The functional architecture of axonal actin

Marie-Jeanne Papandréou[1], Christophe Leterrier[1]

[1] Aix Marseille Université, CNRS, INP UMR7051, 13344 cedex 15, Marseille, France

Correspondence: christophe.leterrier@univ-amu.fr
NeuroCyto, INP CNRS-AMU UMR 7051, Bd Pierre Dramard, 13344 Marseille cedex 15, France

Keywords: axon, cytoskeleton, actin.

Abstract

The cytoskeleton builds and supports the complex architecture of neurons. It orchestrates the specification, growth, and compartmentation of the axon: axon initial segment, axonal shaft, presynapses. The cytoskeleton must then maintain this intricate architecture for the whole life of its host, but also drive its adaptation to new network demands and changing physiological conditions. Microtubules are readily visible inside axon shafts by electron microscopy, whereas axonal actin study has long been focused on dynamic structures of the axon such as growth cones. Super-resolution microscopy and live-cell imaging have recently revealed new actin-based structures in mature axons: rings, hotspots and trails. This has caused renewed interest for axonal actin, with efforts underway to understand the precise organization and cellular functions of these assemblies. Actin is also present in presynapses, where its arrangement is still poorly defined and its functions vigorously debated. Here we review the organization of axonal actin, focusing on recent advances and current questions in this rejuvenated field.

Introduction

The cytoskeleton allows cells to establish, maintain and transform their shape. In neurons, this include cell differentiation, migration, polarization and development of their unique arborization. Axons are very thin (~1 μm), long (up to 1m) and highly branched (>95% of the plasma membrane of a typical neuron) extensions, presenting unique challenges to the cytoskeletal organization (Kevenaar and Hoogenraad, 2015; Leterrier et al., 2017). The axonal architecture must be reliably maintained for decades, but also adapt for optimal functioning in a range of physiological conditions (Jamann et al., 2018). On the one hand, the organization of axonal microtubules and their associated proteins have been extensively studied, although numerous questions remain (Kapitein and Hoogenraad, 2015; Prokop, 2013). On the other hand, actin is well known to support morphogenesis via the dynamic formation of structures such as lamellipodia, filopodia and stress fibers (Blanchoin et al., 2014). Axonal actin has indeed been recognized as a component of dynamic structures in developing axons such as the growth cone and nascent branches, with a less-studied role of structural support in mature axons (Gallo, 2013; Letourneau, 2009). However, this vision has been profoundly transformed by the discovery of new actin structures within axons such as rings, hotspots and trails (Leterrier et al., 2017; Roy, 2016). Most of our knowledge about axonal actin comes from dissociated neuronal cultures; these reductionist models have been invaluable for isolating the core cell-intrinsic processes described here, and a number of them – such as actin rings, hotpsots and trails – have been since described in more integrated models. This review aims at summarizing the current knowledge about axonal actin, with a particular focus on the compartments of mature axons: the axon initial segment, axon shaft and presynaptic boutons. We will concentrate on structural aspects, detailing the different actin assemblies and their established or putative role, leaving mostly aside the myriad of molecular pathways and binding partners that affect actin assembly in various axonal compartments (for reviews see Coles and Bradke, 2015; Menon and Gupton, 2016).

Dynamic actin during axon development

The striking arrangement of enriched actin in structures such as the growth cone has captured the interest of many neuroscientists, from early electron and fluorescent microscopy work (Kuczmarski and Rosenbaum, 1979; Letourneau, 1983; Yamada et al., 1971) to the first live-cell imaging studies (Lin and Forscher, 1995; O'Connor and Bentley, 1993). The intrinsic dynamics of actin polymers, with biased incorporation of actin monomers at the barbed end and disassembly at the pointed end, naturally suggested a role for force generation, morphogenesis and cellular movement (Kirschner, 1980; Wessells et al., 1971). Actin is indeed crucial during axonal development: sprouting of the first neurites and subsequent axonal specification, elongation and branching are driven by dynamic actin-driven processes (Letourneau, 2009). We will only briefly describe these processes, and invite the interested reader to consult more comprehensive reviews on this subject (Fig. 1, Caceres et al., 2012; Omotade et al., 2017; Schelski and Bradke, 2017; Witte and Bradke, 2008).

Role of actin instability and dynamics

Shortly after plating, rounded neurons already exhibit an inhomogeneous actin distribution, with actin enrichment and lamellipodium formation toward the site of the first neurite sprouting (Gärtner et al., 2014; S.-X. Zhang et al., 2016). Importantly, dynamic actin assemblies are required for the initial

stages of neurite growth, as stabilization of actin with jasplakinolide blocks the emergence of neurites, whereas destabilization with latrunculin or cytochalasin accelerates it (Flynn et al., 2012; S.-X. Zhang et al., 2016; Zmuda and Rivas, 2000). Ena/VASP driven filopodium formation and severing by ADF/cofilin are required for remodeling actin during neurite sprouting (Dent et al., 2007; Flynn et al., 2012). The ensuing neurite extension also requires permissive actin instability (Flynn et al., 2012; Lu et al., 2013; Ruthel and Hollenbeck, 2000), although actin disassembly has also been reported to block neurite growth (Chia et al., 2016; Yamada et al., 1970). Axon specification occurs when one neurite starts to grow more rapidly and becomes the unique axon (Dotti et al., 1988), with a length-dependent reinforcement of axonal identity (Lewis et al., 2013; Schelski and Bradke, 2017; Yamamoto et al., 2012). This selective extension depends on the localized destabilization of actin and concurrent stabilization of microtubules within the nascent axon (Bradke and Dotti, 1999; Witte et al., 2008; Zhao et al., 2017). Among the intricate pathways that orchestrates cytoskeletal remodeling during axon specification (Namba et al., 2015; Schelski and Bradke, 2017), actin destabilization depends on a cdc42/Rac/cofilin sequence (Garvalov et al., 2007; Nishimura et al., 2005).

Actin in the growth cone

The growth cone is a specialized structure at the extremity of neurites and axons that drives their growth and navigation during development (Fig. 1A, Lowery and Van Vactor, 2009). An extensive literature is devoted to the organization and role of actin in the growth cone, which will only be briefly summarized here (Dent et al., 2011; Omotade et al., 2017). Actin shapes the tip of the growth cone, with a peripheral lamellipodium from which dynamic filopodia emerge (Fig. 1D, Bray and Chapman, 1985; Korobova and Svitkina, 2008). A rearward flow is established by the assembly of actin filaments at the extremity of the lamellipodium and filopodia coupled with their disassembly in the transition zone inside the growth cone (Schaefer et al., 2002; Suter and Forscher, 2000). Coupling of this rearward treadmilling to the substrate drives the forward advance of the growth cone (Forscher and Smith, 1988). In the central region, bundling by myosin-II creates transverse stress fibers that complement the rearward flow in resisting microtubule entry into the growth cone periphery (Medeiros et al., 2006). Local destabilization within the fibers guides microtubules along filopodia at the periphery, resulting in directed growth (Burnette et al., 2008; Schaefer et al., 2008).

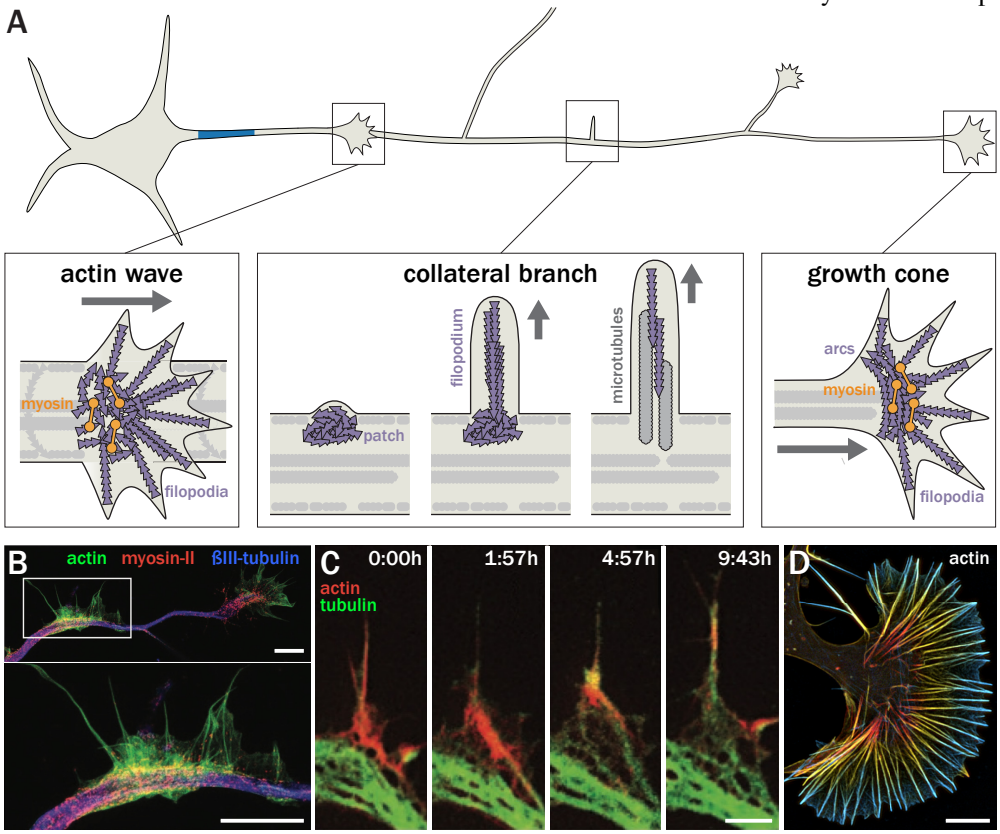

Figure 1: Actin structures in the developing axon

A. The axon of a developing neuron contains dynamic processes enriched in actin structures (purple filaments). Actin waves (left panel) travel along the axon (grey arrow) and contain actin filopodia as well as a denser region crosslinked by myosin-II (yellow). Nascent collateral branches (middle panel) emerge from an actin patch that develop into a filopodium and is later invaded by microtubules as it grows into a branch (grey arrow). Growth cones at the tips of the axon are motile processes (grey arrow) containing a peripheral lamellipodium from which filopodia emerge, and a more central part where myosins (yellow) crosslink and bundle actin into arcs. **B.** STimulated Emission Depletion (STED) image of an actin wave along a developing axon labeled for actin (green), myosin-II (red) and microtubules (ßIII-tubulin, blue). Adapted from (Mortal et al., 2017). Scale bars, 5 µm. **C.** Live-cell imaging sequence (time in hours) showing a collateral branch emerging from an axon with the initial concentration of actin (red) and subsequent entry of microtubules (green). Adapted from (Dent and Kalil, 2001). Scale bar, 5 µm. **D.** Structured Illumination Microscopy (SIM) image of a growth cone labeled for actin, color coded for depth (blue to red, 0 to 1500 nm). Adapted from (Nozumi and Igarashi, 2017). Scale bar, 5 µm.

Actin waves along developing axons

In neurons developing in culture, actin-based waves similar to growth cones traveling at ~3 µm/min along the neurites and nascent axon have been described (Fig. 1A & 1B, Ruthel and Banker, 1998). They are specific to immature, developing neurons and disappear after a few days in culture (Ruthel and Banker, 1999). They have been observed in organotypic slices (Flynn 2009), and also

appear during axonal regeneration (Difato et al., 2011). Waves progress by filament treadmilling and Shootin-1 mediated coupling to substrate-bound L1-CAM (Katsuno and Sakumura, 2015). Waves are driven by actin dynamics, but also depend on microtubule via doublecortin coupling (Ruthel and Banker, 1998; Tint et al., 2009). Actin waves have been proposed to promote axonal growth and branching (Flynn et al., 2009; Ruthel and Banker, 1999), but see (Mortal et al., 2017), by promoting neurite widening and microtubule-based transport (Winans et al., 2016). Interestingly, more waves travel along the neurite that will become the axon in immature multipolar neurons (Flynn et al., 2009). They could also represent a way to transport actin (Flynn et al., 2009; Tomba et al., 2017), although their low frequency (one or two per hour) and developmental downregulation argue against actin waves as the main vehicle for actin transport (Leterrier et al., 2017; see below).

Actin dynamics for axon branching

Actin is also implicated in branching during axonal growth (Armijo-Weingart and Gallo, 2017). Several modes of branching exist, the most commonly studied being collateral branching from the axon shaft (Gallo, 2011; Kalil and Dent, 2014), which is also characterized in vivo (Andersen et al., 2011; Hand et al., 2015). Actin remodeling occurs during the first steps of branching, with the formation of ~1-2 μm actin patches along the axon shaft (Fig. 1A, Ketschek and Gallo, 2010; Loudon et al., 2006; Spillane et al., 2011). Around 20% of these patches give rise to a filopodium resulting from Arp2/3-mediated nucleation followed by bundling (Korobova and Svitkina, 2008; Spillane et al., 2011). Successful conversion of an actin-based filopodium into an axon branch requires invasion by microtubules unbundled from the axon shaft (Dent et al., 1999; Gallo and Letourneau, 1999). Unbundling and microtubule entry into a nascent branch require the downregulation of actomyosin contractility (Fig. 1C, Ketschek et al., 2015). Moreover, crosstalk between actin and microtubules in this crucial step involves additional partners such septin (Hu et al., 2012) and drebrin (Ketschek et al., 2016).

Actin assemblies in the mature axon

By contrast with the dynamic assemblies in developing neurons, actin labeling along the axon shaft itself was initially considered as weak and unremarkable (Letourneau, 2009). Fluorescent labeling showed a uniform distribution of actin with occasional clusters (Kuczmarski and Rosenbaum, 1979; Letourneau, 1983; Spooner and Holladay, 1981), and electron microscopy could resolve a homogeneous submembrane population (Hirokawa, 1982; Tsukita et al., 1986) as well as more intra-axonal short filaments (Bearer and Reese, 1999; Fath and Lasek, 1988). In recent years however, super-resolution microscopy and live-cell imaging have profoundly transformed this view, with the discovery of striking structures along the axon shaft such as the exquisitely organized actin rings or the dynamic hotspots and trails (Fig. 2, Leterrier et al., 2017).

Actin rings

In 2013, Xu et al. used Stochastic Optical Reconstruction Microscopy (STORM), a super-resolution microscopy technique that resolves details down to ~20 nm (instead of ~200 nm for classical epifluorescence, Maglione and Sigrist, 2013) to visualize phalloidin-labeled actin organization in neurons. STORM revealed the presence of submembrane actin rings, oriented perpendicularly to the axonal axis and regularly spaced every ~190 nm (Fig. 2A, Xu et al., 2013). This spacing value corresponds to the length of individual, extended spectrin tetramers (Bennett et al., 1982): spectrin appear as periodic stripes along the axon when labeling the axonal ß2-spectrin and α2-spectrin, or the AIS-specific ß4-spectrin (Fig. 2B & 2C, Huang et al., 2017a; 2017b; Leterrier et al., 2015; Xu et al., 2013). Actin and spectrin thus form a membrane-associated periodic scaffold (MPS) with actin rings connected by head-to-head tetramers of spectrin that bind actin on each side via the aminoterminus of the ß-spectrin subunits (Rasband, 2013; Xu et al., 2013). This periodic scaffold has been observed in living neurons (D'Este et al., 2015; Zhong et al., 2014) and detected in various neuronal types and organisms, including worm, fly, rodent and human (Barabas et al., 2017; D'Este et al., 2016; He et al., 2016). Interestingly, a monodimensional periodic scaffold is also detected along a minority of dendritic segments, along dendritic spine necks (Bär et al., 2016; D'Este et al., 2015; He et al., 2016), and within fine processes of oligodendrocytes (D'Este et al., 2016). In larger dendrites and in the cell body, a hexagonal organization of actin and spectrin is detected (Han et al., 2017), similar to the one found under the erythrocyte plasma membrane (Lux, 2016; Pan et al., 2018). This suggests that the membrane geometry affects the actin/spectrin complex arrangement, with a 1D periodic scaffold in slender cylinders, and a 2D hexagonal pattern along flat membranes.

Actin rings appear first at the proximal axon after two days in culture and are later found along the more distal axon (D'Este et al., 2015; Zhong et al., 2014). Interestingly, appearance of actin rings overlaps with the extinction of actin waves. This could mean that actin monomers that make the rings are brought by actin waves, or at least shows that actin can shift from dynamic to stable structures during axon maturation. However, the molecular mechanism driving the periodic actin/spectrin scaffold formation is still largely unknown (Leite and Sousa, 2016). Once formed, the scaffold is stable and immobile over ~30 minutes, as shown by live-cell PhotoActivation Localization Microscopy (PALM) of photoactivable ß2-spectrin (Zhong et al., 2014). Actin filaments within rings are associated with the capping protein adducin, further suggesting that they are short and stabilized (Xu et al., 2013).

The stability of the periodic actin/spectrin scaffold indicates that it has a structural role: the chain of flexible spectrins and rigid actin rings – similar to a vacuum cleaner hose – could

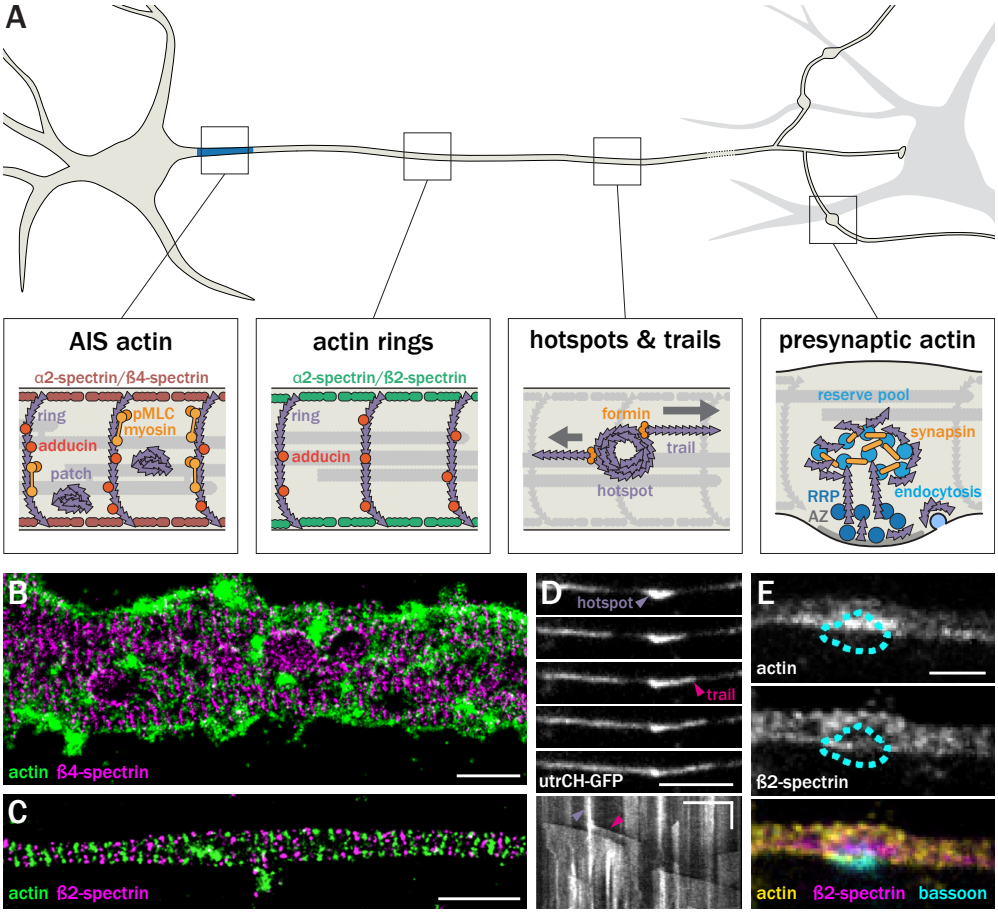

Figure 2: Actin structures in the mature axon

A. The mature axon contains specific actin structures (purple filaments). In the AIS (blue, left panel), submembrane actin rings are spaced every 190 nm by spectrin tetramers composed of α2- and ß4-spectrin (maroon). Actin ring filaments are capped by adducin (red) and associated with phospho-myosin light chain (pMLC) and myosin (yellow). Actin patches are also present inside the AIS. In the more distal axon (middle left panel), the periodic submembrane actin/spectrin scaffold is also present, based on α2/ß2-spectrin tetramers (green). The axon shaft also contains actin hotspots (middle right panel), clusters of actin at the surface of which dynamic trails polymerize (grey arrows) by a formin-dependent mechanism (yellow). In the presynapses contacting target neurons (right panel), actin is present around the reserve pool of synaptic vesicles (light blue) that are linked by synapsin (yellow). Actin also contacts and organizes the readily-releasable pool of synaptic vesicles (RRP, dark blue) at the active zone (AZ), and at endocytosis sites at the periphery of the active zone (cyan). **B.** STORM image of an AIS labeled for actin (green) and ß4-spectrin (magenta). Adapted from (Leterrier et al., 2015). Scale bar, 1µm. **C.** STORM image of a distal axon labeled for actin (green) and the carboxyterminus of ß2-spectrin (magenta). Adapted from (Xu et al., 2013). Scale bar, 1 µm. **D.** Top: live-cell imaging sequence (1 second between each frame) of a neuron expressing the UtrCH-GFP, a probe for filamentous actin. A hotspot (purple arrowhead) generates a trail (magenta arrowhead). Scale bar: 10 µm. Bottom: Kymograph of the axon portion containing the segment shown as a sequence on top. The hotspot and trail seen on the sequence are labeled on the kymograph (purple and magenta arrowheads, respectively). Scale bars, 10 µm (horizontal distance, proximal to distal axon is left to right) and 20s (vertical time, descending). Adapted from (Ganguly et al., 2015). **E.** STED image of a presynapse labeled for actin (yellow on overlay), ß2-spectrin (magenta on overlay) and bassoon (cyan on overlay, dashed region on single channel images). Adapted from (Sidenstein et al., 2016). Scale bar, 0.5 µm.

provide flexibility and resistance to axons, notably in the peripheral nervous system where they are subject to mechanical stress (Xu et al., 2013). Consistent with this mechanoprotective role of the actin/spectrin scaffold, axons of C. Elegans worms lacking ß-spectrin progressively break when the animal is moving (Hammarlund et al., 2007). FRET sensor probes inserted in ß-spectrin show that indeed, axons are under constitutive tension in vivo (Krieg et al., 2014), a finding confirmed by modeling of the axon mechanical properties (Lai and Cao, 2014; Y. Zhang et al., 2017). Complementing the longitudinal contractility provided by spectrins, actin rings are likely to exert a radial myosin-based contractility: adducin depletion does not change the spacing of the rings but leads to larger axon diameters (Leite et al., 2016), as does myosin inhibition (Fan et al., 2017). Interestingly, the phosphorylated form of myosin light chain (pMLC, the activator of contractile myosin-II) was found to associate along actin rings at the axon initial segment (Berger et al., 2018). An interplay between the periodic actin/spectrin scaffold and intra-axonal microtubule bundles is also likely, as microtubule perturbation partially impairs the periodic scaffold (Zhong et al., 2014), and lack of actin rings can exacerbate microtubule defects along the axon (Qu et al., 2016). This interplay exists at the mechanical level, with microtubule bundles providing a resistive force against radial actomyosin contraction (Fan et al., 2017).

Hotspots and trails

In addition to submembrane actin assemblies, a few electron microscopy studies had reported the presence of deeper intra-axonal actin filaments interspersed among microtubules (Bearer and Reese, 1999; Fath and Lasek, 1988; Nagele et al., 1988). Using a GFP-tagged calponin-homology domain of utrophin – a non-perturbing, high fidelity probe for filamentous actin (Burkel et al., 2007; Melak et al., 2017; Patel et al., 2017) – Ganguly et al. observed the dynamics of actin along axons (Ganguly et al., 2015). Beyond the random bidirectional movement previously described (Chetta et al., 2015), they identified two new actin structures: static clusters of actin every 3-4 μm along axon shafts that appear and disappear within minutes called actin hotspots, and filaments up to 10 μm in length that grow in both directions at ~1 μm/s, called actin trails (Fig. 2D). STORM corroborates the presence of

actin clusters and longitudinal filaments along axons (Ganguly et al., 2015). In addition to this initial definition in cultured neurons, hotspots and trails were recently observed in vivo in C. Elegans axons (Sood et al., 2017). Hotspots are likely to be generated by actin polymerization at the surface of static endosomes, as they colocalize with endosomal markers. Trails often appear to start from hotspots, and their extension is formin-dependent (Ganguly et al., 2015).

What are the functions of hotspots and trails? Fast transport of vesicles and organelles along the axon occurs along microtubules, and the presence of hotspots and trails could locally affect this transport. Indeed, actin rich regions can cause local traffic jams of axonal cargoes (Sood et al., 2017). Trails nucleation likely occurs at the endosomal surface, pushing actin filaments away from the hotspot, and this can result in short-range transport of actin itself. Depletion of trails using formin inhibitors results in altered presynaptic function, suggesting that trails could shuttle actin between presynapses (Ganguly et al., 2015). Moreover, the constant growth and of collapse of trails and their bias for the anterograde direction (55% against 45% retrograde) results in the slow transport of actin along the axon (Chakrabarty et al., 2017). Indeed, classic radiolabeling studies have demonstrated that actin is transported with the SCb component of slow transport (1-4 mm/day, Black and Lasek, 1979; Mori and Kurokawa, 1981; Willard et al., 1979), and modeling shows that biased trails-mediated transport could result in similar overall speeds (Chakrabarty et al., 2017).

A particular case: actin at the axon initial segment

The AIS, located along the first 20 to 60 μm of the axon, contains unique actin-based structures (Leterrier, 2018; C. Zhang and Rasband, 2016). The periodic actin/spectrin submembrane scaffold found along axons is present at the AIS, with spectrin tetramers formed by α2- and ß4-spectrin subunits (Fig. 2B, Huang et al., 2017a). In the middle of the spectrin tetramer between actin rings, ß4-spectrin binds to ankyrin G, which anchors a high density of ion channels and cell-adhesion molecules (Leterrier, 2016; Rasband, 2010). This generates a diffusion barrier at the AIS: diffusion of membrane proteins and lipids between the cell body and the distal axon is restricted by the concentration of membrane proteins coupled to the periodic actin/spectrin scaffold (Albrecht et al., 2016; Nakada et al., 2003; Winckler et al., 1999). The AIS also contains actin patches that are readily seen by diffraction-limited imaging of actin probes and electron microscopy (Jones et al., 2014; K. Watanabe et al., 2012). On super-resolution microscopy images, clusters close to the plasma membrane have been proposed to correspond to postsynaptic accumulation, as the AIS is innervated by GABAergic neurons (D'Este et al., 2015). Deeper actin patches have a role in regulating vesicular trafficking to and from the axon (Al-Bassam et al., 2012). These Arp2/3-positive actin clusters are able to immobilize vesicular cargoes in a myosin-dependent manner (Balasanyan et al., 2017; Janssen et al., 2017; K. Watanabe et al., 2012). This is likely an intermediate step in a selective retrieval mechanism at the AIS allowing to reroute mistargeted somatodendritic cargoes before they reach the distal axon (Leterrier, 2018; Nirschl et al., 2017).

Actin at presynapses

Organization of actin at presynapses

Ultimately, electric signals transmitted along the axon are transformed into calcium-dependent release of neurotransmitters on the presynaptic side of chemical synapses (Fig. 2A). Since the first electron microscopy studies (Gray, 1959; Palay, 1956), a huge body of work has detailed the composition and organization of presynapses, culminating with quantitative spatial proteomic approaches (Ackermann et al., 2015; Wilhelm et al., 2014). However, actin is particularly difficult to preserve and label in classical electron microscopy procedures, and this prevented from a clear understanding of actin organization at presynapses (Cingolani and Goda, 2008; Rust and Maritzen, 2015).

A combination of quick-freeze preparation – which minimizes actin destabilization arising from osmium fixation methods – and labeling with myosin fragments or phalloidin identified a network of actin filaments associated with different parts of the presynapse such as the active zone and distinct synaptic vesicle pools (Alonso et al., 1981; Fifková, 1985; Landis et al., 1988; Morales et al., 2000). The active zone is an electron-dense material that lines the presynaptic membrane and contains a cluster of docked synaptic vesicles called the readily releasable pool (RRP) (Alabi and Tsien, 2012; Fowler and Staras, 2015). The active zone is enriched in actin, with filaments associated with RRP vesicles (Hirokawa et al., 1989). Another population of synaptic vesicles, the reserve pool, is located more centrally in the presynapse, where they are linked by synapsin that forms ~30-nm filaments (A. A. Cole et al., 2016; Hirokawa et al., 1989; Siksou et al., 2009). Synapsins connect to 50-100 nm actin filaments organized in strands between and around the synaptic vesicles, or extending from the presynaptic membrane (Landis et al., 1988; Li et al., 2010; Siksou et al., 2007). Immunogold electron microscopy showed little actin labeling within the reserve pool, but more at the periphery, where new synaptic vesicles are generated by endocytosis (Bloom et al., 2003; Pechstein and Shupliakov, 2010).

More recently, optical super-resolution techniques such as STORM and STED, which resolved the periodic actin-spectrin submembrane scaffold along of the axon, have shown that it is interrupted at presynapses (Fig. 2E, He et al., 2016; Sidenstein et al., 2016). Actin enrichment can be observed adjacent to the active zone by super-resolution microscopy, but no precise organization of actin within presynaptic terminals has been determined by these techniques so far, probably because presynaptic actin signal is occluded by the larger enrichment of actin in adjacent postsynaptic spines (Korobova and Svitkina, 2010). Beyond this static vision, live-cell imaging demonstrates that a significant proportion of synaptic actin is

dynamic, with constant cycles of assembly and disassembly in the post- (Fischer et al., 1998) as well as presynaptic compartments (Colicos et al., 2001). Myosins are involved in shaping presynaptic actin (Kneussel and Wagner, 2013): movement of synaptic vesicles within the presynapse depend on the contractile myosin-II, suggesting that actin reorganization can displace vesicles (Peng et al., 2012). By contrast, exchange of synaptic vesicles between neighboring boutons via a vesicle "superpool" (Darcy et al., 2006; Staras et al., 2010) has recently been shown to occur via processive myosin V-mediated transport (Gramlich and Klyachko, 2017).

Role of actin at presynapses

Beyond its architecture, the precise function of actin at presynaptic terminals is a disputed topic. Depending on the models and experimental conditions, actin perturbation can lead to enhanced or impaired synaptic vesicle release (Cingolani and Goda, 2008; Rust and Maritzen, 2015). Depolymerizing drugs such as latrunculin or cytochalasin have been shown to enhance release (Morales et al., 2000; Sankaranarayanan et al., 2003), reduce it (J. C. Cole et al., 2000) or have no effect (Job and Lagnado, 1998; Sakaba and Neher, 2003). This suggests that the presynapse contains distinct actin structures with varying sensitivity to actin-perturbing drugs and contrasted roles at different steps of the synaptic vesicle cycle (Bleckert and Photowala, 2012). Around the reserve pool, peripheral actin could have a corralling effect (Rust and Maritzen, 2015). In addition, actin filaments between the release pool and the readily-releasable pool (RRP) can support vesicle transport, allowing to replenish the RRP after stimulation (Owe et al., 2009; Sakaba and Neher, 2003). Within the RRP at the active zone, actin has been proposed to guide vesicles to the precise sites of exocytosis via a myosin-II dependent mechanism (Lee et al., 2012; Miki et al., 2016). Actin also participates in clustering the active zone machinery and reducing the area of exocytosis sites (Glebov et al., 2017). This leads to more precise and restricted vesicle release and could explain the "barrier" effect observed in a number of previous studies (Rust and Maritzen, 2015).

Vesicular transport of synaptic vesicles to the active zone and their subsequent release upon stimulation is counterbalanced by endocytosis. Several distinct endocytic mechanisms have been described with varying kinetics, dependence on clathrin and/or dynamin, and size of the endocytosed vesicles (Jähne et al., 2015; Kononenko and Haucke, 2015; S. Watanabe et al., 2013). Importantly, all these endocytic pathways depend on actin assembly for proper functioning (Soykan et al., 2017; S. Watanabe et al., 2013; Wu et al., 2016). To clarify the role of actin in vesicle endocytosis and generally in the presynaptic organization and function, it is important to identify the distinct actin nanostructures involved. Here also, a combination of super-resolution microscopy and live-cell imaging should provide decisive structural and molecular insights, allowing to target and observe distinct actin assemblies and tease apart their functions.

Conclusion

Thanks to the recent discovery of rings, hotspots and trails, axonal actin is back in the spotlight. These are exiting times for neuronal cell biologists armed with constantly improving labeling and imaging techniques to observe, quantify and perturb these structures. Future work will undoubtedly answer currently open questions: what are the molecular partners of these assemblies and how do they form? What are the relationships between these structures, and what regulates which actin structure is assembled depending on the time and place in the tight space of the axon? An interesting emerging model in non-neuronal cells is the competition between actin regulators for the formation of distinct structures (lamellipodia, filopodia, stress fibers) (Burke et al., 2014; Davidson and Wood, 2016; Lomakin et al., 2015). This could apply to axonal actin, with the added complexity of a developmental regulation in neurons between dynamic structures and stable assemblies. Finally, The constantly evolving cellular imaging and manipulation capabilities will likely help shine a new light on presynaptic actin, allowing to reconcile the conflicting views of our current knowledge.

References

Ackermann, F., Waites, C.L., Garner, C.C., 2015. Presynaptic active zones in invertebrates and vertebrates. EMBO Reports 16, 923–938. doi:10.15252/embr.201540434

Al-Bassam, S., Xu, M., Wandless, T.J., Arnold, D.B., 2012. Differential trafficking of transport vesicles contributes to the localization of dendritic proteins. Cell Rep 2, 89–100. doi:10.1016/j.celrep.2012.05.018

Alabi, A.A., Tsien, R.W., 2012. Synaptic vesicle pools and dynamics. Cold Spring Harb Perspect Biol 4, a013680. doi:10.1101/cshperspect.a013680

Albrecht, D., Winterflood, C.M., Sadeghi, M., Tschager, T., Noé, F., Ewers, H., 2016. Nanoscopic compartmentalization of membrane protein motion at the axon initial segment. J. Cell Biol. 215, 37–46. doi:10.1083/jcb.201603108

Alonso, G., Gabrion, J., Travers, E., Assenmacher, I., 1981. Ultrastructural organization of actin filaments in neurosecretory axons of the rat. Cell Tissue Res 214, 323–341.

Andersen, E.F., Asuri, N.S., Halloran, M.C., 2011. In vivo imaging of cell behaviors and F-actin reveals LIM-HD transcription factor regulation of peripheral versus central sensory axon development. Neural Dev 6, 27. doi:10.1186/1749-8104-6-27

Armijo-Weingart, L., Gallo, G., 2017. It takes a village to raise a branch: Cellular mechanisms of the initiation of axon collateral branches. Mol. Cell. Neurosci 84, 36–47. doi:10.1016/j.mcn.2017.03.007

Balasanyan, V., Watanabe, K., Dempsey, W.P., Lewis, T.L., Trinh, L.A., Arnold, D.B., 2017. Structure and Function of an Actin-Based Filter in the Proximal Axon. Cell Rep 21, 2696–2705. doi:10.1016/j.celrep.2017.11.046

Barabas, F.M., Masullo, L.A., Bordenave, M.D., A Giusti, S., Unsain, N., Refojo, D., Caceres, A., Stefani, F.D., 2017. Automated quantification of protein periodic nanostructures in fluorescence nanoscopy images: abundance and regularity of neuronal spectrin membrane-associated skeleton. Sci Rep 7, 8206. doi:10.1038/s41598-017-16280-x

Bär, J., Kobler, O., van Bommel, B., Mikhaylova, M., 2016. Periodic F-actin structures shape the neck of dendritic spines. Sci Rep 6, 37136. doi:10.1038/srep37136

Bearer, E.L., Reese, T.S., 1999. Association of actin filaments with axonal microtubule tracts. J. Neurocytol 28, 85–98.

Bennett, V., Davis, J.Q., Fowler, W.E., 1982. Brain spectrin, a membrane-associated protein related in structure and function to erythrocyte spectrin.

Nature 299, 126–131.

Berger, S.L., Leo-Macias, A., Yuen, S., Khatri, L., Pfennig, S., Zhang, Y., Agullo-Pascual, E., Caillol, G., Zhu, M.-S., Rothenberg, E., Melendez-Vasquez, C.V., Delmar, M., Leterrier, C., Salzer, J.L., 2018. Localized Myosin II Activity Regulates Assembly and Plasticity of the Axon Initial Segment. Neuron. doi:10.1016/j.neuron.2017.12.039

Black, M.M., Lasek, R.J., 1979. Axonal transport of actin: slow component b is the principal source of actin for the axon. Brain Res 171, 401–413.

Blanchoin, L., Boujemaa-Paterski, R., Sykes, C., Plastino, J., 2014. Actin dynamics, architecture, and mechanics in cell motility. Physiological Rev 94, 235–263. doi:10.1152/physrev.00018.2013

Bleckert, A., Photowala, H., 2012. Dual pools of actin at presynaptic terminals. J. Neurophysiol. doi:10.1152/jn.00789.2011.—We

Bloom, O., Evergren, E., Tomilin, N., Kjaerulff, O., Low, P., Brodin, L., Pieribone, V.A., Greengard, P., Shupliakov, O., 2003. Colocalization of synapsin and actin during synaptic vesicle recycling. J. Cell Biol. 161, 737–747. doi:10.1083/jcb.200212140

Bradke, F., Dotti, C.G., 1999. The role of local actin instability in axon formation. Science 283, 1931–1934.

Bray, D., Chapman, K., 1985. Analysis of microspike movements on the neuronal growth cone. J Neurosci 5, 3204–3213.

Burke, T.A., Christensen, J.R., Barone, E., Suarez, C., Sirotkin, V., Kovar, D.R., 2014. Homeostatic actin cytoskeleton networks are regulated by assembly factor competition for monomers. Curr. Biol 24, 579–585. doi:10.1016/j.cub.2014.01.072

Burkel, B.M., Dassow, von, G., Bement, W.M., 2007. Versatile fluorescent probes for actin filaments based on the actin-binding domain of utrophin. Cell Motil. Cytoskeleton 64, 822–832. doi:10.1002/cm.20226

Burnette, D.T., Ji, L., Schaefer, A.W., Medeiros, N.A., Danuser, G., Forscher, P., 2008. Myosin II activity facilitates microtubule bundling in the neuronal growth cone neck. Dev. Cell 15, 163–169. doi:10.1016/j.devcel.2008.05.016

Caceres, A., Ye, B., Dotti, C.G., 2012. Neuronal polarity: demarcation, growth and commitment. Curr. Opin. Cell Biol 24, 547–553. doi:10.1016/j.ceb.2012.05.011

Chakrabarty, N., Dubey, P., Tang, Y., Ganguly, A., Ladt, K., Leterrier, C., Jung, P., Roy, S., 2017. Processive movement of actin by biased polymerization: a new paradigm of axonal transport. biorXiv. doi:10.1101/212449

Chetta, J., Love, J.M., Bober, B.G., Shah, S.B., 2015. Bidirectional actin transport is influenced by microtubule and actin stability. Cell. Mol. Life Sci 72, 4205–4220. doi:10.1007/s00018-015-1933-z

Chia, J.X., Efimova, N., Svitkina, T.M., 2016. Neurite outgrowth is driven by actin polymerization even in the presence of actin polymerization inhibitors. Mol. Biol. Cell 27, 3695–3704. doi:10.1091/mbc.E16-04-0253

Cingolani, L.A., Goda, Y., 2008. Actin in action: the interplay between the actin cytoskeleton and synaptic efficacy. Nat. Rev. Neurosci 9, 344–356. doi:10.1038/nrn2373

Cole, A.A., Chen, X., Reese, T.S., 2016. A Network of Three Types of Filaments Organizes Synaptic Vesicles for Storage, Mobilization, and Docking. J Neurosci 36, 3222–3230. doi:10.1523/JNEUROSCI.2939-15.2016

Cole, J.C., Villa, B.R., Wilkinson, R.S., 2000. Disruption of actin impedes transmitter release in snake motor terminals. J. Physiol 525 Pt 3, 579–586.

Coles, C.H., Bradke, F., 2015. Coordinating Neuronal Actin-Microtubule Dynamics. Curr. Biol 25, R677–R691. doi:10.1016/j.cub.2015.06.020

Colicos, M.A., Collins, B.E., Sailor, M.J., Goda, Y., 2001. Remodeling of synaptic actin induced by photoconductive stimulation. Cell 107, 605–616.

D'Este, E., Kamin, D., Göttfert, F., El-Hady, A., Hell, S.W., 2015. STED nanoscopy reveals the ubiquity of subcortical cytoskeleton periodicity in living neurons. Cell Rep 10, 1246–1251. doi:10.1016/j.celrep.2015.02.007

D'Este, E., Kamin, D., Velte, C., Göttfert, F., Simons, M., Hell, S.W., 2016. Subcortical cytoskeleton periodicity throughout the nervous system. Sci Rep 6, 22741. doi:10.1038/srep22741

Darcy, K.J., Staras, K., Collinson, L.M., Goda, Y., 2006. Constitutive sharing of recycling synaptic vesicles between presynaptic boutons. Nat. Neurosci 9, 315–321. doi:10.1038/nn1640

Davidson, A.J., Wood, W., 2016. Unravelling the Actin Cytoskeleton: A New Competitive Edge? Trends Cell Biol 26, 569–576. doi:10.1016/j.tcb.2016.04.001

Dent, E.W., Callaway, J.L., Szebenyi, G., Baas, P.W., Kalil, K., 1999. Reorganization and movement of microtubules in axonal growth cones and developing interstitial branches. J Neurosci 19, 8894–8908.

Dent, E.W., Gupton, S.L., Gertler, F.B., 2011. The growth cone cytoskeleton in axon outgrowth and guidance. Cold Spring Harb Perspect Biol 3. doi:10.1101/cshperspect.a001800

Dent, E.W., Kalil, K., 2001. Axon branching requires interactions between dynamic microtubules and actin filaments. J Neurosci 21, 9757–9769.

Dent, E.W., Kwiatkowski, A., Mebane, L., Philippar, U., Barzik, M., Rubinson, D., Gupton, S., Van Veen, E., Furman, C., Zhang, J., Alberts, A., Mori, S., Gertler, F., 2007. Filopodia are required for cortical neurite initiation. Nat. Cell Biol 9, 1347–1359.

Difato, F., Tsushima, H., Pesce, M., Benfenati, F., Blau, A., Chieregatti, E., 2011. The formation of actin waves during regeneration after axonal lesion is enhanced by BDNF. Sci Rep 1, 183. doi:10.1038/srep00183

Dotti, C.G., Sullivan, C.A., Banker, G.A., 1988. The establishment of polarity by hippocampal neurons in culture. J Neurosci 8, 1454–1468.

Fan, A., Tofangchi, A., Kandel, M., Popescu, G., Saif, T., 2017. Coupled circumferential and axial tension driven by actin and myosin influences in vivo axon diameter. Sci Rep 7, 14188. doi:10.1038/s41598-017-13830-1

Fath, K.R., Lasek, R.J., 1988. Two classes of actin microfilaments are associated with the inner cytoskeleton of axons. J. Cell Biol. 107, 613–621.

Fifková, E., 1985. Actin in the nervous system. Brain Res 356, 187–215.

Fischer, M., Kaech, S., Knutti, D., Matus, A., 1998. Rapid actin-based plasticity in dendritic spines. Neuron 20, 847–854.

Flynn, K.C., Hellal, F., Neukirchen, D., Jacob, S., Tahirovic, S., Dupraz, S., Stern, S., Garvalov, B.K., Gurniak, C., Shaw, A.E., Meyn, L., Wedlich-Soldner, R., Bamburg, J.R., Small, J.V., Witke, W., Bradke, F., 2012. ADF/Cofilin-Mediated Actin Retrograde Flow Directs Neurite Formation in the Developing Brain. Neuron 76, 1091–1107. doi:10.1016/j.neuron.2012.09.038

Flynn, K.C., Pak, C.W., Shaw, A.E., Bradke, F., Bamburg, J.R., 2009. Growth cone-like waves transport actin and promote axonogenesis and neurite branching. Dev. Neurobiol 69, 761–779. doi:10.1002/dneu.20734

Forscher, P., Smith, S.J., 1988. Actions of cytochalasins on the organization of actin filaments and microtubules in a neuronal growth cone. J. Cell Biol. 107, 1505–1516.

Fowler, M.W., Staras, K., 2015. Synaptic vesicle pools: Principles, properties and limitations. Exp. Cell Res 335, 150–156. doi:10.1016/j.yexcr.2015.03.007

Gallo, G., 2013. More than one ring to bind them all: recent insights into the structure of the axon. Dev. Neurobiol 73, 799–805. doi:10.1002/dneu.22100

Gallo, G., 2011. The cytoskeletal and signaling mechanisms of axon collateral branching. Dev. Neurobiol 71, 201–220. doi:10.1002/dneu.20852

Gallo, G., Letourneau, P.C., 1999. Different contributions of microtubule dynamics and transport to the growth of axons and collateral sprouts. J Neurosci 19, 3860–3873.

Ganguly, A., Tang, Y., Wang, L., Ladt, K., Loi, J., Dargent, B., Leterrier, C., Roy, S., 2015. A dynamic formin-dependent deep F-actin network in axons. J. Cell Biol. 104, 20576–417. doi:10.1083/jcb.201506110

Garvalov, B.K., Flynn, K.C., Neukirchen, D., Meyn, L., Teusch, N., Wu, X., Brakebusch, C., Bamburg, J.R., Bradke, F., 2007. Cdc42 regulates cofilin during the establishment of neuronal polarity. J Neurosci 27, 13117–13129. doi:10.1523/JNEUROSCI.3322-07.2007

Gärtner, A., Fornasiero, E.F., Valtorta, F., Dotti, C.G., 2014. Distinct temporal hierarchies in membrane and cytoskeleton dynamics precede the morphological polarization of developing neurons. J. Cell. Sci. doi:10.1242/jcs.149815

Glebov, O.O., Jackson, R.E., Winterflood, C.M., Owen, D.M., Barker, E.A.,

Doherty, P., Ewers, H., Burrone, J., 2017. Nanoscale Structural Plasticity of the Active Zone Matrix Modulates Presynaptic Function. Cell Rep 18, 2715–2728. doi:10.1016/j.celrep.2017.02.064

Gramlich, M.W., Klyachko, V.A., 2017. Actin/Myosin-V- and Activity-Dependent Inter-synaptic Vesicle Exchange in Central Neurons. Cell Rep 18, 2096–2104. doi:10.1016/j.celrep.2017.02.010

Gray, E.G., 1959. Electron microscopy of synaptic contacts on dendrite spines of the cerebral cortex. Nature 183, 1592–1593.

Hammarlund, M., Jorgensen, E.M., Bastiani, M.J., 2007. Axons break in animals lacking beta-spectrin. J. Cell Biol. 176, 269–275. doi:10.1083/jcb.200611117

Han, B., Zhou, R., Xia, C., Zhuang, X., 2017. Structural organization of the actin-spectrin-based membrane skeleton in dendrites and soma of neurons. Proc. Natl. Acad. Sci. U.S.A 114, E6678–E6685. doi:10.1073/pnas.1705043114

Hand, R.A., Khalid, S., Tam, E., Kolodkin, A.L., 2015. Axon Dynamics during Neocortical Laminar Innervation. Cell Rep 12, 172–182. doi:10.1016/j.celrep.2015.06.026

He, J., Zhou, R., Wu, Z., Carrasco, M.A., Kurshan, P.T., Farley, J.E., Simon, D.J., Wang, G., Han, B., Hao, J., Heller, E., Freeman, M.R., Shen, K., Maniatis, T., Tessier-Lavigne, M., Zhuang, X., 2016. Prevalent presence of periodic actin-spectrin-based membrane skeleton in a broad range of neuronal cell types and animal species. Proc. Natl. Acad. Sci. U.S.A 113, 6029–6034. doi:10.1073/pnas.1605707113

Hirokawa, N., 1982. Cross-linker system between neurofilaments, microtubules, and membranous organelles in frog axons revealed by the quick-freeze, deep-etching method. J. Cell Biol. 94, 129–142.

Hirokawa, N., Sobue, K., Kanda, K., Harada, A., Yorifuji, H., 1989. The cytoskeletal architecture of the presynaptic terminal and molecular structure of synapsin 1. J. Cell Biol. 108, 111–126.

Hu, J., Bai, X., Bowen, J.R., Dolat, L., Korobova, F., Yu, W., Baas, P.W., Svitkina, T., Gallo, G., Spiliotis, E.T., 2012. Septin-driven coordination of actin and microtubule remodeling regulates the collateral branching of axons. Curr. Biol 22, 1109–1115. doi:10.1016/j.cub.2012.04.019

Huang, C.Y.-M., Zhang, C., Ho, T.S.-Y., Oses-Prieto, J., Burlingame, A.L., Lalonde, J., Noebels, J.L., Leterrier, C., Rasband, M.N., 2017a. αII Spectrin Forms a Periodic Cytoskeleton at the Axon Initial Segment and Is Required for Nervous System Function. J Neurosci 37, 11311–11322. doi:10.1523/JNEUROSCI.2112-17.2017

Huang, C.Y.-M., Zhang, C., Zollinger, D.R., Leterrier, C., Rasband, M.N., 2017b. An αII Spectrin-Based Cytoskeleton Protects Large-Diameter Myelinated Axons from Degeneration. J Neurosci 37, 11323–11334. doi:10.1523/JNEUROSCI.2113-17.2017

Jamann, N., Jordan, M., Engelhardt, M., 2018. Activity-dependent axonal plasticity in sensory systems. Neuroscience 368, 268–282. doi:10.1016/j.neuroscience.2017.07.035

Janssen, A.F.J., Tas, R.P., van Bergeijk, P., Oost, R., Hoogenraad, C.C., Kapitein, L.C., 2017. Myosin-V Induces Cargo Immobilization and Clustering at the Axon Initial Segment. Front Cell Neurosci 11, 89. doi:10.3389/fncel.2017.00260

Jähne, S., Rizzoli, S.O., Helm, M.S., 2015. The structure and function of presynaptic endosomes. Exp. Cell Res 335, 172–179. doi:10.1016/j.yexcr.2015.04.017

Job, C., Lagnado, L., 1998. Calcium and protein kinase C regulate the actin cytoskeleton in the synaptic terminal of retinal bipolar cells. J. Cell Biol. 143, 1661–1672.

Jones, S.L., Korobova, F., Svitkina, T., 2014. Axon initial segment cytoskeleton comprises a multiprotein submembranous coat containing sparse actin filaments. J. Cell Biol. 205, 67–81. doi:10.1083/jcb.201401045

Kalil, K., Dent, E.W., 2014. Branch management: mechanisms of axon branching in the developing vertebrate CNS. Nat. Rev. Neurosci 15, 7–18. doi:10.1038/nrn3650

Kapitein, L.C., Hoogenraad, C.C., 2015. Building the Neuronal Microtubule Cytoskeleton. Neuron 87, 492–506. doi:10.1016/j.neuron.2015.05.046

Katsuno, H., Sakumura, Y., 2015. Actin Migration Driven by Directional Assembly and Disassembly of Membrane-Anchored Actin Filaments. Cell Rep 12, 648–660. doi:10.1016/j.celrep.2015.06.048

Ketschek, A., Gallo, G., 2010. Nerve growth factor induces axonal filopodia through localized microdomains of phosphoinositide 3-kinase activity that drive the formation of cytoskeletal precursors to filopodia. J Neurosci 30, 12185–12197. doi:10.1523/JNEUROSCI.1740-10.2010

Ketschek, A., Jones, S., Spillane, M., Korobova, F., Svitkina, T., Gallo, G., 2015. Nerve growth factor promotes reorganization of the axonal microtubule array at sites of axon collateral branching. Dev. Neurobiol. doi:10.1002/dneu.22294

Ketschek, A., Spillane, M., Dun, X.-P., Hardy, H., Chilton, J., Gallo, G., 2016. Drebrin coordinates the actin and microtubule cytoskeleton during the initiation of axon collateral branches. Dev. Neurobiol 76, 1092–1110. doi:10.1002/dneu.22377

Kevenaar, J.T., Hoogenraad, C.C., 2015. The axonal cytoskeleton: from organization to function. Front Mol Neurosci 8, 44. doi:10.3389/fnmol.2015.00044

Kirschner, M.W., 1980. Implications of treadmilling for the stability and polarity of actin and tubulin polymers in vivo. J. Cell Biol. 86, 330–334.

Kneussel, M., Wagner, W., 2013. Myosin motors at neuronal synapses: drivers of membrane transport and actin dynamics. Nat. Rev. Neurosci 14, 233–247. doi:10.1038/nrn3445

Kononenko, N.L., Haucke, V., 2015. Molecular mechanisms of presynaptic membrane retrieval and synaptic vesicle reformation. Neuron 85, 484–496. doi:10.1016/j.neuron.2014.12.016

Korobova, F., Svitkina, T., 2010. Molecular architecture of synaptic actin cytoskeleton in hippocampal neurons reveals a mechanism of dendritic spine morphogenesis. Mol. Biol. Cell. doi:10.1091/mbc.E09

Korobova, F., Svitkina, T., 2008. Arp2/3 complex is important for filopodia formation, growth cone motility, and neuritogenesis in neuronal cells. Mol. Biol. Cell 19, 1561–1574. doi:10.1091/mbc.E07-09-0964

Krieg, M., Dunn, A.R., Goodman, M.B., 2014. Mechanical control of the sense of touch by β-spectrin. Nat. Cell Biol 16, 224–233. doi:10.1038/ncb2915

Kuczmarski, E.R., Rosenbaum, J.L., 1979. Studies on the organization and localization of actin and myosin in neurons. J. Cell Biol. 80, 356–371.

Lai, L., Cao, J., 2014. Spectrins in axonal cytoskeletons: Dynamics revealed by extensions and fluctuations. J Chem Phys 141, 015101. doi:10.1063/1.4885720

Landis, D.M., Hall, A.K., Weinstein, L.A., Reese, T.S., 1988. The organization of cytoplasm at the presynaptic active zone of a central nervous system synapse. Neuron 1, 201–209.

Lee, J.S., Ho, W.-K., Lee, S.-H., 2012. Actin-dependent rapid recruitment of reluctant synaptic vesicles into a fast-releasing vesicle pool. Proc. Natl. Acad. Sci. U.S.A 109, E765–74. doi:10.1073/pnas.1114072109

Leite, S.C., Sampaio, P., Sousa, V.F., Nogueira-Rodrigues, J., Pinto-Costa, R., Peters, L.L., Brites, P., Sousa, M.M., 2016. The Actin-Binding Protein α-Adducin Is Required for Maintaining Axon Diameter. Cell Rep 15, 490–498. doi:10.1016/j.celrep.2016.03.047

Leite, S.C., Sousa, M.M., 2016. The neuronal and actin commitment: Why do neurons need rings? Cytoskeleton (Hoboken) 73, 424–434. doi:10.1002/cm.21273

Leterrier, C., 2018. The axon initial segment: an updated viewpoint. J Neurosci 1922–17. doi:10.1523/JNEUROSCI.1922-17.2018

Leterrier, C., 2016. The Axon Initial Segment, 50 Years Later: A Nexus for Neuronal Organization and Function. Curr Top Membr 77, 185–233. doi:10.1016/bs.ctm.2015.10.005

Leterrier, C., Dubey, P., Roy, S., 2017. The nano-architecture of the axonal cytoskeleton. Nat. Rev. Neurosci 18, 713–726. doi:10.1038/nrn.2017.129

Leterrier, C., Potier, J., Caillol, G., Debarnot, C., Rueda-Boroni, F., Dargent, B., 2015. Nanoscale Architecture of the Axon Initial Segment Reveals an Organized and Robust Scaffold. Cell Rep 13, 2781–2793. doi:10.1016/j.celrep.2015.11.051

Letourneau, P.C., 2009. Actin in axons: stable scaffolds and dynamic filaments. Results Probl Cell Differ 48, 65–90. doi:10.1007/400_2009_15

Letourneau, P.C., 1983. Differences in the organization of actin in the growth cones compared with the neurites of cultured neurons from chick embryos. J. Cell Biol. 97, 963–973.

Lewis, T.L., Courchet, J., Polleux, F., 2013. Cell biology in neuroscience: Cellular and molecular mechanisms underlying axon formation, growth, and branching. J. Cell Biol. 202, 837–848. doi:10.1083/jcb.201305098

Li, Y.-C., Bai, W.-Z., Zhou, L., Sun, L.-K., Hashikawa, T., 2010. Nonhomogeneous distribution of filamentous actin in the presynaptic terminals on the spinal motoneurons. J. Comp. Neurol. 518, 3184–3192. doi:10.1002/cne.22374

Lin, C.H., Forscher, P., 1995. Growth cone advance is inversely proportional to retrograde F-actin flow. Neuron 14, 763–771.

Lomakin, A.J., Lee, K.-C., Han, S.J., Bui, D.A., Davidson, M.W., Mogilner, A., Danuser, G., 2015. Competition for actin between two distinct F-actin networks defines a bistable switch for cell polarization. Nat. Cell Biol 17, 1435–1445. doi:10.1038/ncb3246

Loudon, R.P., Silver, L.D., Yee, H.F., Gallo, G., 2006. RhoA-kinase and myosin II are required for the maintenance of growth cone polarity and guidance by nerve growth factor. J. Neurobiol 66, 847–867. doi:10.1002/neu.20258

Lowery, L.A., Van Vactor, D., 2009. The trip of the tip: understanding the growth cone machinery. Nat. Rev. Mol. Cell Biol 10, 332–343. doi:10.1038/nrm2679

Lu, W., Fox, P., Lakonishok, M., Davidson, M.W., Gelfand, V.I., 2013. Initial Neurite Outgrowth in Drosophila Neurons Is Driven by Kinesin-Powered Microtubule Sliding. Curr. Biol. doi:10.1016/j.cub.2013.04.050

Lux, S.E., 2016. Anatomy of the red cell membrane skeleton: unanswered questions. Blood 127, 187–199. doi:10.1182/blood-2014-12-512772

Maglione, M., Sigrist, S.J., 2013. Seeing the forest tree by tree: super-resolution light microscopy meets the neurosciences. Nat. Neurosci 16, 790–797. doi:10.1038/nn.3403

Medeiros, N.A., Burnette, D.T., Forscher, P., 2006. Myosin II functions in actin-bundle turnover in neuronal growth cones. Nat. Cell Biol 8, 215–226. doi:10.1038/ncb1367

Melak, M., Plessner, M., Grosse, R., 2017. Actin visualization at a glance. J. Cell. Sci 130, 525–530. doi:10.1242/jcs.189068

Menon, S., Gupton, S.L., 2016. Building Blocks of Functioning Brain: Cytoskeletal Dynamics in Neuronal Development. Int. Rev. Cell. Mol. Biol 322, 183–245. doi:10.1016/bs.ircmb.2015.10.002

Miki, T., Malagon, G., Pulido, C., Llano, I., Neher, E., Marty, A., 2016. Actin- and Myosin-Dependent Vesicle Loading of Presynaptic Docking Sites Prior to Exocytosis. Neuron 91, 808–823. doi:10.1016/j.neuron.2016.07.033

Morales, M., Colicos, M.A., Goda, Y., 2000. Actin-dependent regulation of neurotransmitter release at central synapses. Neuron 27, 539–550.

Mori, H., Kurokawa, M., 1981. Intra-axonal transport of clathrin and actin. Biomed. Res. 2, 677–685. doi:10.2220/biomedres.2.677

Mortal, S., Iseppon, F., Perissinotto, A., D'Este, E., Cojoc, D., Napolitano, L.M.R., Torre, V., 2017. Actin Waves Do Not Boost Neurite Outgrowth in the Early Stages of Neuron Maturation. Front Cell Neurosci 11, 402. doi:10.3389/fncel.2017.00402

Nagele, R.G., Kosciuk, M.C., Hunter, E.T., Bush, K.T., Lee, H., 1988. Immunoelectron microscopic localization of actin in neurites of cultured embryonic chick dorsal root ganglia: actin is a component of granular, microtubule-associated crossbridges. Brain Res 474, 279–286.

Nakada, C., Ritchie, K., Oba, Y., Nakamura, M., Hotta, Y., Iino, R., Kasai, R., Yamaguchi, K., Fujiwara, T.K., Kusumi, A., 2003. Accumulation of anchored proteins forms membrane diffusion barriers during neuronal polarization. Nat. Cell Biol 5, 626–632.

Namba, T., Funahashi, Y., Nakamuta, S., Xu, C., Takano, T., Kaibuchi, K., 2015. Extracellular and Intracellular Signaling for Neuronal Polarity. Physiological Rev 95, 995–1024. doi:10.1152/physrev.00025.2014

Nirschl, J.J., Ghiretti, A.E., Holzbaur, E.L.F., 2017. The impact of cytoskeletal organization on the local regulation of neuronal transport. Nat. Rev. Neurosci 18, 585–597. doi:10.1038/nrn.2017.100

Nishimura, T., Yamaguchi, T., Kato, K., Yoshizawa, M., Nabeshima, Y.-I., Ohno, S., Hoshino, M., Kaibuchi, K., 2005. PAR-6–PAR-3 mediates Cdc42-induced Rac activation through the Rac GEFs STEF/Tiam1. Nat. Cell Biol 7, 270–277.

Nozumi, M., Igarashi, M., 2017. Vesicular movements in the growth cone. Neurochem. Int. doi:10.1016/j.neuint.2017.09.011

O'Connor, T.P., Bentley, D., 1993. Accumulation of actin in subsets of pioneer growth cone filopodia in response to neural and epithelial guidance cues in situ. J. Cell Biol. 123, 935–948.

Omotade, O.F., Pollitt, S.L., Zheng, J.Q., 2017. Actin-based growth cone motility and guidance. Mol. Cell. Neurosci 84, 4–10. doi:10.1016/j.mcn.2017.03.001

Owe, S.G., Jensen, V., Evergren, E., Ruiz, A., Shupliakov, O., Kullmann, D.M., Storm-Mathisen, J., Walaas, S.I., Hvalby, Ø., Bergersen, L.H., 2009. Synapsin- and actin-dependent frequency enhancement in mouse hippocampal mossy fiber synapses. Cereb. Cortex 19, 511–523. doi:10.1093/cercor/bhn101

Palay, S.L., 1956. Synapses in the central nervous system. J Biophys Biochem Cytol 2, 193–202.

Pan, L., Yan, R., Li, W., Xu, K., 2018. Super-Resolution Microscopy Reveals the Native Ultrastructure of the Erythrocyte Cytoskeleton. Cell Rep 22, 1151–1158. doi:10.1016/j.celrep.2017.12.107

Patel, S., Fok, S.Y.Y., Stefen, H., Tomanić, T., Parić, E., Herold, R., Brettle, M., Djordjevic, A., Fath, T., 2017. Functional characterisation of filamentous actin probe expression in neuronal cells. PLoS ONE 12, e0187979. doi:10.1371/journal.pone.0187979

Pechstein, A., Shupliakov, O., 2010. Taking a back seat: synaptic vesicle clustering in presynaptic terminals. Front Synaptic Neurosci 2, 143. doi:10.3389/fnsyn.2010.00143

Peng, A., Rotman, Z., Deng, P.-Y., Klyachko, V.A., 2012. Differential motion dynamics of synaptic vesicles undergoing spontaneous and activity-evoked endocytosis. Neuron 73, 1108–1115. doi:10.1016/j.neuron.2012.01.023

Prokop, A., 2013. The intricate relationship between microtubules and their associated motor proteins during axon growth and maintenance. Neural Dev 8, 17. doi:10.1186/1749-8104-8-17

Qu, Y., Hahn, I., Webb, S.E.D., Prokop, A., 2016. Periodic actin structures in neuronal axons are required to maintain microtubules. Mol. Biol. Cell 28, 296–308. doi:10.1091/mbc.e16-10-0727

Rasband, M.N., 2013. Cytoskeleton: axons earn their stripes. Curr. Biol 23, R197–8. doi:10.1016/j.cub.2013.01.050

Rasband, M.N., 2010. The axon initial segment and the maintenance of neuronal polarity. Nat. Rev. Neurosci 11, 552–562. doi:10.1038/nrn2852

Roy, S., 2016. Waves, rings, and trails: The scenic landscape of axonal actin. J. Cell Biol. 212, 131–134. doi:10.1083/jcb.201511016

Rust, M.B., Maritzen, T., 2015. Relevance of presynaptic actin dynamics for synapse function and mouse behavior. Exp. Cell Res 335, 165–171. doi:10.1016/j.yexcr.2014.12.020

Ruthel, G., Banker, G.A., 1999. Role of moving growth cone-like "wave" structures in the outgrowth of cultured hippocampal axons and dendrites. J. Neurobiol 39, 97–106. doi:10.1002/(SICI)1097-4695(199904)39:13.0.CO;2-Z

Ruthel, G., Banker, G.A., 1998. Actin-dependent anterograde movement of growth-cone-like structures along growing hippocampal axons: a novel form of axonal transport? Cell Motil. Cytoskeleton 40, 160–173. doi:10.1002/(SICI)1097-0169(1998)40:2<160::AID-CM5>3.0.CO;2-J

Ruthel, G., Hollenbeck, P.J., 2000. Growth cones are not required for initial establishment of polarity or differential axon branch growth in cultured hippocampal neurons. J Neurosci 20, 2266–2274.

Sakaba, T., Neher, E., 2003. Involvement of actin polymerization in vesicle recruitment at the calyx of Held synapse. J Neurosci 23, 837–846.

Sankaranarayanan, S., Atluri, P.P., Ryan, T.A., 2003. Actin has a molecular

scaffolding, not propulsive, role in presynaptic function. Nat. Neurosci 6, 127–135. doi:10.1038/nn1002

Schaefer, A.W., Kabir, N., Forscher, P., 2002. Filopodia and actin arcs guide the assembly and transport of two populations of microtubules with unique dynamic parameters in neuronal growth cones. J. Cell Biol. 158, 139–152. doi:10.1083/jcb.200203038

Schaefer, A.W., Schoonderwoert, V.T.G., Ji, L., Mederios, N., Danuser, G., Forscher, P., 2008. Coordination of actin filament and microtubule dynamics during neurite outgrowth. Dev. Cell 15, 146–162. doi:10.1016/j.devcel.2008.05.003

Schelski, M., Bradke, F., 2017. Neuronal polarization: From spatiotemporal signaling to cytoskeletal dynamics. Mol. Cell. Neurosci 84, 11–28. doi:10.1016/j.mcn.2017.03.008

Sidenstein, S.C., D'Este, E., Böhm, M.J., Danzl, J.G., Belov, V.N., Hell, S.W., 2016. Multicolour Multilevel STED nanoscopy of Actin/Spectrin Organization at Synapses. Sci Rep 6, 26725. doi:10.1038/srep26725

Siksou, L., Rostaing, P., Lechaire, J.-P., Boudier, T., Ohtsuka, T., Fejtova, A., Kao, H.-T., Greengard, P., Gundelfinger, E.D., Triller, A., Marty, S., 2007. Three-dimensional architecture of presynaptic terminal cytomatrix. J Neurosci 27, 6868–6877. doi:10.1523/JNEUROSCI.1773-07.2007

Siksou, L., Triller, A., Marty, S., 2009. An emerging view of presynaptic structure from electron microscopic studies. J. Neurochem 108, 1336–1342. doi:10.1111/j.1471-4159.2009.05888.x

Sood, P., Murthy, K., Kumar, T.V., Nonet, M.L., Menon, G.I., Koushika, S.P., 2017. Cargo crowding at actin-rich regions along axons causes local traffic jams. Traffic. doi:10.1111/tra.12544

Soykan, T., Kaempf, N., Sakaba, T., Vollweiter, D., Goerdeler, F., Puchkov, D., Kononenko, N.L., Haucke, V., 2017. Synaptic Vesicle Endocytosis Occurs on Multiple Timescales and Is Mediated by Formin-Dependent Actin Assembly. Neuron 93, 854–866.e4. doi:10.1016/j.neuron.2017.02.011

Spillane, M., Ketschek, A., Jones, S.L., Korobova, F., Marsick, B., Lanier, L., Svitkina, T., Gallo, G., 2011. The actin nucleating Arp2/3 complex contributes to the formation of axonal filopodia and branches through the regulation of actin patch precursors to filopodia. Dev. Neurobiol 71, 747–758. doi:10.1002/dneu.20907

Spooner, B.S., Holladay, C.R., 1981. Distribution of tubulin and actin in neurites and growth cones of differentiating nerve cells. Cell Motil. 1, 167–178.

Staras, K., Branco, T., Burden, J.J., Pozo, K., Darcy, K., Marra, V., Ratnayaka, A., Goda, Y., 2010. A vesicle superpool spans multiple presynaptic terminals in hippocampal neurons. Neuron 66, 37–44. doi:10.1016/j.neuron.2010.03.020

Suter, D.M., Forscher, P., 2000. Substrate-cytoskeletal coupling as a mechanism for the regulation of growth cone motility and guidance. J. Neurobiol 44, 97–113.

Tint, I., Jean, D., Baas, P.W., Black, M.M., 2009. Doublecortin associates with microtubules preferentially in regions of the axon displaying actin-rich protrusive structures. J Neurosci 29, 10995–11010. doi:10.1523/JNEUROSCI.3399-09.2009

Tomba, C., Braïni, C., Bugnicourt, G., Cohen, F., Friedrich, B.M., Gov, N.S., Villard, C., 2017. Geometrical Determinants of Neuronal Actin Waves. Front Cell Neurosci 11, 86. doi:10.3389/fncel.2017.00086

Tsukita, S., Kobayashi, T., Matsumoto, G., 1986. Subaxolemmal cytoskeleton in squid giant axon. II. Morphological identification of microtubule- and microfilament-associated domains of axolemma. J. Cell Biol. 102, 1710–1725.

Watanabe, K., Al-Bassam, S., Miyazaki, Y., Wandless, T.J., Webster, P., Arnold, D.B., 2012. Networks of polarized actin filaments in the axon initial segment provide a mechanism for sorting axonal and dendritic proteins. Cell Rep 2, 1546–1553. doi:10.1016/j.celrep.2012.11.015

Watanabe, S., Rost, B.R., Camacho-Pérez, M., Davis, M.W., Söhl-Kielczynski, B., Rosenmund, C., Jorgensen, E.M., 2013. Ultrafast endocytosis at mouse hippocampal synapses. Nature 504, 242–247. doi:10.1038/nature12809

Wessells, N.K., Spooner, B.S., Ash, J.F., Bradley, M.O., Luduena, M.A., Taylor, E.L., Wrenn, J.T., Yamada, K., 1971. Microfilaments in cellular and developmental processes. Science 171, 135–143.

Wilhelm, B.G., Mandad, S., Truckenbrodt, S., Kröhnert, K., Schäfer, C., Rammner, B., Koo, S.J., Claßen, G.A., Krauss, M., Haucke, V., Urlaub, H., Rizzoli, S.O., 2014. Composition of isolated synaptic boutons reveals the amounts of vesicle trafficking proteins. Science 344, 1023–1028. doi:10.1126/science.1252884

Willard, M., Wiseman, M., Levine, J., Skene, P., 1979. Axonal transport of actin in rabbit retinal ganglion cells. J. Cell Biol. 81, 581–591.

Winans, A.M., Collins, S.R., Meyer, T., 2016. Waves of actin and microtubule polymerization drive microtubule-based transport and neurite growth before single axon formation. Elife 5, e12387. doi:10.7554/eLife.12387

Winckler, B., Forscher, P., Mellman, I., 1999. A diffusion barrier maintains distribution of membrane proteins in polarized neurons. Nature 397, 698–701.

Witte, H., Bradke, F., 2008. The role of the cytoskeleton during neuronal polarization. Curr. Opin. Neurobiol 18, 479–487.

Witte, H., Neukirchen, D., Bradke, F., 2008. Microtubule stabilization specifies initial neuronal polarization. J. Cell Biol. 180, 619–632.

Wu, X.-S., Lee, S.H., Sheng, J., Zhang, Z., Zhao, W.-D., Wang, D., Jin, Y., Charnay, P., Ervasti, J.M., Wu, L.-G., 2016. Actin Is Crucial for All Kinetically Distinguishable Forms of Endocytosis at Synapses. Neuron 92, 1020–1035. doi:10.1016/j.neuron.2016.10.014

Xu, K., Zhong, G., Zhuang, X., 2013. Actin, spectrin, and associated proteins form a periodic cytoskeletal structure in axons. Science 339, 452–456. doi:10.1126/science.1232251

Yamada, K.M., Spooner, B.S., Wessells, N.K., 1971. Ultrastructure and function of growth cones and axons of cultured nerve cells. J. Cell Biol. 49, 614–635.

Yamada, K.M., Spooner, B.S., Wessells, N.K., 1970. Axon growth: roles of microfilaments and microtubules. Proc. Natl. Acad. Sci. U.S.A 66, 1206–1212.

Yamamoto, H., Demura, T., Morita, M., Banker, G.A., Tanii, T., Nakamura, S., 2012. Differential neurite outgrowth is required for axon specification by cultured hippocampal neurons. J. Neurochem 123, 904–910. doi:10.1111/jnc.12001

Zhang, C., Rasband, M.N., 2016. Cytoskeletal control of axon domain assembly and function. Curr. Opin. Neurobiol 39, 116–121. doi:10.1016/j.conb.2016.05.001

Zhang, S.-X., Duan, L.-H., Qian, H., Yu, X., 2016. Actin Aggregations Mark the Sites of Neurite Initiation. Neurosci Bull. doi:10.1007/s12264-016-0012-2

Zhang, Y., Abiraman, K., Li, H., Pierce, D.M., Tzingounis, A.V., Lykotrafitis, G., 2017. Modeling of the axon membrane skeleton structure and implications for its mechanical properties. PLoS Comput. Biol. 13, e1005407. doi:10.1371/journal.pcbi.1005407

Zhao, B., Meka, D.P., Scharrenberg, R., König, T., Schwanke, B., Kobler, O., Windhorst, S., Kreutz, M.R., Mikhaylova, M., Calderon de Anda, F., 2017. Microtubules Modulate F-actin Dynamics during Neuronal Polarization. Sci Rep 7, 9583. doi:10.1038/s41598-017-09832-8

Zhong, G., He, J., Zhou, R., Lorenzo, D., Babcock, H.P., Bennett, V., Zhuang, X., 2014. Developmental mechanism of the periodic membrane skeleton in axons. Elife 3, 194. doi:10.7554/eLife.04581

Zmuda, J., Rivas, R., 2000. Actin filament disruption blocks cerebellar granule neurons at the unipolar stage of differentiation in vitro. J. Neurobiol 43, 313–328.