CELL BIOLOGY

# Exploring cytoskeletal diversity in neurons

## Cytoskeletal architecture underlies the diversity and function of neuronal compartments

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Often in biology, form follows function. For example, the ability of neurons to receive, process, and transmit information depends on their polarized organization into axons and dendrites. The cytoskeleton and associated motor proteins shape cells and establish spatial organization. Microtubules (MTs) and actin are core components of the cytoskeleton and are assembled through head-to-tail polymerization of α- and β-tubulin heterodimers and actin monomers, respectively, resulting in asymmetric, polarized polymers with two different ends, called plus and minus ends. The spatially regulated polymerization of MTs and actin can drive morphological transitions, such as local protrusion of the plasma membrane, to drive cell migration or the development of specialized extensions, such as axons or dendrites and their branches. In addition, the structural asymmetry of MTs and actin enables cytoskeletal motor proteins (myosin, kinesin, and dynein) to walk toward a specific end of the fibers. Given the extreme dimensions and functional compartmentalization of neurons, such active transport is critical to sort and distribute cellular cargoes. Recent studies have used advanced microscopy to reveal how the cytoskeleton takes many different forms to facilitate local functions in neurons (see the figure).

Actin is strongly enriched in the tip of growing axons, termed growth cones, during development or regeneration and in the small protrusions along dendrites, called spines, that harbor most excitatory synapses. Therefore, research often focused on exploring actin organization and function in relation to axon outgrowth and synaptic organization and plasticity. More recently, techniques that enable diffraction-unlimited microscopy have provided surprising new insights into the organization of the axonal actin cytoskeleton in neurons. Most notably, this revealed a periodic matrix of actin and its cross-linking partner spectrin, alternating in rings spaced ~190-nm apart along the entire axon of hippocampal neurons (*1*). Similar structures have been reported in the cell body and dendrites of different neuronal subtypes (*2*), and it has been shown that they provide mechanical support, maintain axon diameter, and can serve as a diffusion barrier for membrane proteins (*2*).

Other actin-based structures, such as actin-rich patches or "hotspots," have also been identified in axons of hippocampal neurons (*2*–*4*). Different actin nucleators have been implicated in their formation (*3*, *4*), and further away from the cell body, they often form around stationary endosomes (intercellular sorting vesicles) (*4*). Interestingly, more elongated and dynamic actin filaments, termed actin trails, emerge from these distal actin patches (*4*). How these different actin structures contribute to intracellular transport is not fully understood. Myosin-V motor proteins can oppose axonal transport by tethering cargoes onto proximal actin patches, thus serving as a filter for axonal transport (*3*, *5*). Whether myosins can also drive directional transport on actin trails is not known. Furthermore, little is known about the exact organization of actin at the presynapse, the site where neurotransmitter-containing vesicles are released for interneuronal signaling. More work is needed to further resolve the nanoarchitecture of the neuronal actin cytoskeleton, including the polarity of filaments, which determines the direction of motor proteins, and the distribution of actin-binding proteins that can nucleate, stabilize, bundle, or destabilize actin to create functional diversity among different actin networks.

> ***"...the cytoskeleton takes many different forms to facilitate local functions in neurons."***

MTs are often much longer than actin filaments and facilitate long-range transport driven by the kinesin and dynein motor proteins (*6*). Even though each MT is composed of α- and β-tubulin heterodimers, diversity can be generated by the incorporation of different tubulin isotypes, the recruitment of MT-associated proteins (MAPs), and by posttranslational modifications (PTMs), such as detyrosination, polyglutamylation, and acetylation (*6*). Indeed, the neuronal MT cytoskeleton is heterogeneous and features dynamic MTs that are turned over as well as stable MTs that are highly modified. This has led to the tubulin code hypothesis, which proposes that the genetic and chemical diversity of tubulin regulates MT properties and functioning (*6*). For example, PTMs can change the mechanical properties of MTs (*7*) or alter the binding of MAPs (*6*, *8*), which can in turn affect MT stability or activate specific motor proteins. Consequently, by recruiting motor proteins that prefer specific MT subsets, cargoes could ensure delivery to the proper compartment, such as axons, dendrites, dendritic spines, or growth cones. Moreover, dynamic MTs recruit specific proteins to their growing plus end that can, for example, establish local signaling networks that promote actin remodeling and result in outgrowth or branching (*6*). Nevertheless, in most cases the exact functions of different MT populations are still unknown. In addition, how MTs with different properties emerge and coexist is unclear.

In many cell types, MTs are generated by a MT organizing center (MTOC), such as the centrosome, where γ-tubulin ring complexes (γ-TuRCs) and other MAPs nucleate tube-like structures that then quickly elongate through polymerization at the plus end. This results in a radial array, with most MTs pointing toward the cell periphery. In such cells, minus-end–directed dynein drives retrograde transport toward the cell center, whereas outward, anterograde transport is driven by the mostly plus-end–directed kinesin family members. Nonetheless, in developing neurons the centrosome quickly loses its role as MTOC, suggesting that most MTs are nucleated throughout the whole cell (*9*). Local MT formation requires mechanisms to stabilize labile minus ends (*6*), which could be achieved by nucleating MTs from structures to which they remain anchored. For example, Golgi outposts have been proposed as potential sites of noncentrosomal nucleation in fruitfly (*Drosophila melanogaster*) neurons, although active mispositioning of these structures did not alter MT organization (*10*). Understanding the birth and fate of neuronal MTs requires dissecting the frequency, spatial distribution, and mechanisms of local MT nucle-

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ation. In systems with small numbers of MTs, such as axons in the nematode worm *Caenorhabditis elegans*, careful analysis of intensity patterns, recently used to determine MT density and length, might also reveal nucleation events (*11*). However, for more dense MT arrays, the development of live-cell markers that highlight nucleation is needed.

Local nucleation also requires additional mechanisms to control the proper orientation of newly formed MTs within the existing cytoskeleton. Because MT orientation determines motor protein directionality, one would assume that efficient long-range transport requires that most MTs in a cellular compartment have similar orientations. Indeed, MTs are largely uniformly oriented plus-end–outward in the axons, ensuring that kinesin activation results in anterograde cargo transport, whereas retrograde transport is driven by dynein. Recent work has shown that this uniform organization depends on the augmin complex, which can position γ-TuRCs along existing MTs so that newly nucleated MTs have the same orientation (*12*). In addition, TRIM46 (tripartite motif 46) is important for generating parallel MT bundles near the axon initial segment (AIS), a specialized zone involved in the generation of action potentials and filtering of membrane proteins and intracellular cargoes (*13*).

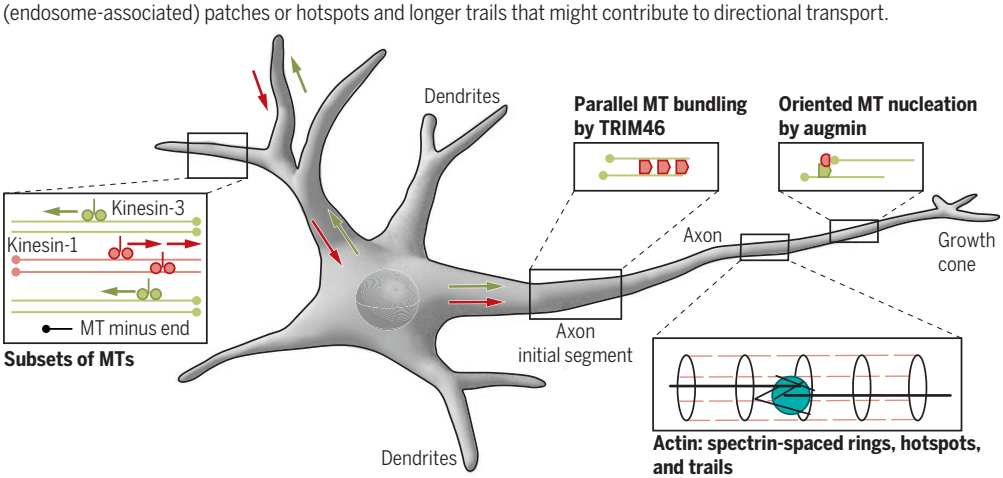
In fruitflies and worms, dendrites also have a uniform MT array, but oriented oppositely relative to the axon. In such systems, kinesins drive axon-selective transport, whereas dynein drives transport into dendrites (*14*). Remarkably, in dendrites of mammalian cells MTs have mixed orientations. How do motor proteins navigate such a network to still ensure directional transport? Recent work has addressed this question by introducing a new super-resolution technique, called motor-PAINT (point accumulation for imaging in nanoscale topography), that enabled mapping both MT orientations and PTMs (*15*). This revealed that although overall both orientations are equally abundant, MTs locally cluster into bundles with a more uniform orientation. This ensures that motor proteins will mostly persist in the direction dictated by the bundle orientation, even when they occasionally switch to neighboring MTs. In addition, stable and modified MTs were predominantly oriented minus-end outward, whereas dynamic MTs were mostly oriented plus-end out (*15*). Because these MT subsets facilitate transport by distinct kinesins, this creates an overall inward or outward bias for different plus-end–directed kinesins. Whereas kinesin-1 would mostly move inward over stable MTs, kinesin-3 would move outward over dynamic MTs. These findings explain why some kinesins only transport cargoes to axons, whereas others target both axons and dendrites.

Despite these important insights, why certain motor proteins selectively interact with specific MTs remains unresolved. Motor proteins could recognize specific combinations of PTMs and/or MAPs or particular features of the MT lattice. Recent progress in the purification of tubulin isotypes and the reconstitution of different PTMs now allows for teasing out the differential effects of PTMs and MAPs on MT dynamics, mechanics, and MT-based transport (*7*, *8*, *16*). Such controlled in vitro reconstitution experiments will hopefully lead to a structural understanding of motor protein selectivity. Similar experiments might also provide hints toward the mechanisms through which MT heterogeneity is established. Why do some MTs become modified and stabilized and others not? How can MTs with different chemical properties have opposite orientations? Resolving these questions will also require visualization of the developmental dynamics of different MT subsets, both at the time scale of single-MT turnover (minutes to hours) as well as the time scale of dendrite differentiation (several days). Here, developing markers for live-cell imaging of different subsets would provide exciting new opportunities.

Although frequently studied independently, actin and MTs often are functionally connected and also interconnect with other cytoskeletal structures, such as neurofilaments and septins, the functions of which are much less explored. In addition, these cytoskeletal components often interact with different organelles, including endosomes, the Golgi apparatus, and the endoplasmic reticulum. How such interactions contribute to the compartment-specific shaping of the heterogenous neuronal cytoskeleton or the communication between organelles is an important topic for future studies. Although mammalian cytoskeletal studies have largely focused on cultured, dissociated neurons, it will be important to explore cytoskeletal form and function in more intact model systems in vivo. This might also enable unraveling the mechanisms by which cytoskeletal organization and intracellular transport are affected during neuronal degeneration and suggest avenues for modulation. ■

## Examples of cytoskeletal network diversification in a neuron

Oppositely oriented microtubules (MTs) in dendrites have different properties and recruit different plus-end–directed motor proteins. Parallel MT organization in the axon is established through bundling by TRIM46 in the axon initial segment and by augmin-mediated oriented nucleation. Actin in the axon is organized in rings that provide mechanical support, as well as (endosome-associated) patches or hotspots and longer trails that might contribute to directional transport.





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