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Neuronal polarity: remodeling microtubule organization

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Cell polarization generates morphological and functional asymmetry, and is crucial for the development and proper functioning of many cell types. Recent data have revealed that the microtubule cytoskeleton is a major determinant in the establishment and maintenance of neuronal polarity. Microtubules provide the structural basis for neuronal polarization, because of their intrinsic properties including inherent polarity. Moreover, the polarized microtubule network also forms the basis for selective cargo trafficking into axons and dendrites. Here we review recent studies examining the molecular processes that control microtubule remodeling and polarized cargo sorting, and propose that changes in microtubule organization play an instructive role in the initial polarization.

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Introduction

Neurons are classical examples of highly polarized cells [1]. They typically have one long process to transmit information (axon) and several relatively short processes to receive information (dendrites). Previous studies have identified several signaling factors that are of critical importance for neuronal polarization [2]. Most of these signaling molecules impinge on the intracellular cytoskeleton [3]. Several studies have demonstrated that microtubule cytoskeleton determines initial polarization, as axon formation correlates with microtubule remodeling [4,5]. Axon initiation and outgrowth is characterized by the formation of uniform parallel microtubule bundles with their plus-ends pointing outward towards the growth cone [6]. It was also shown that microtubule stabilization is sufficient to induce axon formation [7]. Subsequently, increased microtubule stability leads to polarized cargo trafficking and further axon elongation [8]. Therefore, both local stabilization and bundling of parallel microtubules are most likely key processes driving neuronal polarization. In this review we discuss how polarized microtubule arrays are generated and how they contribute to selective cargo trafficking into axons and dendrites.

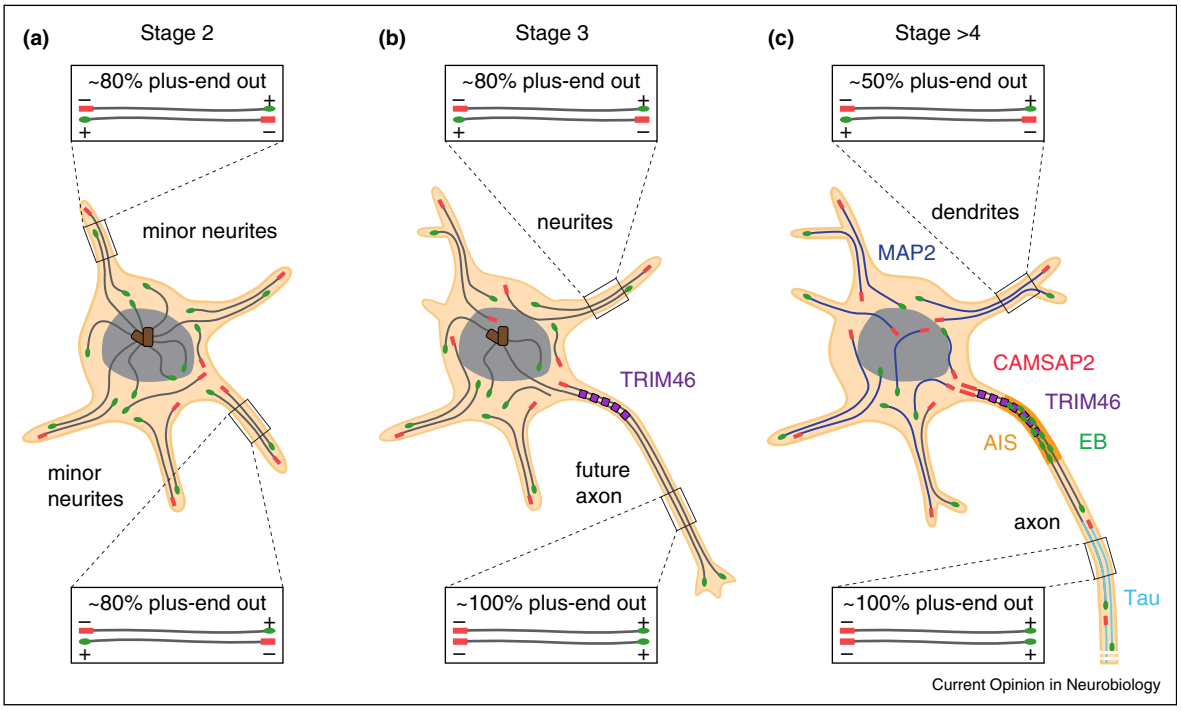
Two distinct microtubule organizations in one neuron

It is well known that in mature neurons, the microtubule organization is different between axons and dendrites [4,9]. The hook-decoration technique was originally used to determine the orientation of neuronal microtubules by electron microscopy [6]. Using this approach, it was found that in axons, microtubules are typically arranged with their plus-ends out, whereas in dendrites, a mixed microtubule polarity (plus-end and minus-end out) is observed (Figure 1a–c). The different microtubule orientations were confirmed using fluorescently labeled microtubule plus-end binding proteins (so-called +TIPs) in different neuronal cell types from distinct *in vitro* and *in vivo* model systems [10,11]. Live-cell imaging in combination with laser-induced severing revealed that microtubule orientations are approximately equally mixed throughout the dendritic processes [11]. These data showed that, in mammalian neurons, microtubules have an antiparallel organization in mature dendrites (Figure 1c). In contrast, uniform minus-end out microtubules are the signature of dendrites in *Drosophila* and *Caenorhabditis elegans* neurons [12–14]. Motor protein-based microtubule guidance and sliding are likely mechanisms to bias the orientations of microtubule arrays in dendrites of invertebrate neurons [15,16]. However, it remains an open question how mixed microtubule orientations in mammalian dendrites are set up and why dendritic microtubules in mammalian and invertebrate neurons have a different organization.

Microtubule remodeling and polarized cargo trafficking

During early stages of neuronal development, microtubules of mixed polarity (20% minus-end out) were detected in the minor neurites of non-polarized stage 2 neurons [11] (Figure 1a). In stage 3 neurons, the orientation of microtubules in axons drastically changes directly after axon outgrowth: the majority of axonal microtubules are stable and oriented with their plus-end out [7,11] (Figure 1b). These data suggest a model in which stabilization and bundling of parallel microtubules in the newly forming axon is a critical process underlying neuronal polarization. Microtubule rearrangement also directly influences the sorting of cargo into either axons or dendrites [8,17]. It has been shown that uniform plus-end out microtubules in axons facilitate the selective sorting of kinesin-driven vesicles, whereas the minus-end out population within the mixed microtubule arrays allows dynein motors to drive cargo specifically into

Figure 1



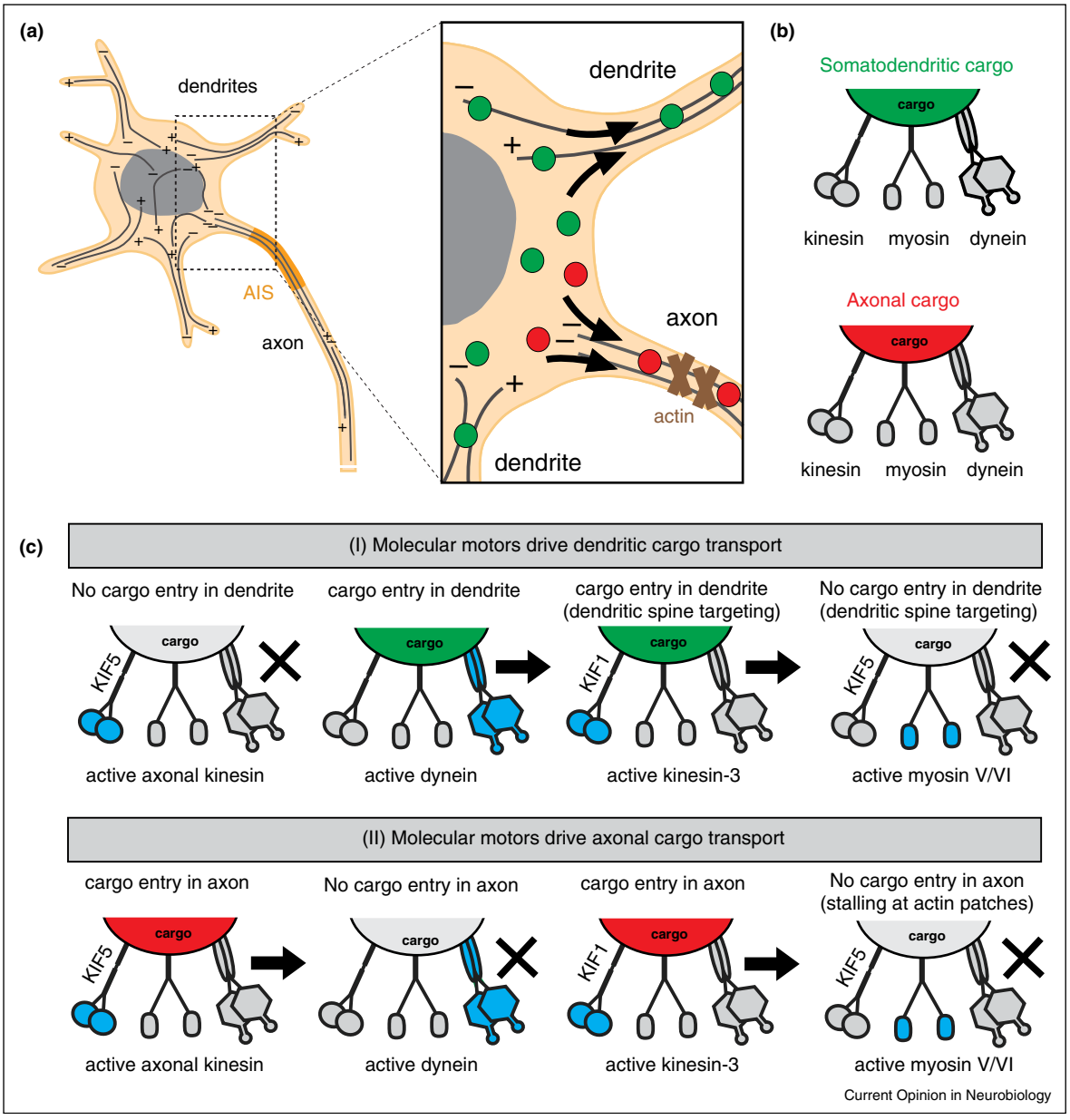
Microtubule reorganization during neuronal polarization and axon formation. The morphological changes of hippocampal neurons in culture have been defined by distinct neurodevelopmental stages and specific events of neuronal polarization. Shortly after plating, hippocampal neurons form lamellipodia around the cell body (stage 1). This is followed by the generation of several minor neurites (stage 2) **(a)**. Formation of the axon at stage 3 is the initial step in breaking the morphological symmetry of neurons, and polarity is established at the transition between stages 2 and 3 **(b)**. TRIM46 localizes to the proximal region of the future axon and forms plus-end out microtubule arrays. During stage 4, the remaining minor neurites develop as dendrites and the axon initial segment (AIS) is assembled at the proximal axon **(c)**. The axonal microtubules are decorated by tau, whereas MAP2 becomes enriched in the somatodendritic compartment. The microtubule 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. While EB proteins are usually associated with growing MT plus ends, they have also been found to bind along the microtubule lattice in the AIS. The microtubule organization is indicated at the various developmental stages as percentages of plus-end out microtubules.

dendrites [18,19] (Figure 2a–c). Loss-of-function experiments in *Drosophila* and *C. elegans* neurons emphasize the importance of polarized microtubule organization and selective kinesin-driven and dynein-driven cargo transport mechanisms in controlling axon and dendrite identity, respectively [8,17]. Systematic analysis of the ~45 kinesin motors demonstrated that the majority of 'cargo translocating' kinesin motors transport vesicles selectively into axons [20]. At the same time, none of the kinesin motors drive cargo selectively to dendrites. Only five members of the kinesin-3 (KIF1A/B/C) and kinesin-4 (KIF21A/B) families can target both the axon and dendrites [21]. Microtubule binding protein doublecortin-like kinase 1 (DCLK1) is required for kinesin-3-dependent cargo trafficking into dendrites [20,22], suggesting that additional regulatory cues and/or local signals are important for specific kinesin motors to drive dendritic cargo transport. Interestingly, within dendrites, recruitment of kinesin-3 (KIF1C) or myosin V motors facilitates dendritic spine targeting [23].

Polarized cargo sorting at the proximal axon

Previous studies have suggested that somatodendritic cargoes are selectively halted at the proximal axon, whereas cargoes transporting axonal vesicles are allowed to proceed through the axon initial segment (AIS) into more distal parts [24]. However, recent data suggested that the AIS is not critically important for selective cargo filtering [25•,26•]. It was suggested that cargo sorting and trafficking in hippocampal neurons does not occur at the AIS, but rather at a pre-axonal region at the base of the AIS [25•]. In addition, selective transport was already detected during early neuronal development, shortly after axon formation but before the AIS is formed [26•]. The vesicle-sorting function of the AIS may depend on the actin cytoskeleton [24]. Distinct organizations of actin filaments have been reported in the AIS and along the axon shaft [27•,28–30]. At the AIS, actin patches may act as a barrier for entry of somatodendritic vesicles by slowing down or halting myosin-V/VI positive vesicles [28] (Figure 2a–c). These data suggest a model in which polarized cargo sorting in neurons

Figure 2



Basic mechanisms of polarized cargo sorting. **(a)** Schematic diagram showing the microtubule organization, axonal (red) and somatodendritic (green) cargos in a polarized neuron. Actin patches at the axon initial segment (AIS) selectively prevent passage of myosin-bound cargos into the distal axon. **(b)** Schematic diagram of three molecular motors attached to axonal (red) and somatodendritic (green) cargoes. Cargo sorting along the polarized microtubule cytoskeleton will largely depend on the dominant motor type (tug-of-war mechanism) or the signaling pathways that control specific motor activity (regulatory mechanism). **(c)** Polarized sorting of cargos depends on the ability of vesicles to recruit and/or activate the appropriate motor (blue). (I) In this model, dynein or non-selective kinesin-3/KIF1 motors drive cargo transport over mixed microtubule arrays into dendrites. (II) All kinesin motors, like kinesin-1/KIF5 and kinesin-3/KIF1, can drive cargo transport over uniform, plus-end out microtubule arrays into axons. Dynein hitchhikes on kinesin-driven cargos and is responsible for retrograde axonal transport. Cargos that have (active) myosin motors attached will stop at actin-rich patches.

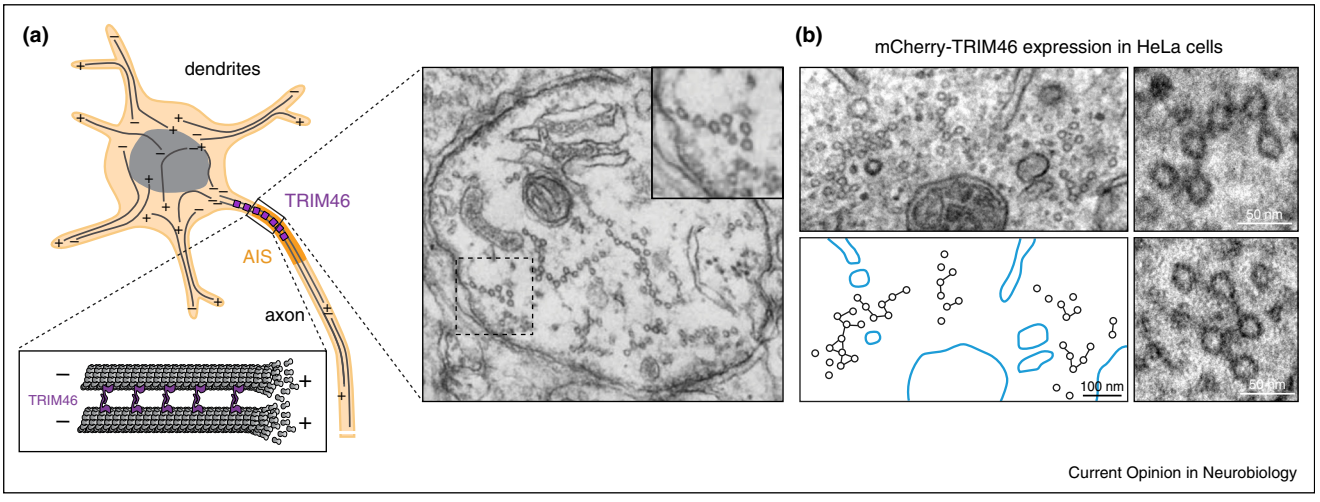
depends on the polarized organization of the microtubule cytoskeleton, and on the ability of the vesicles to acquire an appropriate set of motor proteins. Cargos that bind axonal kinesins can traverse through the proximal axon to the distal axon, while those that also have (active) myosin attached cannot pass through (Figure 2c). Activation of dynein at the AIS will trigger cargo reversal and induce retrograde axonal transport back to the cell body. Cargos with dynein and/or non-selective kinesin motors, such as kinesin-3, can target dendrites (Figure 2c). The AIS may

not be essential in this process but could play a regulatory role in modulating motor activity [31,32]. Future studies should focus on signaling proteins that control cargo reversals at the AIS. Furthermore, Ankyrin G links the AIS cortex directly to microtubules via an interaction with end-binding (EB) proteins [33], suggesting that the AIS may organize a (sub)population of EB-positive microtubules close to the membrane and indirectly control cargo trafficking [34] (Figure 1c).

Parallel microtubule bundles are required for axon formation

Axon initiation and outgrowth is characterized by the formation of parallel microtubule bundles with their plus-ends pointing outward [6]. These data imply that specific microtubule-associated proteins localize to the newly specified axon, where they establish uniform microtubule orientation. Using autoimmune antibodies that label the proximal axon [35,36], a new microtubule-associated protein, named TRIM46, was identified [37••]. TRIM46 belongs to the tripartite motif containing (TRIM) protein family of ubiquitin E3 ligases [38], although ubiquitin ligase activity and putative substrates for TRIM46 have not been reported. Instead, TRIM46's localization partly overlaps with the AIS, where it forms plus-end out microtubule bundles. As such, TRIM46 is required for neuronal polarity and axon specification *in vitro* and *in vivo* [37••]. TRIM46 is also an early marker for neuronal polarization because it localizes to the future axon before axon extension and AIS assembly. Moreover, expression of TRIM46 in heterologous cells induces the formation of bundles of closely spaced microtubules linked by thin cross-bridges (Figure 3a and b), which closely resemble the microtubule fascicles seen on transverse electron microscopy sections at the AIS [39–41]. Recent evidence demonstrated that the microtubule minus-end targeting protein CAMSAP2, which protects the minus-ends from depolymerization [42], is critical for axon specification and that CAMSAP2 is enriched in the very first part of the axon [43•] (Figure 1c). Here, CAMSAP2 may create a local pool of stabilized, non-centrosomal microtubules to promote plus-end out microtubule growth in axons of developing hippocampal neurons. Consistent with the importance of non-centrosomal microtubules in neurons, it has been shown that the centrosome loses its function as a microtubule-organizing center during early stages of neuronal development [44] (Figure 1a–c). Further microtubule lattice stabilization might be controlled by post-translational modifications (PTMs) of tubulin [45]. For instance, it was found that microtubules containing polyaminated tubulin are resistant to depolymerization and that polyaminated tubulin increases microtubule stability in neurons [46]. Alternatively, microtubule-interacting proteins can also stabilize microtubule arrays [9]. For example, mammalian partition defective 3 (mPar3), a key component of the Par polarity complex that controls neuronal polarization, directly regulates microtubule stability and organization [47]. We propose a model in which both local microtubule stabilization and the formation of uniform microtubule bundles in the axonal shaft induces axon formation. These stable microtubule arrays may provide the mechanical forces to drive axon elongation in neurons [48,49]. Axonal outgrowth can be further promoted by directional transport along uniform microtubule arrays, which may act as delivery tracks for critical organelles or polarity factors that contribute to axonal fate. A recent study suggested that

Figure 3



TRIM46 forms parallel microtubule fascicles. **(a)** Schematic diagram showing the microtubule organization and TRIM46 (purple) localization in polarized neurons. Microtubule fascicles with electron-dense cross bridge are present in the proximal axon. **(b)** Representative electron microscopy images of HeLa cells expressing mCherry-TRIM46. Figure (a) (right panel) was previously published and reproduced with permission [41]. Figure (b) was previously published and reproduced with permission [37••].

increased retrograde transport of proteasomes during axonal outgrowth establishes asymmetric proteostasis and leads to local changes in protein concentration [50].

Discussion

Similar to other polarized cell types, neuronal polarization results from the dynamic reorganization of the inherently polar neuronal cytoskeleton along the axis of polarity [9]. It is now becoming clear that several cytoskeletal features underlie axon initiation and elongation, including actin filament destabilization, local microtubule stabilization, the formation of parallel microtubule bundles and the polarized flow of membrane cargoes [5]. However, much less is known about how the actin and microtubule regulators interact and how they are controlled to give rise to a polarized neuron [51]. Moreover, the mechanisms that generate non-centrosomal microtubules are largely unknown. Golgi outposts in *Drosophila* neurons have been reported to locally act as sites of non-centrosomal microtubule nucleation [52,53]. Another mechanism to create non-centrosomal microtubules is the severing of pre-existing microtubules [54]. A recent study highlights the functional overlap between the γ-tubulin binding protein ninein and CAMSAP/Patronin protein families in controlling non-centrosomal microtubule assembly in *C. elegans* [55••]. In addition, anchoring dynein to the cortex in *Drosophila* S2 cells results in movement of minus-end out microtubules towards the cell body [56], suggesting a potential mechanism for non-centrosomal microtubule sorting. Recent advances in microscopy techniques and imaging tools will help to further visualize non-centrosomal microtubules and their dynamics [10,57,58]. Alterations in microtubule organization and transport processes have been described in several neurodegenerative diseases [59,60]. A better understanding of the mechanisms underlying polarized microtubule organization and selective cargo trafficking will surely advance our insight into injury and disease of the nervous system.

Conflict of interest statement

Nothing declared.

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- •• of outstanding interest

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