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Current Opinion in Neurobiology

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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Current Opinion in Neurobiology 2016, **39**:1–7

This review comes from a themed issue on **Cellular neuroscience**

Edited by **Bettina Winckler** and **Mikael Simons**

For a complete overview see the Issue and the Editorial

Available online 2nd March 2016

http://dx.doi.org/10.1016/j.conb.2016.02.003

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.

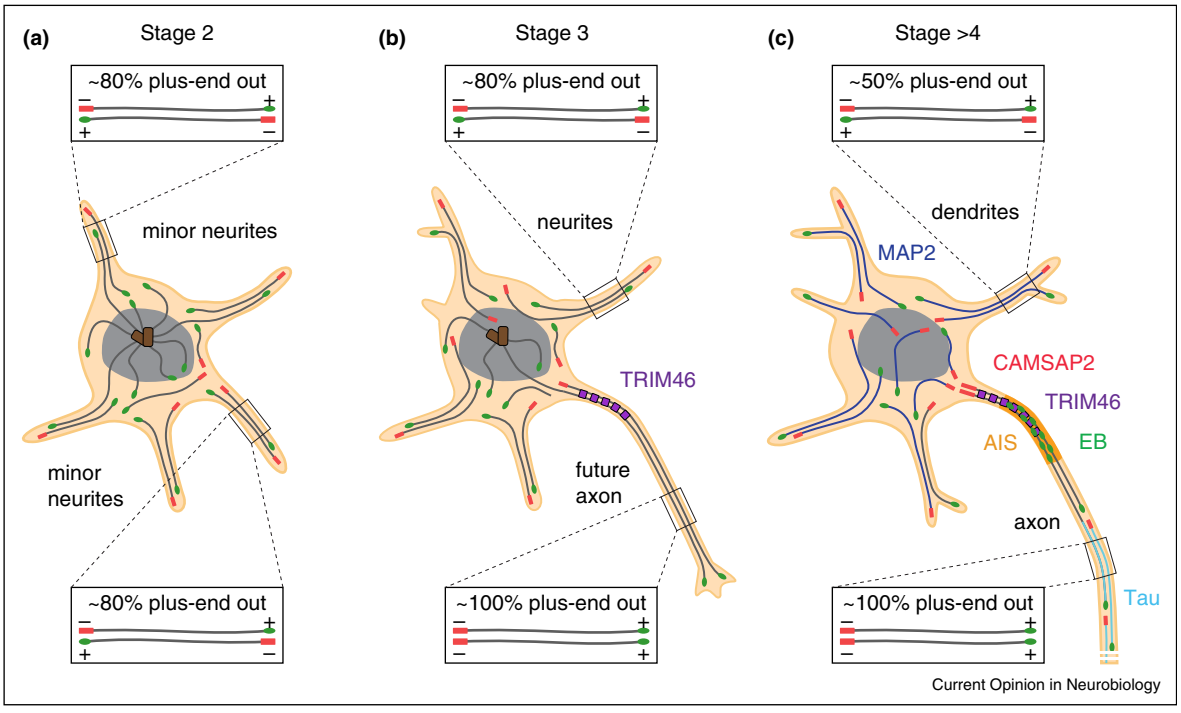
Two distinct microtubule organizations in one neuron

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