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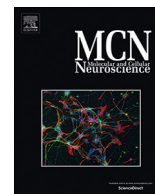
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The functional architecture of axonal actin

Marie-Jeanne Papandréou, Christophe Leterrier*

Aix Marseille Université, CNRS, INP UMR7051, 13344 cedex 15, Marseille, France



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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.

1. Introduction

The cytoskeleton allows cells to establish, maintain and transform their shape. In neurons, these 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, hotspots 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).

2. 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 fluorescence 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

* Corresponding author at: INP CNRS-AMU UMR 7051, Bd Pierre Dramard, 13344 Marseille cedex 15, France.
E-mail address: christophe.leterrier@univ-amu.fr (C. Leterrier).

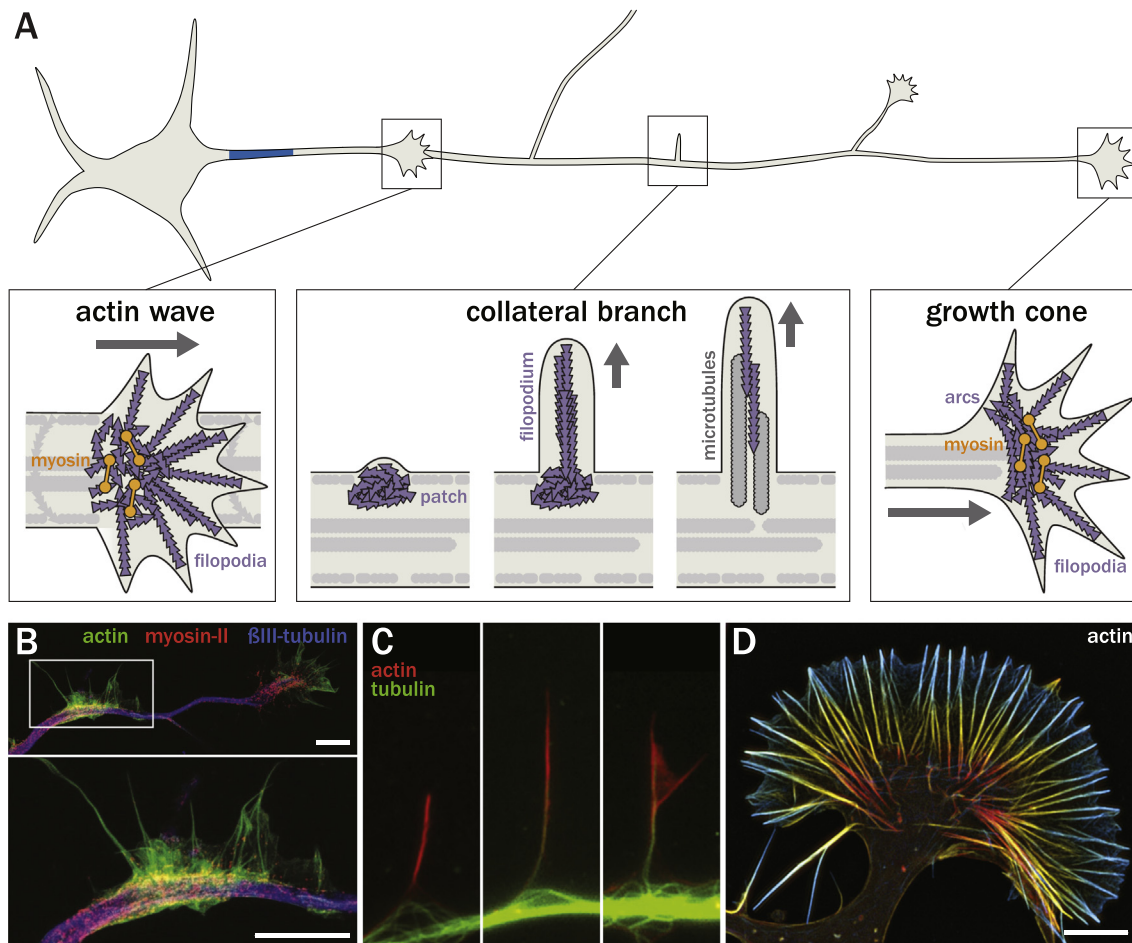


Fig. 1. Actin structures in the developing axon.

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 (β III-tubulin, blue). Adapted from (Mortal et al., 2017). Scale bars, 5 μ m. C. Three stages of collateral branch formation: the initial formation of an actin filopodia (red, left) and subsequent entry of microtubules (green, middle) and appearance of an actin rich growth cone at the new tip (red, right). Individual images of different neurons, adapted from (Pacheco and Gallo, 2016). 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 μ m. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

subject (Caceres et al., 2012; Omotade et al., 2017; Schelski and Bradke, 2017; Witte and Bradke, 2008) (Fig. 1).

2.1. 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; 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; 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). These contrasted results suggest that a balance between actin polymerization

and disassembly is required for neurite extension, and that actin structures with distinct sensitivities to various drug treatments and doses could play different roles in this regard. 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 local dynamics of actin and concurrent stabilization of microtubules within the nascent axon (Bradke and Dotti, 1999; Witte et al., 2008; Zhao et al., 2017). Intricate pathways orchestrate the cytoskeletal remodeling during axon specification, and we refer to interested reader to recent reviews on this (Namba et al., 2015; Schelski and Bradke, 2017).

2.2. 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 (Lowery and Van Vactor, 2009) (Fig. 1A). 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;

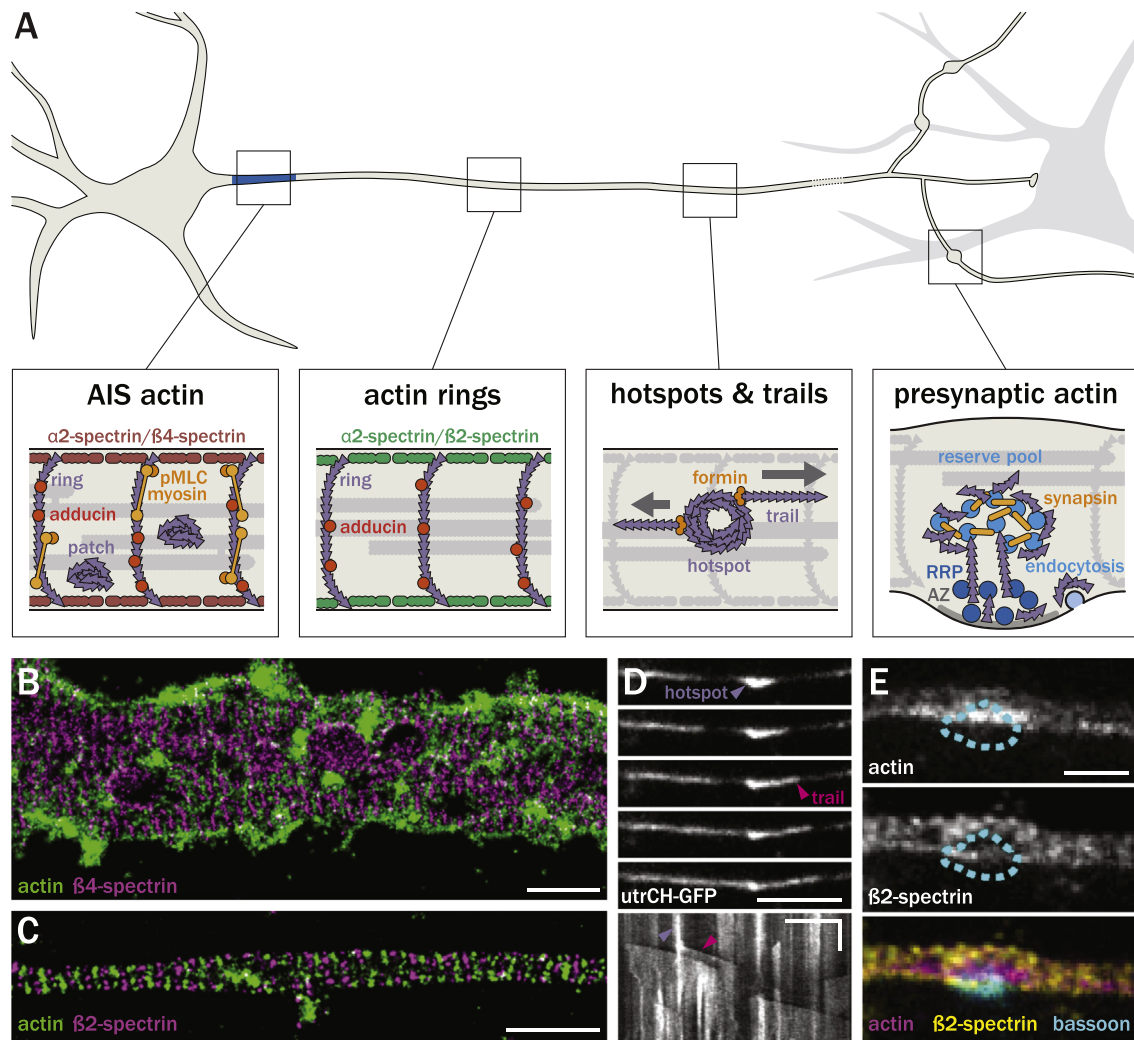


Fig. 2. Actin structures in the mature axon.

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 $\alpha 2$ - and $\beta 4$ -spectrin (maroon). Actin ring filaments are capped by adducin (red) and associated with phospho-myosin light chain (pMLC) and myosin that may organize along rings (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 $\alpha 2$ / $\beta 2$ -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). Note that protein lengths and axonal dimensions are not drawn to scale in these diagrams. **B.** STORM image of an AIS labeled for actin (green) and $\beta 4$ -spectrin (magenta). Adapted from (Leterrier et al., 2015). Scale bar, 1 μ m. **C.** STORM image of a distal axon labeled for actin (green) and the carboxyterminus of $\beta 2$ -spectrin (magenta). Adapted from (Xu et al., 2013). Scale bar, 1 μ m. **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 μ m. 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 μ m (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), $\beta 2$ -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 μ m. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Omotade et al., 2017). Actin shapes the tip of the growth cone, with a peripheral lamellipodium from which dynamic filopodia emerge (Bray and Chapman, 1985; Korobova and Svitkina, 2008) (Fig. 1D). 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 treadmill 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).

2.3. Actin waves along developing axons

In neurons developing in culture, actin-based waves similar to growth cones traveling at $\sim 3 \mu$ m/min along the neurites and nascent axon have been described (Ruthel and Banker, 1998) (Fig. 1A & 1B). 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 et al., 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 and contain myosin-II and myosin-Ib (Iuliano et al., 2018; Mortal et al., 2017), 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).

2.4. 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 $\sim 1\text{--}2\ \mu\text{m}$ actin patches along the axon shaft (Ketschek and Gallo, 2010; Loudon et al., 2006; Spillane et al., 2011) (Fig. 1A). 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 (Ketschek et al., 2015) (Fig. 1C). 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).

3. 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 (Leterrier et al., 2017) (Fig. 2).

3.1. Actin rings

In 2013, Xu et al. used Stochastic Optical Reconstruction Microscopy (STORM), a super-resolution microscopy technique that resolves details down to $\sim 20\ \text{nm}$ (instead of $\sim 200\ \text{nm}$ for classical epifluorescence, (Maglione and Sgrist, 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 $\sim 190\ \text{nm}$ (Xu et al., 2013) (Fig. 2A). This spacing value corresponds to the length of individual, extended spectrin tetramers (Bennett et al., 1982; Brown et al., 2015): spectrin appear as periodic stripes along the axon when labeling the axonal $\beta 2$ -spectrin and $\alpha 2$ -spectrin, or the AIS-specific $\beta 4$ -spectrin (Huang et al., 2017a, 2017b; Leterrier et al., 2015; Xu et al., 2013) (Fig. 2B & 2C). 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 aminoterminal of the β -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 mono-dimensional 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 $\sim 30\ \text{min}$, as shown by live-cell PhotoActivation Localization Microscopy (PALM) of photoactivable $\beta 2$ -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 stable actin rings could 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 β -spectrin progressively break when the animal is moving (Hammarlund et al., 2007). At rest, spectrin tetramers are $\sim 50\text{--}70\ \text{nm}$ long, whereas in axons their $\sim 180\ \text{nm}$ indicates that they are fully extended, and thus under tension like extended springs (Brown et al., 2015). FRET sensor probes inserted in β -spectrin show that indeed, spectrins are extended and under tension along axons (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 tension of extended 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).

3.2. 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, 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 $\sim 1 \mu\text{m}/\text{s}$, called actin trails (Ganguly et al., 2015) (Fig. 2D). Super-resolution microscopy corroborates the presence of actin clusters and longitudinal filaments along axons (Ganguly et al., 2015; Unsain et al., 2018). In addition to this characterization in cultured neurons, hotspots and trails were recently observed *in vivo* in *C. Elegans* axons (Sood et al., 2018). 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., 2018). 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 collapse of trails and their bias for the anterograde direction (55% against 45% retrograde) may result 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).

3.3. 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 $\alpha 2$ - and $\beta 4$ -spectrin subunits (Huang et al., 2017a) (Fig. 2B). In the middle of the spectrin tetramer between actin rings, $\beta 4$ -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; 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; Lewis et al., 2009). 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; Watanabe et al., 2012). This immobilization likely precedes retrograde transport to the cell body via dynein, a mechanism allowing to reroute mistargeted somatodendritic cargoes before they reach the distal axon (Franker et al., 2016; Leterrier, 2018; Nirschl et al., 2017).

4. Actin at presynapses

4.1. 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 (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 (He et al., 2016; Sidenstein et al., 2016) (Fig. 2E). 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).

4.2. 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, drugs that perturb actin organization can lead to enhanced or impaired synaptic vesicle release (Cingolani and Goda, 2008; Rust and Maritzen, 2015). Depolymerizing drugs such as la-trunculin or cytochalasin have been shown to enhance release (Morales et al., 2000; Sankaranarayanan et al., 2003), reduce it (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 corraling 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), with the participation of a formin/Rho peripheral actin scaffold (Deguchi et al., 2016). 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.

5. Conclusion

Thanks to the recent discovery of rings, hotspots and trails, axonal actin is back in the spotlight. These are exciting 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.

Declarations of interest

None.

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