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Neuron
Review

Building the Neuronal Microtubule Cytoskeleton

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http://dx.doi.org/10.1016/j.neuron.2015.05.046

Microtubules are one of the major cytoskeletal components of neurons, essential for many fundamental cellular and developmental processes, such as neuronal migration, polarity, and differentiation. Microtubules have been regarded as critical structures for stable neuronal morphology because they serve as tracks for long-distance transport, provide dynamic and mechanical functions, and control local signaling events. Establishment and maintenance of the neuronal microtubule architecture requires tight control over different dynamic parameters, such as microtubule number, length, distribution, orientations, and bundling. Recent genetic studies have identified mutations in a wide variety of tubulin isotypes and microtubule-related proteins in many of the major neurodevelopmental and neurodegenerative diseases. Here, we highlight the functions of the neuronal microtubule cytoskeleton, its architecture, and the way its organization and dynamics are shaped by microtubule-related proteins.

Introduction

The formation of complex nervous systems requires cytoskeleton-based processes that coordinate proliferation, migration, and differentiation of neurons. Neuronal cells undergo major developmental changes as they migrate, develop axons and dendrites, and establish synaptic connections. The structural organization and dynamic remodeling of the neuronal cytoskeleton contribute to all these morphological and functional changes in neurons. Along with the actin cytoskeleton, the assembly, organization, and remodeling of the microtubule (MT) cytoskeleton are essential to successfully complete all the different stages of neuronal development (Figures 1A and 1B) (Barnes and Polleux, 2009; Kuijpers and Hoogenraad, 2011; Marín et al., 2010). MTs either provide tracks for intracellular transport (Hirokawa et al., 2010; Maday et al., 2014), set up local cues to position organelles (de Forges et al., 2012), act as signaling devices (Akhmanova and Steinmetz, 2008), or generate cellular forces (Subramanian and Kapoor, 2012) (Figures 1C and 1D).

Reflecting the importance of the MT cytoskeleton in neuronal development, MT defects cause a wide range of nervous system abnormalities and several human neurodevelopmental disorders have been linked to altered microtubule-mediated processes. Mutations in microtubule-related genes encoding for microtubule-associated proteins (MAPs) (e.g., Tau), MT severing proteins (e.g., spastin), microtubule-based motor proteins (e.g., dynein, kinesin), and motor associated regulators (e.g., dynactin, doublecortin, and lis1) are associated with various neurodevelopmental problems (Lipka et al., 2013; Reiner and Sapir, 2013). In addition, impairment of axonal transport in mature neurons is a common factor in many of the major neurodegenerative diseases, including the motor neuron diseases amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (Millecamps and Julien, 2013) and neuroinflammatory diseases such as multiple sclerosis (Sorbara et al., 2014). More direct evidence supporting the involvement of the MT cytoskeleton in neurodegenerative diseases comes from genetic studies identifying mutations in various tubulin family members (Franker and Hoogenraad, 2013; Tischfield et al., 2011). However, many of the molecular mechanisms regarding the assembly of microtubule-based structures remain largely unknown, and we still do not understand how the complex MT arrays are built and maintained in neurons.

Over the last decades, the neuronal MT architecture has been studied in detail using electron microscopy and live cell imaging (Baas and Lin, 2011; Conde and Cáceres, 2009). Imaging dynamic MTs has greatly advanced our understanding of the complex remodeling and reorganization of MT during neuronal development and plasticity processes (Hoogenraad and Bradke, 2009). Recent advances in super resolution fluorescence microscopy have revealed that the organization of the neuronal cytoskeleton is even more complex than previously thought (D'Este et al., 2015; Xu et al., 2013; Yau et al., 2014; Zhong et al., 2014) (Figure 1B). On the other hand, results from structural, biochemical, and in vitro reconstitution approaches have increased the mechanistic understanding of MT assembly and dynamics (Akhmanova and Steinmetz, 2008; Brouhard and Rice, 2014; Subramanian and Kapoor, 2012). Several other studies have described signaling pathways and molecular mechanisms that control the assembly, dynamics, and stabilization of MT arrays during neurodevelopment (Arimura and Kaibuchi, 2007). Here, we review the basic functions of the neuronal cytoskeleton and its main architectural features, as well as the many molecular processes that together control MT organization and dynamics during the different stages of neuronal development.

Microtubule Basics

The MT core structure is built from heterodimers of α- and β-tubulin, which bind in a head-to-tail fashion to form structurally polarized linear protofilaments and associate laterally to form a hollow tube with an outer diameter of ~25 nm (Akhmanova and Steinmetz, 2008) (Figure 1D). De novo MT formation in cells is typically nucleated by the γ-tubulin ring complex (γ-TuRC) that templates the formation of MTs (Kollman et al., 2011). MTs switch between phases of growth, and disassembly is a process

named dynamic instability, which allows individual MTs to explore cellular regions and retract in case they do not find the proper environment (Howard and Hyman, 2009). MT dynamics is regulated by the intrinsic properties of tubulin dimers within the lattice. Free tubulin binds GTP, which (for β-tubulin) is hydrolyzed shortly after incorporation into the MT lattice. Because GDP tubulin tends to destabilize the lattice, stable growth is believed to depend on the presence of a cap of GTP-tubulin at the MT plus end. In this GTP-cap model, loss of the cap will result in rapid disassembly, called a catastrophe. While many questions remain about the stochastic switching between MT assembly and disassembly, a structural model for how GTP hydrolysis destabilizes the MT has recently been proposed (Alushin et al., 2014).

Although both MT ends can grow and depolymerize, the dynamics of the two MT ends are very different (Akhmanova and Hoogenraad, 2015). The plus end, terminated by β-tubulin, grows faster, undergoes catastrophe more frequently and is a crucial site for regulating MT dynamics (Akhmanova and Steinmetz, 2008). MT dynamics is regulated by plus-end tracking proteins (+TIPs), which accumulate at the ends of growing MTs and control different aspects of neuronal development and function (Hoogenraad and Bradke, 2009). End binding (EB) proteins are among the core +TIP complex components as they can autonomously track growing MT plus ends and bind numerous other +TIPs, such as MT motors, actin-associated proteins, and signaling factors, through a conserved basic and serine-rich region containing a core SxIP motif (Honnappa et al., 2009; Jiang et al., 2014). In addition, several other +TIPs target the plus-end independent of EB proteins (Akhmanova and Steinmetz, 2008). Fluorescently tagged EB proteins are widely used to track growing MTs and provide an efficient tool to probe the MT organization in neuronal cells in vitro and in vivo (Jaworski et al., 2009; Kleele et al., 2014; Stone et al., 2008). However, the assembly of microtubule-based structures not only depends on +TIPs but also requires the coordinated actions of many additional regulatory factors such as neuron specific tubulin isotypes, post-translational modifications (PTMs), motor proteins, and various MAPs (Janke and Kneussel, 2010; Subramanian and Kapoor, 2012).

Largely on the basis of their mode of action, microtubule-related proteins can be roughly divided into five groups (Figure 1D). The first group comprises proteins that bind to MT ends and regulate their dynamics, such as +TIPs and minus-end targeting proteins (−TIPs) (Akhmanova and Hoogenraad, 2015; Akhmanova and Steinmetz, 2008). The second group contains proteins that bind to the MT lattice and can stabilize or crosslink MTs (Dehmelt and Halpain, 2005; Subramanian and Kapoor, 2012). The third group includes proteins that directly modulate MT numbers, such as regulators of nucleation (Kollman et al., 2011) and enzymes that sever pre-existing MTs (Roll-Mecak and McNally, 2010; Sharp and Ross, 2012). The fourth group comprises the kinesin and dynein family members that can generate forces and induce directional movement along MTs (Hirokawa et al., 2010). The fifth set comprises tubulin folding cofactors and tubulin-modifying enzymes that through PTMs can generate distinct MT subtypes (Hammond et al., 2008; Janke and Kneussel, 2010; Szolajska and Chroboczek, 2011).

Functions of the Neuronal Microtubule Cytoskeleton

Neuronal MTs guide intracellular transport and induce morphological changes during the various phases of neuronal development and synapse formation. In this section, we will briefly highlight the most important neuronal processes that depend on the MT cytoskeleton.

Microtubules Guide Intracellular Transport

The extreme dimensions of neurons necessitate active transport mechanisms to properly distribute many different cellular components and to establish robust signaling pathways from the synapse to the soma and vice versa (Hirokawa et al., 2010; Maday et al., 2014). The development and maintenance of axonal and dendritic processes and the formation and dynamics of synaptic structures all depend on proper intracellular transport. Microtubule-based motors of the kinesin and cytoplasmic dynein families drive the transport of many types of neuronal cargo, including organelles, synaptic vesicle precursors, neurotransmitter receptors, cell adhesion molecules, cell signaling molecules, and mRNAs (Hirokawa et al., 2010; Kardon and Vale, 2009). In addition, various mechanisms operate to ensure the delivery of cargo to the correct location. Cargo-adaptor proteins, regulatory molecules, and local signaling pathways play important roles in proper cargo loading, anchoring, motility, and delivery in neurons (Maday et al., 2014). The specific organization of the MT cytoskeleton also provides selective transport routes for the sorting of cargo into either axons or dendrites (Kapitein and Hoogenraad, 2011; Rolls, 2011). Recent studies demonstrated that the selective presence of minus-end out-oriented MTs in dendrites enables the minus-end directed motor dynein to selectively transport cargoes into dendrites (Kapitein et al., 2010). In addition, plus-end directed motor kinesin-1 has been shown to selectively transport cargoes into the axon, despite the presence of plus-end out-oriented MTs in dendrites (Nakata and Hirokawa, 2003). Axon selectivity appears mediated by specific properties of stabilized and/or modified axonal MTs, as treatment with the MT stabilizing agent paclitaxel results in non-polarized targeting to both axons and dendrites (Kapitein et al., 2010). Nevertheless, the exact MT properties that guide certain kinesins to axons have remained poorly understood (Hammond et al., 2010; Huang and Banker, 2012; Konishi and Setou, 2009; Nakata et al., 2011).

Microtubules Establish Morphological Transitions

MTs also play important roles during the morphological transitions that occur during neuronal development, such as neurite initiation, migration, polarization, and differentiation. MTs contribute to these processes by facilitating transport to specific sites, by providing mechanical forces, or by acting as local signaling platform.

Neuronal Migration. Neuronal migration in the developing brain involves a complex sequence of motile and morphogenetic events (Marín et al., 2010). Neurons extend a leading process, translocate the nucleus into the leading process, and eliminate its trailing process, which leads to the net movement of the cell. All these steps are driven by both actin and MT dependent forces (Cooper, 2013). Actin dynamics promotes neuronal migration by protrusive polymerization of actin at the leading process and/or propulsive contractions at the cell rear. The MT cytoskeleton in migrating neurons is anchored

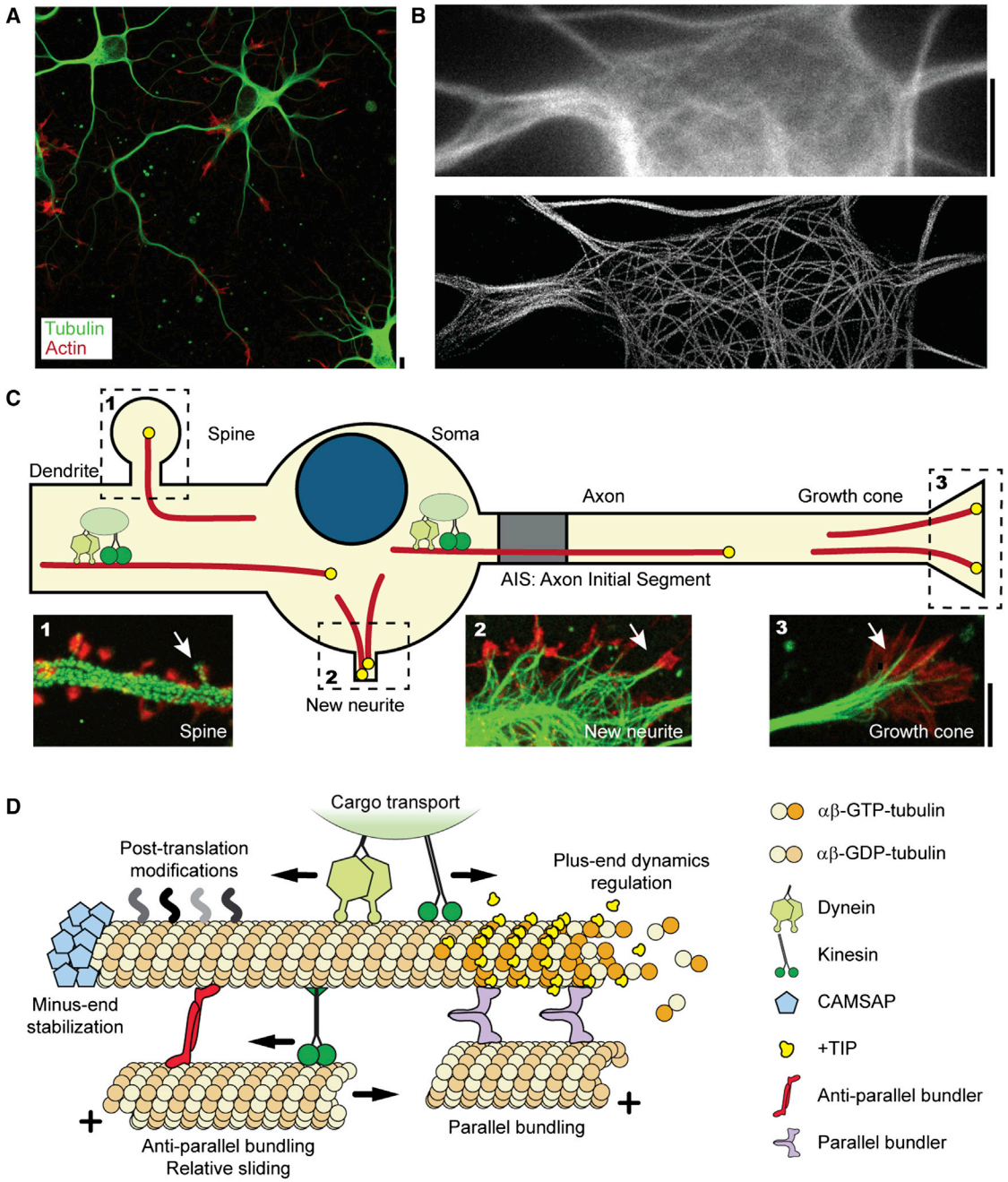

Figure 1. Neuronal Microtubule, Their Functions, and Associated Proteins

(A) Cultured rat hippocampal neurons at day in vitro 6 (DIV6), stained for tubulin (green) and actin (red). Scale bars, 5 μm.

(B) DIV5 neuron stained for tubulin and imaged using conventional widefield microscopy or single-molecule localization microscopy (SMLM). Data from Yau et al. (2014).

(C) Cartoon illustrating the different functions of neuronal microtubules. Zooms show microtubule organization in dendritic spines (1), emerging neurites (2), and growth cones (3) of cultured rat hippocampal neurons. (1) Zoom of dendritic spines of neurons at DIV56 expressing the actin marker Lifeact (red) and the microtubule growth marker MT+TIP. For the MT+TIP, an overlay of 48 frames is shown to highlight the trajectories in the dendritic shaft, including spine entries. (2 and 3) Zooms of neurite formation (2) and a growth cone (3) from a cultured rat hippocampal neurons at DIV6, stained for tubulin (green) and actin (red). Arrows indicate overlap between the actin and microtubule cytoskeleton. Scale bars, 5 μm.

(D) Cartoon illustrating how different microtubule-related proteins interact with microtubules.

to the centrosome, extends into the leading process, and forms a cage-like structure around the nucleus (Rivas and Hatten, 1995). Cytoskeletal forces at the tip of the leading edge may then pull the centrosome into the proximal part of the leading process, thereby moving the nucleus in the direction of migration.

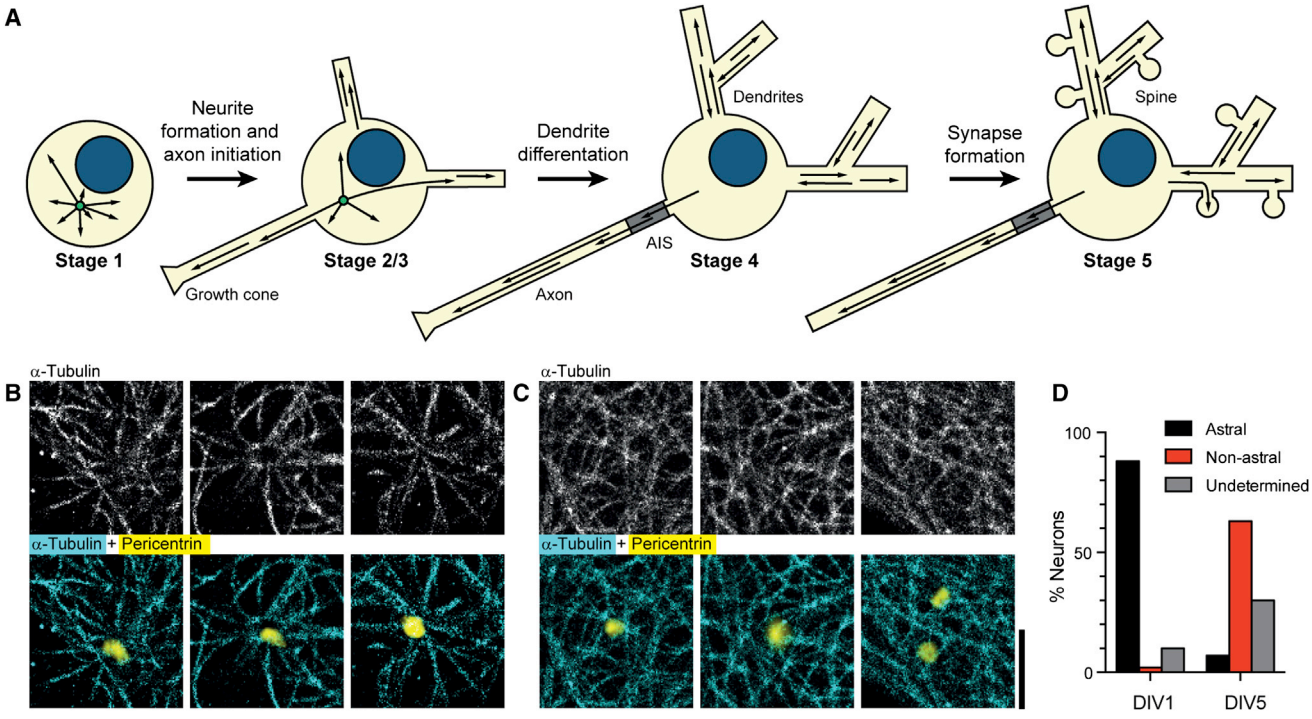

Figure 2. Changes in Microtubule Organization during Development

(A) After their final division, neurons transit through several developmental stages and the MT cytoskeleton has a pivotal role at all stages. During these stages, the MT organization changes from a radially centrosome-based and largely plus-end outward-oriented network to an acentrosomal network with uniform orientations in the axon and mixed orientation in dendrites.

(B and C) Three zooms of microtubule organization around the centrosome for three neurons at DIV1 (B) and DIV5 (C), obtained using SMLM. Bottom: overlay of SMLM microtubule image with conventional widefield image of pericentrin. Scale bars, 2 μm.

(D) Occurrence of astral and non-astral microtubule organization for neurons at DIV1 (n = 50 neurons) or DIV5 (n = 43 neurons). Data are from Yau et al. (2014).

Neurite Initiation and Outgrowth. Neuronal polarization begins with the breakage of the symmetric shape of a round, newborn neuron by minor neurite formations (Cáceres et al., 2012) (Figure 2A). These extending neurites contain bundled MTs and an actin-rich growth cone. Several studies have demonstrated that both MTs and actin filaments mediate the pushing and pulling forces that contribute to membrane protrusions (Dent et al., 2011). A model has been proposed in which neurite initiation and outgrowth depends on the local increase in actin dynamics in combination with stabilization of MTs (Flynn et al., 2012).

Axon Differentiation. MT stabilization plays a key role in the initial specification of the axon during neuronal polarization (Hoogenraad and Bradke, 2009). Local stabilization of MTs using a photoactivatable analog of the microtubule-stabilizing drug taxol induces axon formation in unpolarized neurons (Witte et al., 2008). Consistently, treatment of cultured neurons with low doses of taxol restricts MT dynamics to process tips and leads to the formation of multiple axons (Witte et al., 2008). Local MT stabilization in morphologically unpolarized neurons may also offer an explanation for the increased membrane traffic and selective targeting of kinesin motors that precedes axon formation. Kinesin-1 shows a higher affinity for stabilized and/or modified MTs and could select axon-specific tracks required for polarized trafficking (Kapitein et al., 2010; Nakata and Hirokawa, 2003; Nakata et al., 2011). Therefore, increased MT stability in the future axon may lead to kinesin-mediated polarized membrane flow and contribute to determining the site of axon formation. The endogenous mechanism responsible for local MT stabilization remains largely unknown. Hypothetically, internal signals like Golgi positioning, centrosome localization, or local self-promoting cytoskeleton assembly could initiate a local imbalance inside the MT network and stabilize MTs in only one of the neurites.

Axon Elongation and Regeneration. Imaging MT plus ends has advanced our understanding of the complex remodeling and reorganization of MTs in the growth cone during axon elongation (Dent et al., 2011). The MT cytoskeleton participates in functional interactions with adhesion complexes and actin, and numerous +TIPs are required for modulating MT dynamics and stability to influence growth-cone steering (Prokop, 2013). In addition, the delivery of organelles and cytoskeletal elements to the tip of the axon and MT assembly in the growth cone have been proposed as mechanisms to drive growth cone advance and axonal outgrowth. In addition to MT assembly, translocation of the whole MT bundle in the axon may contribute to axon elongation (Suter and Miller, 2011). Recent studies found that translocation presumably occurs because MTs are sliding apart either through pulling or pushing forces generated by molecular motors (Lu et al., 2013; Roossien et al., 2014). In addition to normal elongation, axon regrowth after injury also critically depends on the MT

cytoskeleton (Baas and Ahmad, 2013; Bradke et al., 2012; Chisholm, 2013; Hur et al., 2012). For example, drug-induced MT stabilization after injury reduces scar formation and promotes axon outgrowth (Hellal et al., 2011; Ruschel et al., 2015)

Dendritic Spine Morphodynamics and Synapse Functioning. The MT cytoskeleton also contributes to synapse formation in several systems. At the *Drosophila* neuromuscular junction (NMJ), the conversion of a motile growth cone into a presynaptic terminal is associated with the formation of MT loops in the growth cone (Roos et al., 2000). In addition, dynamic MTs contribute to the structural changes of dendritic spines, the postsynaptic membrane protrusions that encompass most excitatory synapses in the mammalian brain (Hu et al., 2008; Jaworski et al., 2009). MT entries into spines are regulated by both neuronal activity and brain-derived neurotrophic factor (BDNF). BDNF treatment of cultured hippocampal neurons prolongs MT invasions into dendritic spines (Hu et al., 2011), whereas a protocol for inducing NMDA-dependent long-term depression (LTD) resulted in a rapid loss of MT dynamics in dendrites and spines (Kapitein et al., 2011). Current evidence suggests that MT entry is associated with transient changes in spine shape, such as the formation of spine head protrusion and spine enlargement (Hoogenraad and Bradke, 2009). Although not demonstrated directly, it is likely that microtubule-dependent motors use dynamic MT entries to drive postsynaptic cargos into spines.

Architecture of the Neuronal Microtubule Cytoskeleton

Neurons have a very dense MT network (Figures 1A and 1B). Axonal cross sections typically contain ~10–100 MTs, which are often organized in bundles. However, the neuronal MT cytoskeleton is also very heterogeneous. MTs in different parts of the cell may differ in orientations, stability, modifications, and associated proteins. Remarkably, the precise organization of the neuronal MT network has remained largely unresolved. The detection of specific MT modifications and MAPs using electron microscopy remains challenging, and also the complete three-dimensional reconstruction of MT organization from thin sections is time-consuming and error prone. Conventional fluorescence microscopy has allowed the selective labeling of different microtubule-related targets, but cannot resolve individual MTs within the tightly bundled MT arrays found in neurons. Nevertheless, fluorescence microscopy techniques that offer resolutions beyond the diffraction limit (Huang et al., 2009) are starting to reveal novel insights (Figure 1B). In this section, we will summarize our current knowledge on the main properties of the neuronal MT network. In the following section, we will then review the molecular mechanisms that establish this organization.

Centrosomal versus Non-centrosomal Organization

In many cell types, most MTs are nucleated at a microtubule-organizing center (MTOC), such as the centrosome (Bettencourt-Dias and Glover, 2007). However, MTs can also be generated at other positions, such as the Golgi apparatus (Efimov et al., 2007), or along existing MTs (Sánchez-Huertas and Lüders, 2015), giving rise to a MT network where not all minus ends are associated with a central organization center. When newly polarized neurons start to differentiate, the centrosome first acts as an active MTOC, but over time this activity attenuates and the centrosome-dependent MT organization is almost completely lost (Stiess et al., 2010) (Figures 2A and 2B). The centrioles remain in the cell body and most likely serve as a basal body for subsequent cilia formation (Berbari et al., 2007). Serial section electron microscopy studies have previously shown that MTs are not anchored to the centrosome and often free at both ends in differentiated neurons (Yu and Baas, 1994). More recent analysis of MT organization using super-resolution fluorescence microscopy confirmed the loss of radial MT organization around the centrosome during early neuronal development and the presence of non-centrosomal MTs (Yau et al., 2014).

Microtubule Orientations in Axons and Dendrites

Because most MTs do not emerge from a central MTOC, their relative orientations can vary. Early electron microscopy on cross sections of cultured hippocampal neurons used the hook-decoration technique to observe MT orientations and found that MTs in axon and dendrites have different patterns of MT orientations (Baas and Lin, 2011). Whereas uniformly plus-end out-oriented MTs were observed in axons of various types of cultured vertebrate neurons, proximal dendrites contained non-uniformly oriented MTs that were found to be roughly half plus end out and half minus end out (Baas et al., 1988; Burton, 1988). These different MT orientations in axons and dendrites were confirmed using GFP-tagged EB3 in living hippocampal and Purkinje cell cultures (Stepanova et al., 2003). In *Drosophila* and *C. elegans* neurons, MTs in axons are also arranged with their plus ends distal to the cell body. However, in dendrites most MTs are arranged with their minus ends distal to the cell body, although mixed orientation MTs have also been observed (Maniar et al., 2012; Stone et al., 2008). It is thought that differences in the MT cytoskeleton contribute to polarized trafficking to axons and dendrites, for example, by allowing dynein to act as a anterograde motor in dendrites (Kapitein and Hoogenraad, 2011; Rolls, 2011). Although these results suggest that the presence of minus-end out-oriented MTs could be a fundamental property of dendrites, the number of examined neuronal cell types and model organisms is still too limited to warrant this conclusion. Nevertheless, evidence for mixed MT arrays in the living mouse brain have been presented both by second-harmonic generation microscopy and by live-cell imaging of MT growth (Kleele et al., 2014; Kwan et al., 2008).

Microtubule Stability and Length

Visualizing MT plus ends demonstrated that MTs remain dynamic (i.e., alternating between growing and shrinking states) in both dendrites and axons even after the neurons become fully mature (Kleele et al., 2014; Maniar et al., 2012; Stone et al., 2008). However, neurons also have many stable MTs that are resistant to depolymerizing drugs (Baas and Black, 1990). Importantly, these categories are not completely mutually exclusive, because stable MTs might still be able to grow and shrink at their plus end, while the rest of lattice is protected against depolymerization. Indeed, is has been shown that many axonal MTs have stretches with more PTMs near their minus end (Baas and Black, 1990), suggesting that these stretches are longer lived than the remainder of the MT. Nevertheless, the number of estimated MTs per neuron is typically many times higher than the number of growing plus ends, suggesting that many plus ends of stable MTs could be non-dynamic. This idea is

consistent with the relatively slow MT turnover in both axons and dendrites. Differences in MT stability will also result in differences in MT length, but little is known about the precise distribution of MT length in axons and dendrites. Early electron microscopy work has reported an average length of 100 μm in axons of sensory neurons (Bray and Bunge, 1981), whereas later serial section reconstructions reported an average length of about 4 μm in developing neurons (Yu and Baas, 1994).

Microtubule Diversity—Tubulin Isotypes

Despite the high level of structural conservation, several genetic, biochemical, and biophysical properties can generate MT diversity. In neuronal cells, MT diversity is generated by the expression of different α- and β-tubulin genes, referred to as tubulin isotypes, the generation of PTMs, and the regulation of protofilament number (Brouhard and Rice, 2014; Janke and Kneussel, 2010). Tubulin isotypes have subtle differences in their amino acid sequences in the carboxy-terminal tails, which stick out from the MT lattice. The combination of the different tubulin isotypes provides a potential for encoding patterns on the MT surface and generating functional MT heterogeneity. It has been proposed that MTs with a specific isotype composition are present in specialized cells and can carry out unique functions. For instance, the β-tubulin isotype-III (TUBB3) is exclusively expressed in the nervous system, but it has remained unclear whether specific isotypes preferentially polymerize together. Evidence from earlier experiments in non-neuronal cells suggests this is not the case (Lopata and Cleveland, 1987). Recently a large number of mutations in various α/β-tubulin isotypes have been linked to a wide range of human neurodevelopmental and neurodegenerative disorders (Tischfield et al., 2011). For example, exome-wide rare variant analysis recently identified α-tubulin isotype-IVA (TUBA4A) mutations associated with familial ALS (Smith et al., 2014). Mutations in several other tubulin family members have been described to cause various neurological diseases (Franker and Hoogenraad, 2013). These disease-causing tubulin alterations disturb many different neuronal MT functions, such as the stability of α/β-tubulin heterodimers, their incorporation into MTs, and MT dynamics and function, as well as their motor protein and/or MAP interactions (Cederquist et al., 2012; Niwa et al., 2013). Moreover, mutations in the gene encoding the tubulin-specific chaperone E (TBCE) involved in formation of α/β-tubulin heterodimers cause a human developmental disorder called hypoparathyroidism, mental retardation, and facial dysmorphism syndrome (HRD). In mice, mutations in TBCE lead to a reduced number of MTs in distal axons and cause progressive motor neuronopathy (Franker and Hoogenraad, 2013). Future studies of specific tubulin-related mutations may provide novel fundamental insights into how subtle alterations of the MT cytoskeleton can lead to functional aberrations in neurons.

Microtubule Diversity—Post-translational Modifications

The carboxy-terminal tails of tubulin acquire further variations by undergoing PTMs after their incorporation into the MT polymer (Hammond et al., 2008; Janke and Kneussel, 2010). Longer lived MTs are expected to collect more modifications, and conversely, such modifications might contribute to the stability of these MTs, for example, by preventing the activity of MT depolymerases or severing enzymes or by recruiting stabilizing proteins. Well-known PTMs involve removal of the C-terminal tyrosine of α-tubulin, further cleavage to Δ2-tubulin, acetylation, polyglutamylation, phosphorylation, and polyglycylation. Importantly, several enzymes involved in removal or addition of specific groups have recently been identified (Janke and Kneussel, 2010), although the mechanisms that control the heterogeneous modification of subsets of MTs have largely remained elusive. Interestingly, many modifications are enriched in specific parts of the neurons (Hammond et al., 2010), although their exact functions in these regions are still unclear. Certain modifications, such as polyglutamylation, have been hypothesized to recruit specific MAPs or selectively guide motor proteins, but most studies have so far only reported relatively mild differences between the motile properties of kinesins on reconstituted MTs with specific modification mimics (Sirajuddin et al., 2014). It is also important to note that many PTM enzymes, such as polyglutamylases, acetyltransferases, and deactylases, do not only act on microtubules but have many different cellular substrates. For instance, histone deacetylases (HDACs) have been shown to control neurite outgrowth and axonal regeneration via transcription-dependent activities (Cho and Cavalli, 2014).

Microtubule Diversity—Protofilament Number

Neuronal MTs appear structurally similar to those found in all other cell types. MTs with 13 protofilaments are found most frequently, but also different numbers of protofilaments have been found in various neuronal cell types. Axonal MTs of crayfish and lobster nerve cords have 12 protofilaments, whereas in *C. elegans* most MTs have 11 protofilaments, with the notable exception of the touch receptor neuron, where MTs have 15 protofilaments (Chalfie and Thomson, 1982). Interestingly, protofilaments are expected to exhibit a helical supertwist in MTs with less or more than 13 protofilaments (Ray et al., 1993). Given that kinesin-1 typically follows the protofilament axis, this could result in spiraling motility over the microtubule surface (Ray et al., 1993). The microtubule-nucleating gamma-tubulin ring complex assembles into rings with 13-fold rotational symmetry to form a template for MTs with 13 protofilaments (Kollman et al., 2011). Recent studies found that MT PTMs are also involved in controlling protofilament numbers. In *C. elegans* α-tubulin acetyltransferase (α-TAT) MEC-17 mutants, polymorphic MTs consisting of 10–16 protofilaments are seen in touch receptor neurons (Cueva et al., 2012; Topalidou et al., 2012). In addition, Doublecortin, a MAP expressed during the early stages of neuron development and mutated in cases of human cortical malformations, is unique in its ability to recognize and stabilize 13-protofilament MTs (Brouhard and Rice, 2014). These observations suggest that both MT modifications and MAPs can determine protofilament number. In contrast, the precise neuronal function of the variation in protofilament numbers remains unclear, with models ranging from MT stabilization and MT bundling to regulating specific transport routes (Cueva et al., 2012; Liu et al., 2012; Topalidou et al., 2012).

Microtubule Organization at the Axon Initial Segment

The AIS located at the proximal axon segment has been shown to function as a diffusion barrier for both cytoplasmic and membrane proteins and as a gate keeper for axon specific cargo transport (Leterrier and Dargent, 2014; Rasband, 2010). The MTs in the proximal axon are thought to play an important role

in these processes and have several unique features. It has been well-documented that the proximal axon contains fascicles of MTs, which are groups of closely spaced MTs linked by thin cross bridges. The MT fascicles are seen on transverse electron microscopy sections and used as morphological marker to identify the AIS. The MT minus-end binding protein CAMSAP2, which stabilizes non-centrosomal microtubules, is enriched in the very first part of the axon, but absent from the AIS (Yau et al., 2014). While EB proteins are usually associated with growing MT plus ends, they have been found to bind all along the MT lattice in the proximal axon and to interact with the main AIS scaffold protein ankyrinG (Leterrier et al., 2011; Nakata and Hirokawa, 2003). In addition, accumulations of small stretches of non-hydrolyzed GTP-tubulin have been described in the proximal axon (Nakata et al., 2011), suggesting that these microtubule structures play a role in the enrichment of EBs at the AIS. Although the precise relationship between the AIS architecture and axonal MTs remains unclear, the link might be important for filtering of intracellular transport or controlling retrograde diffusion of Tau from the axon to the soma (Li et al., 2011).

Mechanisms that Shape the Neuronal Microtubule Cytoskeleton

As discussed above, distinct groups of microtubule-related proteins work together to control MT organization and dynamics in neurons. The combined actions of structural MAPs, +TIPs, kinesin and dynein motors, and various other factors provide the mechanisms for the spatiotemporal control of the MT architecture and remodeling. Here we will review the current knowledge on the mechanisms that control formation of new MTs, the regulation of MT stability, and the establishment of arrays of distinct orientations.

Formation of New Microtubules

Centrosomal Nucleation. γ-Tubulin assembles into multi-subunit γTuRCs that provide a structural template to nucleate the polymerization of new MTs from α- and β-tubulin subunits, but is not itself incorporated into that polymer (Kollman et al., 2011). Although γ-tubulin is enriched at centrosomes, experiments in non-neuronal cells have shown that γ-tubulin-mediated nucleation can occur both centrosomally and non-centrosomally (Bettencourt-Dias and Glover, 2007). In contrast, it has long been speculated that in neurons, non-centrosomal MTs are generated by cutting them loose from the MTOC. Although some experimental evidence is described, this process has never been directly observed in neurons (Baas and Lin, 2011). Instead, recent work has shown that the centrosome is not required for MT organization and neuronal development. Functional studies in *Drosophila* and mice have shown that neurons lacking an active centrosome display a normal MT network and have proper axon outgrowth and neuronal morphology (Basto et al., 2006; Nguyen et al., 2011; Stiess et al., 2010). These studies suggest that the centrosome is not required as a source for new MTs in neurons.

Non-centrosomal Nucleation. Because the centrosome is dismantled during neuronal differentiation, γ-tubulin and other centrosomal proteins may redistribute from the centrosome to the cytoplasm where they form new sites of MT nucleation (Kuijpers and Hoogenraad, 2011). In fact, γ-tubulin and other pericentriolar material were found in both dendrites and axons in *Drosophila* and rodent neurons and are important at the different stages of neuronal differentiation (Nguyen et al., 2014; Ori-McKenney et al., 2012; Yau et al., 2014). Further evidence for non-centrosomal nucleation in neurons comes from live-cell imaging of +TIPs, where multiple comets emanated from specific sites, indicating these MTs may be nucleated from a common structure located within axons and dendrites (Nguyen et al., 2014; Ori-McKenney et al., 2012; Yau et al., 2014). A number of potential nucleation sites have been identified in non-neuronal cell types, including the nuclear envelope of myotubes, the plasma membrane of polarized epithelia, the Golgi apparatus, and melanosomes in pigment cells (Kuijpers and Hoogenraad, 2011).

Golgi-derived MTs have been proposed to be functionally distinct MTs that establish a polarized MT network and organize directional trafficking toward the front of the motile cell. Dispersed and fragmented Golgi membranes can still form new MTs, indicating that individual Golgi stacks contain the necessary machinery for MT nucleation (Efimov et al., 2007). Since Golgi positioning and microtubule-mediated membrane delivery are both important events during neuronal polarization (Bradke and Dotti, 1997; Gärtner et al., 2012), a similar Golgi-dependent MT polarization mechanism may occur during the early stages of axon formation. In young neurons, visualizing Golgi-associated MT nucleation is a challenge because the Golgi apparatus is located in the vicinity of the centrosome, and the MT density in the cell body precludes identification of single MT nucleation sites. One approach is to depolymerize MTs with nocodazole and visualize the MT nucleation sites after washout of the drug (Stiess et al., 2010). This procedure revealed non-centrosomal nucleation in the soma of young neurons, but whether all new MTs originated from the Golgi remains unclear.

In more differentiated neurons, the neuronal Golgi apparatus is composed of Golgi stacks in the cell body and discrete Golgi outposts in a subpopulation of dendrites (Horton et al., 2005; Quassollo et al., 2015; Ye et al., 2007). Golgi outposts have been reported to locally nucleate MTs and shape dendrite morphology in *Drosophila* dendrites of Dopamine neurons (Ori-McKenney et al., 2012). Similar to fibroblast cells (Rivero et al., 2009), Golgi-dependent nucleation in these neurons requires γ-tubulin and the centrosomal protein AKAP450. Interestingly, MT plus ends grew from the Golgi outposts in particular directions, suggesting a possible role for local nucleation in establishing specific MT orientations. A more recent study confirms the role for local MT nucleation in neurons, but does not support the idea that non-centrosomal nucleation occurs at the Golgi complex (Nguyen et al., 2014). When Golgi outposts were dragged out of dendrites using an activated kinesin, γ-tubulin remained in dendrites and the MT organization was only mildly affected. Although other organelles, such as recycling endosomes and mitochondria did not correlate with MT nucleation points (Ori-McKenney et al., 2012), it is possible that γ-tubulin in neurons is associated with the plasma membrane or some other intracellular membrane such as the endoplasmic reticulum.

Moreover, non-centrosomal MT nucleation does not need to be membrane dependent. Analogous to F-actin cytoskeleton,

new MTs can be generated at the lattice of existing MTs by branch nucleation (Sánchez-Huertas and Lüders, 2015). An important factor in this process is the microtubule-associated hetero-octameric protein complex named augmin or HAUS (Lawo et al., 2009). Augmin binds to the MT lattice, recruits γ-tubulin, and thereby promotes the centrosome-independent MT nucleation during cell division. This process is mostly studied during cell division and an interphase role for this branching-type nucleation has not yet been identified in animal cells.

Cutting Pre-existing Microtubules. Another mechanism to create more MTs is by cutting of pre-existing MTs using severing enzymes (Roll-Mecak and McNally, 2010; Sharp and Ross, 2012). Three classes of microtubule-severing enzymes have been identified, named katanin, spastin, and fidgetin, which are all part of the AAA large superfamily of P loop ATPases involved in protein unfolding and disaggregating activities (Frickey and Lupas, 2004). All severing enzymes are highly expressed in the nervous systems and particularly katanin and spastin are described to have an impact on neuronal morphology and axon regeneration. MT severing by katanin and spastin is particularly important for the formation of axonal branches and dendritic development (Yu et al., 2008). Several studies have also uncovered an important role for MT severing in the outgrowth of motor neurons, the formation of NMJs, and axon regeneration (Mao et al., 2014; Stone et al., 2012). Consistent with the axonal defects in various model systems, spastin mutations have been directly linked to axonal pathologies in the human neurodegenerative disease called hereditary spastic paraplegia (HSP) (Fink, 2013). Although most disease mutations in spastin either inactivate or downregulate severing activity (Evans et al., 2005), some recent studies report gain-of-function effects on MT dynamics (Solowska et al., 2014). Because MT severing by spastin could either reduce MT mass through destruction of pre-existing MT or increase MT mass through creating of novel seeds that can then elongate, a direct comparison between spastin activity and the number of neuronal MTs is ambiguous. For example, both spastin overexpression and loss-of-function mutations in *Drosophila* motor both show fewer MT bundles within the NMJ (Sherwood et al., 2004).

Katanin exists as a heterodimer of a catalytic subunit (p60) and targeting/regulatory subunit (p80) and is widely distributed throughout the neuron and severs MTs in both axons and dendrites. *Drosophila* and mammalian neurons also contain an additional katanin-p60-like protein (Kat-60L1) (Roll-Mecak and McNally, 2010). Recent data showed that *Drosophila* Kat-60L1 promotes MT growth and dendritic stability during early neuronal development, while at later stages it has an opposite function and controls net MT disassembly during dendritic pruning (Lee et al., 2009; Stewart et al., 2012). This suggests that MT susceptibility may change the activity of katanin-mediated MT severing at different developmental stages. There are several lines of evidence that show how specific MAPs and PTMs can control MT severing (Sharp and Ross, 2012). It is, for example, reported that tau binding to MTs protects them against katanin-mediated severing (Qiang et al., 2006). An interesting hypothesis is that the MT defects observed in tauopathies, such as Alzheimer's disease, may result from elevated severing of axonal MTs as they lose their tau binding (Sudo and Baas, 2011). Spastin activity is not strongly affected by tau, but is enhanced on polyglutamylated MTs (Lacroix et al., 2010). A more recent study expands these finding and connects the pathological missorting of tau in Alzheimer's disease with spastin-mediated MT severing (Zempel and Mandelkow, 2014). In this model, missorted tau promotes the translocation of polyglutamylase TTLL6 into dendrites, where it induces polyglutamylation of MTs and triggers the subsequent recruitment of spastin and severing of MTs (Zempel et al., 2013). Interesting in this respect is that kinesin motility is somewhat sensitive for MT polyglutamylation and alterations in PTMs may influence cargo transport in neurons (Ikegami et al., 2007; Sirajuddin et al., 2014). However, building a coherent model of how MT severing and PTMs influence MT remodeling and intracellular trafficking will require additional work.

Microtubule Stabilization

Minus-End Stabilization. MT severing will produce two new MTs, which can both function as seeds for new outgrowth. Nevertheless, newly created minus ends are typically unstable. Recently, a new family of MT minus-end binding proteins, named CAMSAP/Patronin/Nezha has been characterized and found to specifically recognize MT minus ends and stabilize MTs against depolymerization (Akhmanova and Hoogenraad, 2015) (Figures 1D and3A). In *Drosophila*, the CAMSAP family member, named Patronin, associates with free MT minus ends and inhibits their disassembly by the action of the kinesin-13 MT depolymerase (Goodwin and Vale, 2010). In mammals, all three CAMSAP family members recognize growing MT minus ends and CAMSAP2 and CAMSAP3 form stretches that are stably deposited on the MT lattice generated by minus-end polymerization (Hendershott and Vale, 2014; Jiang et al., 2014). These stable CAMSAP stretches may stabilize MTs to serve as seeds for MT re-growth (Figure 3A). Indeed, repetitive MT plus-end growth and shrinkage was also observed from CAMSAP2 stretches in neurons (Yau et al., 2014). Consistent with the idea that stable non-centrosomal MTs have a prominent role in neuronal development, CAMSAP2 is required for neuronal polarity, axon specification, and dendritic branch formation in vitro and in vivo (Yau et al., 2014). In worms, loss of the CAMSAP homolog PTRN-1 caused defects in neurite morphology and synaptic vesicle localization (Marcette et al., 2014; Richardson et al., 2014) and also affected the axonal regeneration after injury (Chuang et al., 2014). Interestingly, MT severing contributes to disassembly of CAMSAP-decorated MT stretches and acts antagonistically to CAMSAP-mediated MT stabilization (Jiang et al., 2014).

Lattice Stabilization. It is well known that neurons contain multiple MT subclasses that differ in stability. A class of short and stable MTs might be constructed from specially modified forms of tubulin (Janke and Kneussel, 2010) or by decoration with specific MT stabilizing proteins, such as microtubule-associated protein 6 (MAP6) or stable tubule only polypeptide (STOP) (Guillaud et al., 1998). Detyrosination and acetylation of α-tubulin correlate with MT stability in many systems, but in vitro these modifications do not confer a measurable change in MT stability. Recently, a new post-translational modification of tubulin was identified that directly confers stability to MTs (Song et al., 2013). Biochemical characterization of stable MT fractions demonstrated that polyamination of tubulin is a major modification directly involved in stabilization of neuronal MTs. The

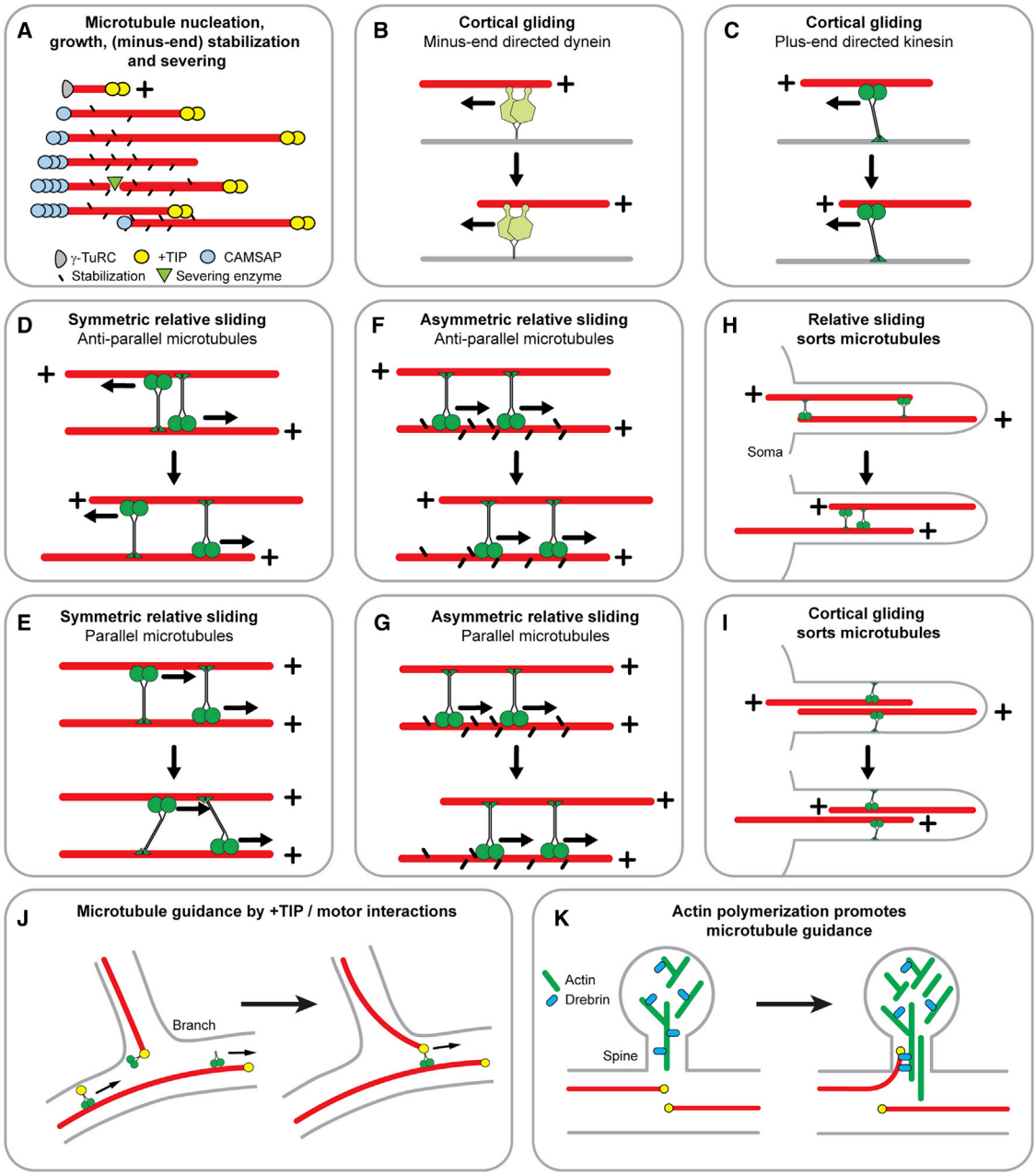

Figure 3. Processes Involved in Microtubule Stabilization and Orientation

(A) Dynamics of microtubules (red line) after nucleation by γ-TuRC: minus-end stabilization by CAMSAP, fast plus-end growth regulated by +TIPs, and slow lattice stabilization by MAPs and PTMs. In addition, severing enzymes can cut microtubules, resulting in a dynamic plus end and a novel minus end that will also be stabilized by CAMSAP.

(B and C) Expected microtubule movements driven by cortically attached minus- (B) or plus-end (C) directed motors.

(D and E) Expected microtubule movements for anti-parallel (D) and parallel (E) aligned microtubules when symmetrically crosslinked by motors with additional microtubule binding domains.

(F and G) Expected microtubule movements when the motor domains of a sliding motor preferentially bind to a subset of microtubules decorated by specific modifications or MAPs.

(H and I) Promoting a minus-end out microtubule organization by relative sliding (H) or cortical gliding (I) driven by a plus-end directed kinesin.

(J) Microtubule guidance by +TIP associated motor proteins at dendritic branch points.

(K) Microtubule guidance by a local actin network in dendritic spines.

covalent attachment of polyamines, such as putrescine, spermidine, and spermine, to tubulin is catalyzed by transglutaminase and adds an unusual positive charge to tubulin. The most commonly studied PTMs are either acidic (phosphorylation and glutamylation) or charge neutral (acetylation and detyrosination) and do not directly confer stability to MTs but, rather,

accumulate on long-lived MTs (Janke and Kneussel, 2010). MTs containing polyaminated tubulins are resistant to depolymerization, and inhibiting polyamine synthesis or transglutaminase activity significantly decreases MT stability in neurons. Polyamination may not only provide stability of short MT fragments but also allow them to act as small ''transportable'' MT organizing complexes in neurons. Nevertheless, the transport of MT fragments and the relevance for neuronal functions has been controversial for many years.

The CLASP family of MT plus-end binding protein has been described to locally stabilize MTs at various subcellular locations. CLASPs are known to promote MT growth by binding plus ends and promoting rescues (Akhmanova and Steinmetz, 2008). For instance, CLASPs are present at the Golgi apparatus and found to be critical players in controlling local MT nucleation (Efimov et al., 2007). It has been proposed that CLASPs at the Golgi membranes are able to coat newly polymerized MT regions, prevent their disassembly, and allow them to serve as seeds for polymerization. Since γ-tubulin is required for de novo MT nucleation, it is likely that CLASPs are not directly involved in the formation of new MTs, but in stabilizing pre-existing MT seeds. Consistent with this model, attachment of CLASP to mitochondrial membranes exhibited no potential for MT nucleation (Efimov et al., 2007). CLASP2 is enriched in neuronal tissues and found to be involved in axon outgrowth and neuronal polarity by acting as local MT stabilizer (Beffert et al., 2012; Lee et al., 2004).

Spatial Arrangement of Microtubules

Orienting Microtubules. How do neurons establish and maintain the specific orientations of MTs in axons and dendrites? In the case of parallel axonal arrays, their initial parallel orientations might directly follow from the central centrosomal nucleation, resulting in MTs growing into neurites with their plus end out (Figure 2). When centrosomal activity decreases during later stages of development, other mechanisms might be required. The high levels of CAMSAP2 found at the base of the axon may create a local pool of stabilized MT minus ends and promote plus-end out-oriented MT growth in axons. Importantly, the extent of de novo axonal MT nucleation, which would require mechanisms to properly orient the newly formed MTs, is not known. If instead most MTs would be formed by severing existing MTs, their orientations would be correct from the start. In addition, shorter MTs, as well as newly nucleated MTs, could be properly oriented by bundling them to existing MTs in a parallel orientation. Some tau isotypes could act as parallel bundlers, and also the minus-end motor protein kinesin-14, which has MT binding domains in addition to their motor domain, might preferentially crosslink parallel MTs (Braun et al., 2009).

The selective removal of oppositely oriented MTs would also contribute to parallel bundle formation. Dynein anchored to either the cortex or to other MTs will induce sliding of MTs fragments with their plus end leading (Figure 3B). Minus-end out-oriented MTs will therefore be pushed back to the cell body and removed from the axon. Indeed, knockdown of dynein in *Drosophila* neurons increases the number of plus ends growing toward the cell body (Zheng et al., 2008). Moreover, rapid motility (1 μm/s) of MTs has been directly observed after photobleaching long axonal stretches in rat neurons expressing or injected with fluorescent tubulin (Wang and Brown, 2002). However, in these experiments the orientation of the moving fragments has never been directly determined. In addition, if such MT movements occur frequently, this should lead to rapid retrograde motility of plus-end associated comets, but such events are rarely observed, suggesting that only a specific subset of non-dynamic MTs undergo rapid movements (Ma et al., 2004).

In dendrites, local nucleation could explain the existence of minus-end out MTs (Nguyen et al., 2014; Ori-McKenney et al., 2012). Alternatively, MTs nucleated elsewhere could be brought in with their minus end pointing outward. This could be achieved by sliding motors that induce movement between oppositely oriented MT pairs and thereby can bring in minus-end out MTs over the pre-existing plus-end out-oriented MTs (Figures 3C–3I). Such sliding motors, such as Kinesin-5 and possibly Kinesin-6, are known to operate in dividing cells to form the microtubule-based mitotic spindle (kinesin-5) or anaphase midzone (kinesin-6), often in cooperation with passive non-motor bundlers that promote antiparallel bundling, such as PRC1 (Subramanian and Kapoor, 2012). Consistently, several papers have reported a role for the Kinesin-6 MKLP1 in the formation of the anti-parallel dendritic MT array (Lin et al., 2012). In principle, because force is generated on both MTs, this mechanism could also lead to retrograde motility of plus-end out-oriented MTs and thereby deplete them from the dendrites (Figures 3H and 3I). However, this would only work if these MTs are not connected to other structures and if the sliding speed is faster than the MT growth rate, which appears not to be the case for Kinesin-6 and Kinesin-5.

Recent work has shown that the nearly uniform dendritic MT orientation of *C. elegans* DA9 and PHC neurons is almost completely reversed upon knockdown of the Kinesin-1 homolog *unc-116* (Yan et al., 2013). A model was proposed in which the additional MT binding site in the tail region of the Kinesin-1 facilitates the crosslinking and sliding of anti-parallel MTs (Figure 3D). Alternatively, Kinesin-1 could be attached to the cortex or other immobile structures to drive MT sliding with the minus end leading (Figures 3C and 3D). In both cases, the sliding speed will be faster than the MT polymerization rate, and therefore these mechanisms could really deplete the dendrite from plus-end outward MTs, because their growth cannot catch up (Figures 3H and 3I). Again, such retrograde transport of polymerizing MTs should result in rapid retrograde motility of plus-end associated comets, which has so far not been reported.

In dendrites with uniform minus-end out polarity, branch points also pose a challenge, because MTs should grow exclusively into the primary branch that leads to cell body. Work in *Drosophila* proposed that growing MTs approaching a branch are guided toward the plus ends of pre-existing MTs by a Kinesin-2 that interacts with the growing plus end through EB1 and APC (Figure 3J) (Mattie et al., 2010). Subsequent in vitro reconstitution experiments have demonstrated that kinesins interacting with dynamic plus ends through EB proteins can indeed establish MT guidance (Chen et al., 2014; Doodhi et al., 2014).

Motor-based MT guidance and sliding, possibly combined with passive bundling in preferred orientations, thus appear versatile strategies to bias the overall polarity orientations of MT arrays (Subramanian and Kapoor, 2012). Interestingly, Kinesin-1 based relative sliding of MTs has also been proposed to provide

the driving force for initial neurite outgrowth in cultured neuron-like cells from *Drosophila* (Lu et al., 2013). This model implicates that the protrusive MTs are being pushed outward with their minus end leading, unless the binding of the Kinesin-1 motor domains is somehow biased toward the guidance MT (Figures 3D–3G). Recent work reported that, in more mature neurons, such rapid Kinesin-1 based MT sliding is reduced by the slow bipolar sliding motor MKLP1 (Kinesin-6) (del Castillo et al., 2015).

Bundling, Spacing, and Spine Entries. Most neuronal MTs appear to be heavily bundled. Tau and MAP2 are two abundant neuronal MAPs that can induce MT bundling (Dehmelt and Halpain, 2005). MAP2 is exclusively located to dendrites, where it decorates stable MTs, whereas tau is present in both axons and dendrites, but is enriched in the distal axon. The mechanisms underlying these polarized distributions are largely unknown. Interestingly, expressing tau and MAP2 in non-neuronal cells induces the formation of MT bundles with a distinct spacing between MTs of 20 nm and 65 nm, respectively (Chen et al., 1992). This spacing nicely corresponds with the spacing found between MTs in dendrites (65 nm) and small caliber axons without neurofilaments (20 nm), suggesting that tau and MAP2 are involved in the proper spatial organization of the neuronal MTs (Chen et al., 1992). Whether tau and MAP2 promote MT bundling in parallel or anti-parallel orientations, respectively, is currently unknown.

Despite the extensive MT bundling, growing MTs frequently leave the dendritic shaft to penetrate into dendritic spines (Jaworski et al., 2009). Mechanically, MTs behave as relatively rigid rods and therefore tend to grow in straight lines (Hawkins et al., 2010), suggesting that specific mechanisms underlie spine entries. Indeed, recent work reported that MT entries depend on the actin cytoskeleton and the actin-binding protein Drebrin A, which was suggested to link MT plus ends to actin (Merriam et al., 2013) (Figure 3K). Local calcium transients were found to promote spine entries by increasing actin polymerization, suggesting that MT spine entries are regulated by synaptic activity.

Conclusions and Outlook

The formation of a complex nervous system requires microtubule-mediated processes that coordinate proliferation, migration, and differentiation of neuronal cells. Therefore, it is not surprising that many neurodevelopmental problems and neurodegenerative disorders are caused by deficiencies in microtubule-related genes. Advances in discovery of microtubule-targeting agents (MTAs) and synthesis of small molecules that modulate microtubule-based processes might offer new therapeutic paradigms to treat neurological defects and intervene in neurodegenerative processes. Probably the most striking example to date is the positive effects of MTAs on axon regeneration (Baas and Ahmad, 2013; Bradke et al., 2012; Chisholm, 2013; Gornstein and Schwarz, 2014). For instance, treatment with MT stabilizing drugs such as taxol and epothilone B has recently been shown to decrease fibrotic scar formation and promote axon regeneration after spinal cord injury (Hellal et al., 2011; Ruschel et al., 2015). Particularly, this dual effect of MT stabilizing agents is important for treating axon regeneration. In addition, MT stabilizing agents, such as epothilone D, were found to reduce axonal dysfunctioning and Alzheimer-like pathology in aged tau transgenic mice (Zhang et al., 2012). Although these MTAs are potentially useful for treating nerve injury and neurodegenerative diseases, they are now primarily used for anticancer therapies and have been shown to cause unwanted side effects in the rest of the body. Importantly, however, MTAs were used 20-fold less concentrated in the axon regeneration experiments compared with cancer treatments. One critical challenge for nervous-system-directed therapies is finding ways to specifically retain MTAs in the nervous system to allow for prolonged drug activity where needed. On the other hand, future therapeutic strategies may focus on interventions at the level of MT associated proteins or related signaling pathways. Further advances in understanding the function of microtubule-related proteins and regulatory mechanism of MTs in both scar-forming fibroblasts and axons may lead to improved targeting and development of therapeutic interventions for nerve injury and neurodegenerative diseases.

During the past decade numerous advances have been made in identifying microtubule-related processes and signaling pathways that control the neuronal cytoskeleton. We have, however, just begun to understand the fundamental properties of the neuronal MT network and basic molecular mechanisms that establish this complex organization in various neuronal model systems. A major challenge for the future is to determine the multiple conserved and variable molecular players that associate with the MT cytoskeleton and directly control MT organization and remodeling. For instance, it will be important to further decipher how the axonal and dendritic MT arrays assemble and actually function and how microtubule-related proteins participate in establishing neuronal polarity and control synaptic plasticity. Moreover, the mechanisms that generate non-centrosomal MTs in both axons and dendrites are largely unknown. In addition, it will be critical to determine which microtubule-based cues drive axon-selective trafficking. Finally, it will be important to study neuronal MT organization and remodeling under conditions that more closely resemble the in vivo situation. Organotypic brain slice cultures allow live cell imaging in combination with electrophysiological recordings, local drug applications, and/or photostimulation. Recent developments in optogenetic techniques allow light-activated control of protein-protein interactions inside neurons (van Bergeijk et al., 2015) and are a useful tool to explore local MT functions in different model systems.

ACKNOWLEDGMENTS

We would like to thank Dr. Anna Akhmanova, Dr. Martin Harterink, and Dr. Anaël Chazeau for very helpful comments on the manuscript and Roderick Tas and Dr. Philip Schätzle for providing images. This work was supported by the Netherlands Organization for Scientific Research (NWO-VIDI to L.C.K. and NWO-VICI to C.C.H.), the Netherlands Organization for Health Research and Development (ZonMW-TOP to C.C.H.), and the European Research Council (ERC Starting Grant to L.C.K. and ERC Consolidator Grant to C.C.H.).

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