brain [33]. It is somewhat unclear how microtubule acetylation and/or αTAT1 would regulate neurite growth. Although acetylation does not affect the growth of stabilized microtubules in vitro, loss of αTAT1 in vivo correlates with an increase in microtubule growth frequency in neurons [27,30,33]. Notably, αTAT1 increases microtubule growth frequency in NIH 3T3 cells by destabilizing dynamic microtubules [34], which may hint at how αTAT1 restrains microtubule growth and neurite growth in developing neurons. While the effects of PTMs, such as acetylation, on microtubules in vitro and in cell lines have been characterized, there is still much to learn about the roles that these modifications play in regulating microtubules in neurons in vivo.

Microtubules regulate cargo trafficking

Microtubules serve a fundamental role as the “roads” that molecular motors traverse to deliver cargo. The unique morphological and functional polarity of neurons pose particular challenges to the accurate and precise delivery of cargos. In addition, axons and dendrites often extend over large territories, requiring molecular motors to sustain transport over significant distances. In humans this is perhaps best highlighted in motor neurons, where cargos originating in the cell body are reliably delivered to specific destinations up to a meter away. Meeting these transport challenges are multiple kinesin family members and dynein, which mediate transport to the plus-and minus-ends of microtubules, respectively. Given the uniform plus-end-distal orientation of axonal microtubules, it follows that kinesins move cargo to axon terminals. In contrast, dendritic cargos are typically moved out of the cell body by dynein and, in mammalian neurons, a subset of kinesins. However, kinesins and dynein are active within both compartments, so tight regulation of microtubule-based transport must exist to ensure the proper delivery of cargo to its subcellular destination. MAPs, microtubule PTMs, as well as other motor-binding proteins play important roles in selectively regulating the association of motors with microtubules [23,35–37]. Exciting new progress has been made in delineating the relationships between MAPs that have opposing effects on different motors (e.g. kinesin-1 versus kinesin-3), providing insight into the MAP-based regulation of motor activity [38]. In addition, recent work has highlighted the role of kinesin autoinhibition in proper axon outgrowth,the localization of cargo to dendrites, and in the regulation of synapse formation, including synapse size and density [39–41]. It will be of great interest to determine how the patterning of the microtubule cytoskeleton intersects with the regulation of motor activity to direct the selective transport of the many different axonal and dendritic cargos that are needed for proper neuronal activity.

Microtubule-based transport in dendrites

In addition to compartment-specific differences in microtubule polarity, the PTMs that decorate microtubules can also vary between and within axons as well as dendrites. This patterning of microtubules by different combinations of PTMs as well as tubulin isotypes is referred to as the “tubulin code” [23,42]. Recent work has revealed a striking correlation between microtubule polarity and the tubulin code in the dendrites of cultured hippocampal neurons [43] (Figure 1). “Motor-paint,” super-resolution microscopy coupled with motor particle tracking, showed that dendritic microtubules of the same polarity are bundled

together, marked with distinctive PTMs, and are preferred tracks for different motor families. Microtubules oriented with their plus-ends towards the cell body are acetylated and preferentially traversed by kinesin-1 whereas plus-end-distal microtubules are heavily tyrosinated and bound by kinesin-3. Consistent with these findings, acetylated microtubules in cultured cells are also preferentially bundled together to promote kinesin-1 motility [44]. Kinesin- 1 and kinesin-3 were previously reported to differentially prefer either acetylated or tyrosinated microtubules, respectively [45–48]. Notably, kinesin-3 is recruited to tyrosinated microtubules in hippocampal dendrites by the MAP doublecortin-like kinase 1 (DCLK1), suggesting a potential coordination between microtubule polarity, the tubulin code, and MAPs [47]. Like kinesin-3, the microtubule minus-end-directed motor dynein carries cargo into dendrites and preferentially binds tyrosinated microtubules in vitro and in mammalian axons [49,50]. However, the findings of Tas et al. would suggest that dynein travels on acetylated microtubule tracks into dendrites [43]. Thus, it is likely that these microtubule PTMs may bias, but do not absolutely determine, the tracks that motors traverse.

In addition to DCLK1, kinesin-mediated transport into the dendrites of mammalian neurons is regulated by a microtubule-associated septin, Septin 9 [51]. Septins are GTP-binding proteins that can self-assemble into a variety of structures, including filaments [52]. Septin 9 localizes to microtubules in proximal dendrites and selectively allows kinesin-3, but not kinesin-1, to directly enter dendrites [51]. Septin 9 also regulates transport to hippocampal dendrites by directly binding to the kinesin-2 motor KIF17 [53]. Unlike its effects on kinesin-1 and kinesin-3, Septin 9 modulates the interaction of KIF17 with its cargo but does not disrupt KIF17 motility. Thus, Septin 9 has the ability to regulate transport through distinct mechanisms. It is unclear whether or how Septin 9 function intersects with other MAPs or microtubule PTMs, although another septin, Septin 7, has been implicated in regulating microtubule acetylation by recruiting HDAC6 to microtubules in mammalian neurons [54]. While knocking-down Septin 9 does not perturb microtubule organization or neuronal architecture, its effects on cargo transport indicate that Septin 9 plays an integral role in the functional polarity of neurons.

How is microtubule trafficking locally regulated to mediate dendritic function? In hippocampal neurons, growing microtubules have been caught transiently entering dendritic spines, the actin-enriched protrusions that contain excitatory synapses [55–57] (Figure 1D). These polymerizing microtubules are used by kinesin-3 to carry synaptotagmin IV, which regulates exocytosis, into spines via a so-called “direct deposit” mechanism [58]. The entry of microtubules into spines and delivery of synaptotagmin IV is enhanced by BDNF-mediated stimulation. BDNF stimulation also regulates the kinesin-4 family member KIF21B, which both transports cargo and regulates microtubule plus-end dynamics [59–61]. Evidence suggests that the motor shifts to a predominant cargo transport mode upon BDNF stimulation [60]. This interplay between motors, cytoskeletal dynamics, and neuronal activity is integral to learning and memory.

Microtubule-based transport in axons

Axons are comprised of different domains: the proximal axon controls cargo entry and the axon shaft is a highway for the delivery of cargo to synapses at the axon terminal.

Specialized microtubule and actin cytoskeletons in the proximal axon regulate cargo trafficking by affecting motor behavior to permit the selective entry of axonal, not dendritic, cargos [62]. In hippocampal axons, the proximal axon is divided into two zones, a pre-axonal exclusion zone and axon initial segment (AIS), both of which regulate cargo entry. To exclude dendritic cargo, the dynein cofactor Ndel1, which is enriched at the AIS, “catches” dendritic cargo and transfers the cargo to dynein for transport to dendrites [63] (Figure 1E). The fly ortholog of Ndel1, NudE, likely carries out a similar role in restricting the entry of Golgi into axons [64]. Ndel1 activity at the AIS is regulated by CDK5 phosphorylation, and enrichment of the CDK5 activator p35 at the AIS provides spatial control over Ndel1 and dynein function in axons [2]. Kinesin activity is also both positively and negatively regulated in the proximal axon. When bound to dendritic cargo, the kinesin-2 motor KIF17 initially targets the AIS, where it remains bound to microtubules until dynein is recruited to shuttle the cargo and KIF17 to dendrites [65]. Thus, proper targeting of a cargo depends on the coordination of motor activity, in particular the coordination of motors that pull cargos towards opposite ends of a microtubule. In the proximal axon of mammalian sensory neurons, MAP2 inhibits kinesin-1 but not kinesin-3, hence restricting the entry of dense core vesicles (DCVs) that are not bound by both motors [66] (Figure 1D). Once DCVs have made it past the proximal axon, the motors coordinate their activity for efficient transport to the axon terminal [66,67].

Beyond the proximal axon, the main axonal microtubule cytoskeleton is composed of overlapping microtubule polymers. Pioneering work in worm neurons revealed that microtubule length and density set the upper rate limit of efficient transport [68]. Using a combination of light and electron microscopy, the authors correlated axonal microtubule organization and cargo trafficking. This correlative analysis also enabled the development of a powerful fluorescence-based approach to quantify microtubule length and organization along the axon in neurons in vivo and in different genetic mutants. At the axon terminal, microtubule stability and dynamics shape terminal morphology as well as the formation and remodeling of synapses. In a class of worm neurons, an increase in microtubule dynamics drives synapse remodeling by affecting kinesin-mediated transport and does so independently of changes in morphology [69].

Concluding Remarks

Neurons are incredibly complex cells that rely on a highly differentiated microtubule cytoskeleton to carry out the specialized tasks of signal reception and transmission. Yet we still know little of the mechanisms that diversify microtubule function and regulate the dynamic remodeling of microtubule networks that underlie neuronal shape and create distinct functional domains. Understanding the intricacies of microtubule organization and dynamics and their interplay with cargo transport is difficult. However, new technologies are making it easier to visualize and probe microtubules, motors, and MAPs in neurons. One central challenge is to translate what is known about the activity of purified microtubules, MAPs, and motors in vitro into understanding their function and regulation in neurons in vivo. This can be done, in part, by leveraging genome engineering techniques to introduce function-probing mutations in native tubulin proteins, MAPs, and molecular motors. Genome engineering has made it easier to mutate and tag endogenous RNAs and proteins,

thus eliminating potential over-expression artifacts, and has facilitated the simultaneous manipulation of multiple genes. Moreover, the development of new optogenetic approaches will assist in probing microtubule function with spatial and temporal precision. In addition to these functional approaches, advances in cryo-electron microscopy hold the exciting promise of gaining high-resolution images of microtubules and their associated proteins in neurons [70]. These new tools will aid in achieving a molecules-to-cells understanding of how microtubules and transport are locally regulated to mediate signal reception and transmission.

Acknowledgements

We thank Erik Dent (University of Wisconsin-Madison), Dena Johnson-Schlitz, Josephine Werner, and other members of the Wildonger lab for helpful feedback and discussions. This work was supported by the National Institutes of Health [R01 NS102385].

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Highlights

- Regulation of non-centrosomal microtubule organization in neurons
- Role of resilient acetylated microtubules in mechanosensation
- Patterning microtubule tracks to deliver cargo to the right place at the right time

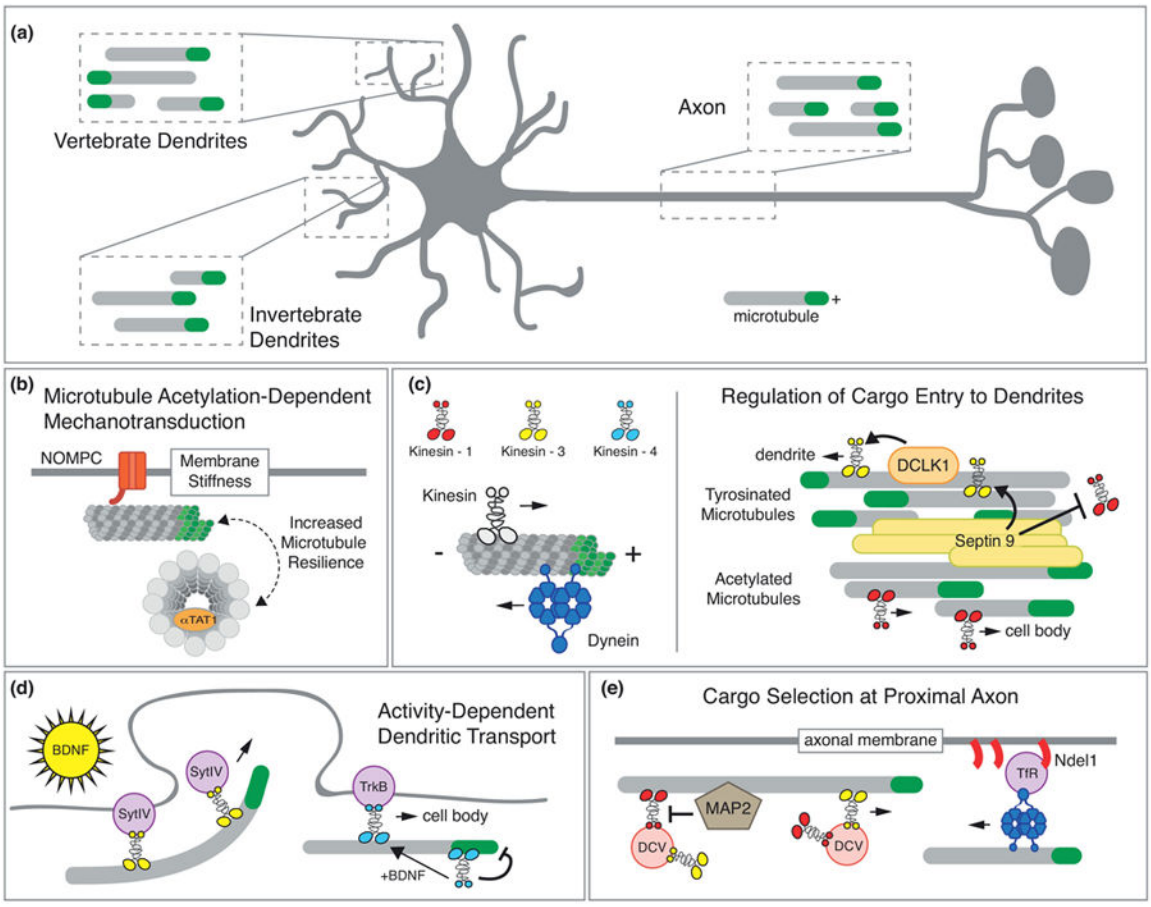

Figure 1. Microtubules carry out diverse roles within neurons.

Microtubules within axons and dendrites are distinctly organized. Axonal microtubules are uniformly oriented with their plus-ends positioned distal, or away from the cell body. Microtubules in the dendrites of invertebrate neurons are largely oriented with their plus-ends toward the cell body whereas microtubules in vertebrate dendrites typically have a mixed polarity. (B) αTAT1 acetylates α-tubulin at lysine 40, which is located within the microtubule lumen. Acetylated microtubules regulate mechanotransduction mediated by transmembrane channels, such as NOMPC, by tuning cell membrane stiffness and stabilizing microtubules. (C) Microtubules serve as the roads for plus-end-directed kinesin family members (top) and the minus-end-directed dynein motor complex (bottom). Transport into dendrites is controlled by microtubule organization, PTMs, and MAPs. In hippocampal neurons, microtubules of the same polarity are bundled together and marked by distinct post-translational modifications (tyrosination, acetylation). DCLK1 promotes the motility of kinesin-3 motors into dendrites along bundles of tyrosinated microtubules. Septin 9 selectively enhances kinesin-3 motility while preventing kinesin-1 from walking into dendrites. Instead, dendrite-localized kinesin-1 returns to the cell body along distinct tracks of acetylated microtubules. (D) BDNF stimulates microtubules to transiently grow into dendritic spines where they provide the tracks for the delivery of SytIV vesicles by kinesin-3 motors. BDNF likewise triggers the transport of TrkB vesicles by kinesin-4, a motor that also regulates microtubule plus-end dynamics. (E) MAP2 in the proximal axon blocks

kinesin-1-mediated transport of DCVs and thus selects for DCVs carried by both kinesin-1 and kinesin-3, which coordinately transport DCVs to the axon terminal. The dynein cofactor Ndel1 is anchored in the proximal axon where it ‘catches’ dendritic cargoes, such as the transferrin receptor (TfR), and primes their transport by dynein into dendrites.