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Neurons rely on a unique organization of their cytoskeleton to build, maintain and transform their extraordinarily intricate shapes. After decades of research on the neuronal cytoskeleton, it is exciting that novel assemblies are still discovered thanks to progress in cellular imaging methods. Indeed, super-resolution microscopy has revealed that axons are lined with a periodic scaffold of actin rings, spaced every 190 nm by spectrins. Determining the architecture, composition, dynamics, and functions of this membrane-associated periodic scaffold is a current conceptual and technical challenge, as well as a very active area of research. This short review aims at summarizing the latest research on the axonal periodic scaffold, highlighting recent progress and open questions.

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Introduction

Axons are the long cellular extensions that connect neurons and form nerve fibers. By a conservative estimate, neuronal information runs through ~160 000 km of axons in our brain alone, not counting peripheral axons that can individually reach a length of one meter. Development of this elaborate circuitry and maintenance during a whole lifetime is a daunting task, as evidenced by the ~40% drop in total axon length at age 80 [1]. The unique morphology and functions of axons rely on specific cytoskeletal assemblies of microtubules, intermediate filaments and actin [2–4]. Among these, the organization of actin along the axon shaft has long been overlooked, as it was unremarkably uniform when seen by optical microscopy, and difficult to visualize by electron microscopy [5]. Only the advent of super-resolution optical microscopy could resolve the unique actin structures along axons, starting with the discovery of the submembrane scaffold of actin rings periodically spaced by spectrins [6]. In this short review, I will summarize recent research about the architecture and functions of this membrane-associated periodic scaffold (MPS), as well as highlight open questions: its mechanism of formation, its relationship with other cytoskeletal assemblies along axons, and how progress in cellular imaging can tackle them.

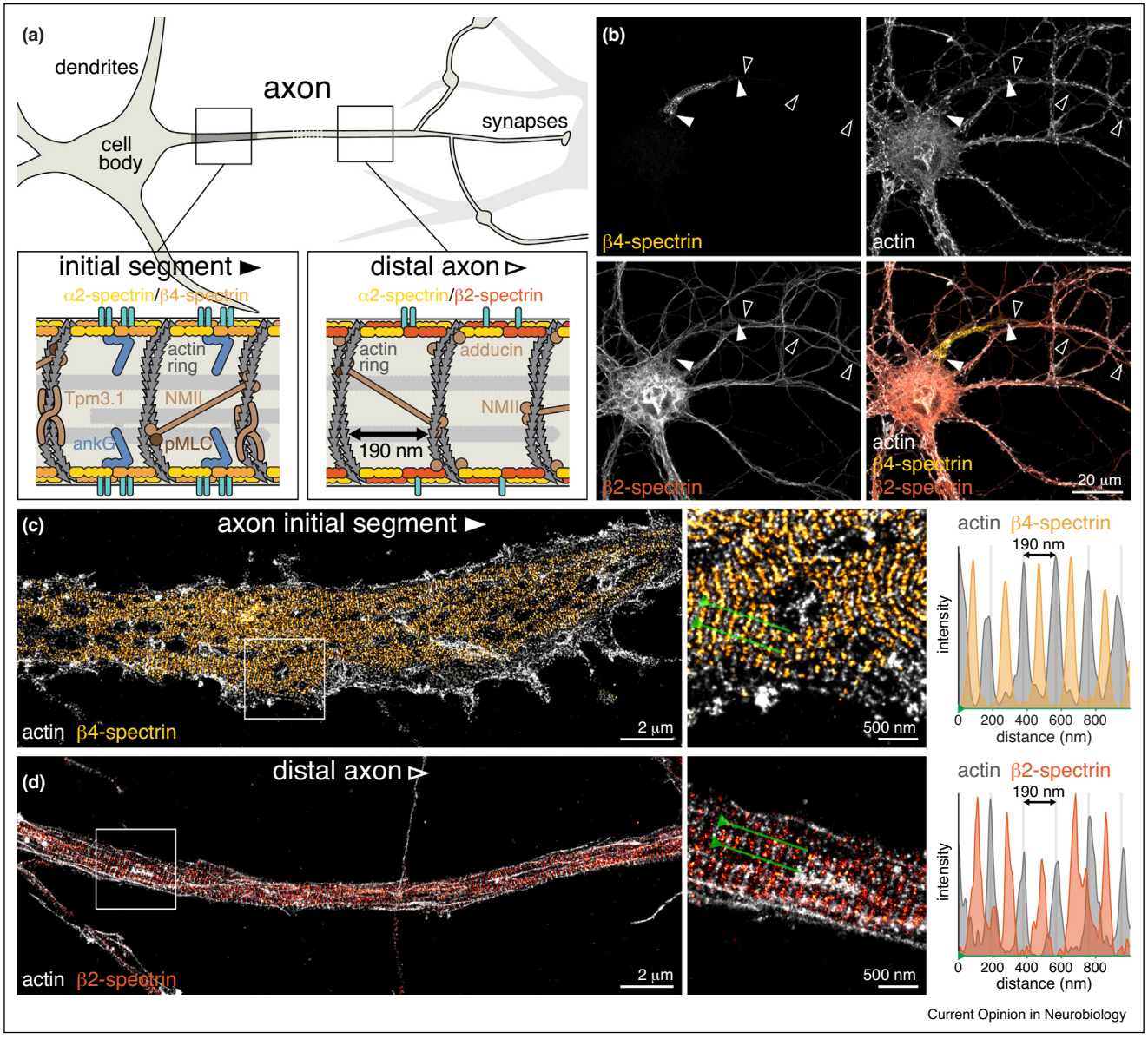
Ubiquity of the axonal MPS and how to look at it

The MPS is present along the whole axon shaft and composed of submembrane, circumferential actin rings regularly spaced every 190 nm by tetramers of spectrin that connect two adjacent rings (Figure 1a) [7]. It can be thought of as a mono-dimensional, tubular analog to the bi-dimensional hexagonal pattern of spectrin and short actin filaments lining the erythrocyte plasma membrane [8]. Originally discovered in hippocampal cultured neurons and brain slices [6], the MPS has since been described in all neuronal types and organisms studied so far [9,10]. It is present along unmyelinated but also myelinated axons, both at and between nodes of Ranvier [9,11,12]. A periodic organization of actin rings spaced by spectrin can also be detected in parts of dendrites, notably spine necks, but is far less systematic [13,14,15••].

The close apposition of rings every 190 nm makes them invisible to classical optical microscopy, which is limited to a ~250 nm resolution (Figure 1b). They were visualized for the first time in 2013 [6] using Single Molecule Localization Microscopy (SMLM), a super-resolution technique relying on sequential imaging of single fluorophores to build an image that attains ~20 nm in resolution [16]. Following this initial description, SMLM has remained a method of choice to map the composition and organization of the MPS using variants such as Stochastic Optical Reconstruction Microscopy (STORM, see Figure 2c) [17,18••,19•], photoactivated Localization Microscopy (PALM) that is compatible with live cells [13], or DNA-Point Acquisition in Nanoscale Topography (PAINT) that allows straightforward multi-color imaging (Figure 1c–d) [20,21••].

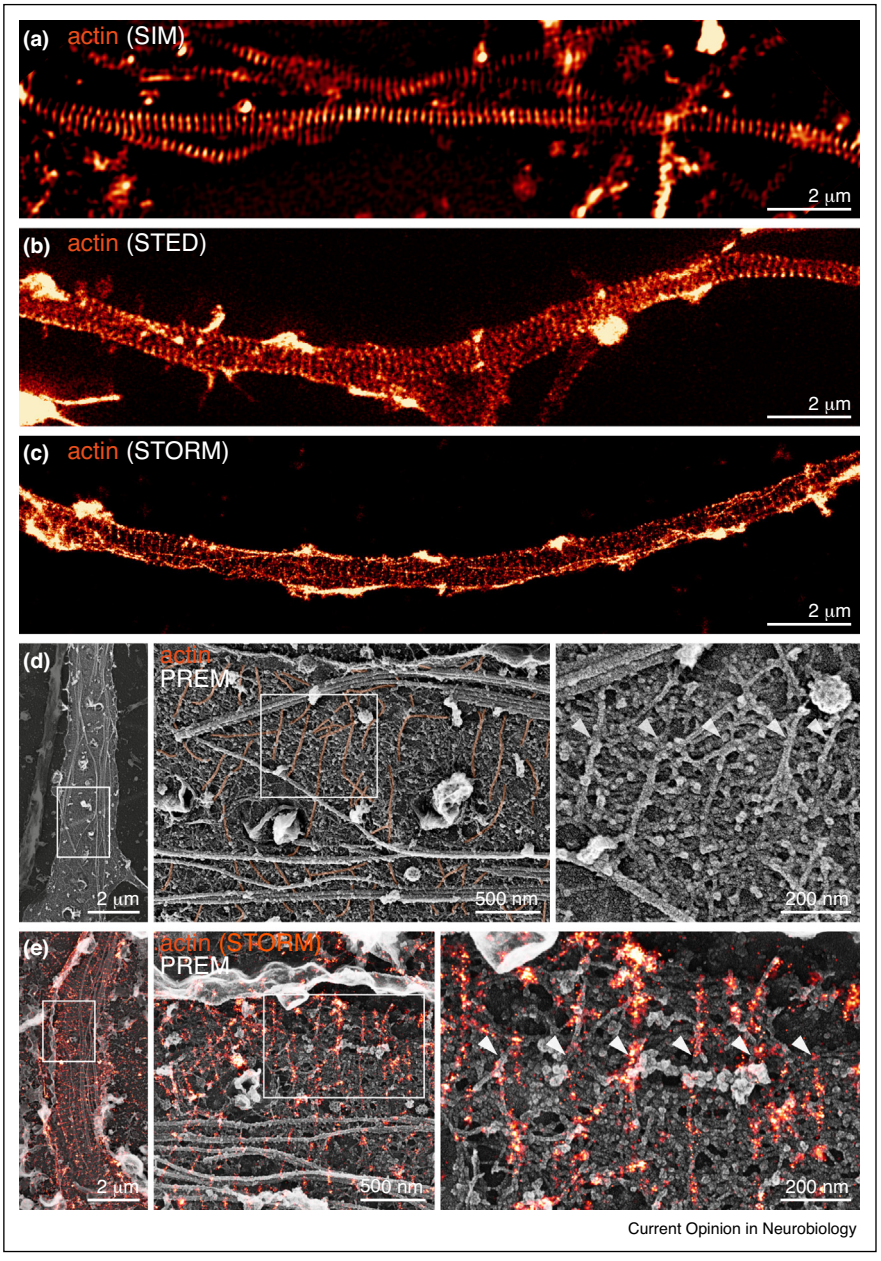
Other super-resolutive techniques [16] have successfully visualized the MPS: Structured Illumination Microscopy (SIM), with a ~120 nm typical resolution, can image the overall 190 nm periodicity along axons with larger fields of view and faster speeds (Figure 2a) [22,23•]. Notably, the first published image of the axonal MPS was a SIM image of the scaffold protein ankyrin at the Drosophila

Figure 1



Molecular organization of the membrane-associated periodic scaffold (MPS).
(a) Cartoon of a neuron showing its dendrites, cell body (top right) and axon that make synapses onto a downstream neuron (top left). The axon comprises the axon initial segment (AIS, gray) and the distal axon. Bottom, organization of the MPS in the AIS (bottom left) and distal axon (bottom right). The MPS is made of actin rings (gray) spaced every 190 nm by spectrin tetramers. In the AIS, the spectrin tetramers are made from α2-spectrin (yellow) and ß4-spectrin (light orange). Ankyrin G (ankG, blue) binds the center of the spectrin tetramer and anchors ion channels and cell adhesion molecules (light blue) at the membrane. In the distal axon, spectrin tetramers are made of α2-spectrin and ß2-spectrin (orange). Tropomyosin Tpm3.1 (brown) associates with the AIS actin rings, while assemblies of phospho-myosin light chain (pMLC, dark brown) and myosin heavy chains (brown) can crosslink actin rings. Along the distal axon, adducin (brown) associates with the actin rings. **(b)** Diffraction-limited image of a mature cultured hippocampal neuron stained for ß4-spectrin (AIS, arrowheads, yellow on overlay), ß2-spectrin (distal axon, outlined arrowheads, orange on overlay) and actin (gray on overlay). **(c)** Two-color DNA-PAINT image actin (gray) and ß4-spectrin (yellow) along the AIS. The ß4-spectrin antibody recognizes the C-terminus of ß4-spectrin, that is, the center of the spectrin tetramer. The resolution of DNA-PAINT (~20 nm) resolves the 190-nm periodic appearance of the MPS, with acting rings interspaced by ß4-spectrin bands (zoom image and intensity profile along the green line on the right). **(d)** Two-color Point Accumulation in Nanoscale Topography (PAINT) image actin (gray) and ß2-spectrin (yellow) along the distal axon. The ß2-spectrin antibody recognizes the C-terminus of ß2-spectrin, that is, the center of the spectrin tetramer. PAINT resolves the 190-nm periodic appearance of the MPS, with acting rings interspaced by ß2-spectrin bands (zoom image and intensity profile along the green line on the right). (b–d) Are adapted from Ref. [20], with permission.

Figure 2



Actin rings visualized by super-resolved and electron microscopy in living or fixed cells.
(a) Image from a 4-frame movie (1 frame every 20 s) of axons labeled for actin using silicon-rhodamine (SiR)-actin and imaged live by Structured Illumination Microscopy (SIM). Data from Ref. [34•], provided by the authors with permission. **(b)** Image of an axon labeled for actin using SiR-actin and imaged live by Stimulated Emission-Depletion microscopy (STED). Data from Ref. [24], provided by the authors with permission. **(c)** Stochastic Optical Reconstruction Microscopy (STORM) image of an axon from a fixed cell labeled for actin using phalloidin. **(d)** PREM image of an unroofed and fixed axon showing the MPS with actin braids (arrowheads on zoom, right) embedded in a spectrin mesh. **(e)** Correlative STORM of actin (phalloidin labeling, orange) and PREM of an unroofed and fixed axon showing that the actin rings images by STORM are the actin braids seen by PREM (arrowheads on zoom, right). (a–e) Are images obtained from cultured hippocampal neurons. (d–e) Are adapted from Ref. [20], with permission.

neuromuscular junction, resolving a 200-nm structured lattice [24]. The ~60 nm resolution obtained by Stimulated Emission Depletion (STED) microscopy has also been used to visualize the MPS (Figure 2b) [9,14,25–28]. Expansion Microscopy (ExM), where the sample is physically expanded after embedding into a hydrophilic gel, can also visualize periodic spectrins along the MPS [29]. Although classic actin labels are not compatible with ExM

protocols, new reagents could potentially be used to directly visualize actin rings by ExM [30].

Composition of the MPS

In addition to actin, a number of MPS components have been identified (Figure 1a). The spectrins connecting actin rings are head-to-head, 190-nm long tetramers of two α-spectrins and two ß-spectrins [31]. The only α-spectrin expressed in neurons, α2-spectrin, is present all along the axon [12,20,32]. At the axon initial segment (AIS, the first 20–50 μm of the axon), α2-spectrin associates with ß4-spectrin to form tetramers (Figure 1b–c), whereas in the more distal axon, it associates with ß2-spectrin (Figure 1b & d) [6,17]. In the AIS, membrane proteins (sodium and potassium voltage-gated channels, cell adhesion molecules CAMs) are anchored to the MPS via interaction of the scaffold protein ankyrin G with the spectrin tetramers, and are thus also periodically organized along the plasma membrane [6,17].

Adducin, which caps the short actin filaments found in the spectrin hexagonal lattice of erythrocytes, associates with actin rings along the axon [6,26], but is less present along its proximal part [21••,33]. There, AIS actin rings are decorated by tropomyosin Tpm3.1, which could have a role in stabilizing them [23•]. Actin rings have also recently been characterized as actomyosin structures: a concentration of phospho-myosin light chain (pMLC), activator of non-muscle myosin (NM-II), was found along the actin rings at the AIS [34•], and NM-II itself attaches to rings along the whole shaft (Figure 1a) [19•,35•]. Recent reports have described the association of activated membrane receptors (the cannabinoid G-protein coupled receptor CB1, L1-CAM, receptor tyrosine kinases FGFR1/TrkB) and downstream signaling proteins such as Src at actin rings after their activation [18••,36]. It is likely that more MPS components and partners remain to be discovered, for example using unbiased methods such as BioID tagging and mass spectrometry [37].

Molecular organization of the MPS

Until recently, only optical super-resolved technique had been able to reveal the MPS, so the arrangement of MPS components at the molecular scale remained speculative. By analogy with the actin/spectrin hexagonal lattice of erythrocytes [8], the presence of adducin associated with axonal actin rings suggested that these rings were made of short, capped filaments bundled into rings [6,7]. Polarized-SIM analysis of signal from axonal actin led to propose that the short filaments were longitudinal along the axon, and laterally assembled into rings [38]. The ultrastructural detail of EM is needed to answer such a question, but decades of EM studies could not discern the submembrane periodic scaffold [2]. It is likely that classical thin-section EM cannot easily contrast actin assemblies made of a few filaments - and more recent cryoEM approaches also failed to visualize the MPS along axons [39,40].

An EM method that can visualize the cytoskeleton with great contrast and resolution is platinum-replica EM (PREM), where the topography of cells is transferred to a platinum-carbon replica before being examined by transmission EM. PREM of axons after membrane extraction by detergents only revealed short, disorganized actin filaments with no periodic structures [33]. Recently, PREM after mechanical unroofing — removal of the dorsal part of cultured neurons and their axons by ultrasonic pulses — was shown to preserve the MPS and its periodic organization [21••]. Along the ventral plasma membrane of the axon, PREM views showed numerous instances of submembrane actin ring fragments, regularly spaced every 190 nm, and connected by a mesh of spectrin (Figure 2d). Moreover, high magnification views revealed that actin rings are made of two long actin filaments arranged in a braid, with typically 0.5–1 μm of continuous braid being visible within a dense mesh. Identification of actin and spectrin by immunogold-PREM and correlative SMLM/PREM confirmed that the actin rings seen by super-resolution microscopy are indeed the braided, long actin filaments seen by PREM (Figure 2e) [21••]. Unroofing necessarily break rings into fragments, preventing the visualization of their intact arrangement around the axon circumference. Techniques like cryo-electron tomography may help resolving whole actin rings at the ultrastructural level in the future [41].

The braids of two long filaments that make actin rings are unique structures that have never been observed in other cells or compartments, opening many questions. Are actin braids made of two antiparallel or parallel actin filaments? Do they polymerize together, or are they assembled from individual filaments? How can long, stiff actin braids - actin filaments have a persistence length of ∼10 μm - curve into ∼1 μm diameter rings along the axon circumference? *In vitro* work has recently shown how calponin homology domains of actin-associated proteins can induce strong individual filament curvature, and this could be relevant for axonal actin rings [42]. Rings made of long filaments also question the assumed role of adducin at actin rings: more than a protein capping short filaments like in erythrocytes, it could primarily help lateral association of filaments with spectrins [43,44].

Relationship to other axonal cytoskeleton assemblies

What is the relationship between the MPS and other cytoskeletal assemblies in the axon? Interplay with intermediate filaments has not been explored, but microtubules disassembly can destabilize the MPS at least partially [6,13,22]. Conversely, defect in microtubule bundling and axonal swellings have been observed in drosophila axons lacking an MPS [22]. Disentangling

indirect and direct interplay between axonal actin and microtubules is difficult, as drugs targeting the cytoskeleton have acute pleiotropic effects, and shRNA or genetic deletion strategies can only be observed over long term. Ideally, localized and acute strategies such as optically-controlled constructs or drugs should be used to overcome this challenge.

Another open question is the interplay between the MPS and dynamic, intra-axonal actin structures such as actin hotspots (minute-lived, immobile actin clusters that appear and disappear inside the axon shaft) and actin trails (fast, filamentous assemblies nucleated from hotpots that elongate over several μm and disappear within seconds) [45]. Interestingly, most actin hotspots are contacting the MPS on axonal cross-section from 3D SMLM data [46]. More generally, it would be important to get a better understanding of the axonal 'actin economy', that is, the distribution of the available actin pool between dynamic (growth cone, hotspots, trails) and structural (actin rings) assemblies along axons. Finally, the MPS usually stops at presynapses [10,47], but how is this pause established and maintained, and how is presynaptic actin itself organized, remain elusive [5]. Microtubules nucleation mostly occurs away from the centrosome in neurons [48], and their nucleation near at boutons [49,50] could play a role in shaping the MPS around presynapses.

Mechanism of assembly and dynamics

How is the MPS assembled during development? In neuronal cultures, it appears just after axon differentiation at two days *in vitro* (div). The first assembled MPS components from 2 div on are ß2-spectrin and actin – notably, actin rings are easier to detect in these immature neurons using the actin probe silicon-rhodamine (SiR)-actin imaged by STED [25], than using phalloidin imaged by SMLM [6,13]. Actin and spectrin seem mutually interdependent for MPS assembly and integrity: acute actin perturbation and depletion of spectrin both disassemble the MPS or prevent its assembly. Adducin appears later, at around 6 div [13], and its presence is not necessary for the MPS to form [26]. A fundamental question here is what drives MPS assembly. Considering the 2D hexagonal lattice in erythrocytes and the 1D periodic lattice in neurons, one can speculate that core component of the MPS are able to auto-organize, driven by the local geometry (flat membrane versus tubular extension). *In vitro* reconstitution of a minimal actin/spectrin system within defined membrane geometries (flat, spherical or tubular membrane) would allow to test auto-assembly hypotheses of MPS components.

Assembly of the MPS first happens along the proximal axon at 2 div, before spreading forward toward the distal axon [13]. Of note, even in mature neurons, the very distal part of the axon before the growth cone is usually devoid of a detectable MPS. Beyond this proximo-distal sequence, not much is known about the mechanism of MPS assembly: is it continuously propagating like a polymer from the assembled part toward the distal axon, or are there multiple seeds of proto-MPS that grow and coalesce into a single scaffold? Live-cell imaging of the MPS could answer such questions but is difficult, because super-resolution imaging requires high illumination intensities that restrict the possibility of long-term imaging [16]. The MPS has been observed in living cells by PALM of photoactivable spectrin [13], or STED [25] and SIM [35•] of actin probes (Figure 2a–b), but only for a handful of frames and a limited time (seconds to minutes), precluding the study of slow processes such as assembly or structural plasticity. Moreover, it is challenging to visualize the low amount of actin within rings in living cells, as live-cell actin probes such as SiR-actin or LifeAct can over-stabilize filaments and induce actin bundles [15••]. A related open question is the nature and dynamics of MPS assembly during axon regeneration, which can be induced by stabilizing microtubules [51,52].

Live-cell and drug treatment experiments have nevertheless brought key insights about the MPS paradoxical dynamics: on the one hand, the MPS is a static and stable scaffold, as suggested by the half-life of spectrins in neurons that attains 5–10 days [53,54]. PALM of ß2-spectrin shows no significant movement of rings within 30 minutes, and FRAP only very slow turnover of spectrins [13]. Although actin rings can be disassembled by actin-targeting drugs, they are very stable in the AIS [17,21••,55] and resistant to actin assembly perturbators along the whole shaft [19•]. PREM images show rings as actin braids deeply embedded in a spectrin mesh, in line with their high stability and slow turnover. On the other hand, the MPS can be profoundly remodeled within a few hours in physiopathological conditions. MPS disassembly occurs along dorsal root ganglion axons after only three hours of NGF withdrawal, upstream of the axonal degeneration events that occur later in this model [56,57]. It was also shown that MPS remodeling can occur to regulate physiological signaling by GPCRs, CAMs and RTKs [18••]. After activation, these membrane proteins associate at the center of the spectrin tetramers in between actin rings, condensing with downstream proteins of the ERK signaling pathway. Signaling itself induces a negative feedback loop of calpain-driven disassembly of the MPS after ~1 hour, driving signaling extinction. Even faster rearrangements have recently been demonstrated, allowing the transport of large cargoes through axons of smaller diameter: a myosin-dependent enlargement/opening of rings occurs during cargo passage, before the axon shrinks back to its original diameter within seconds [35•]. These results reveal that beyond its long-term stability, the MPS is exquisitely regulated, and the structural mechanisms of this short-term plasticity remain to be fully explored.

Cellular functions of the MPS

What are the cellular roles of the MPS for the axon physiology? Given the MPS strikingly regular organization and the cable-like morphology of axons, one of the first functional hypothesis was mechanical support to axons, notably peripheral axons that undergo intense stress during body movements. The association of rigid actin rings connected by spring-like spectrins may form a mechanically resistant, yet flexible, scaffold [6]. Indeed, discovery of the MPS provided a structural basis to the earlier finding that *C. elegans* mutant for ß-spectrin have more fragile axons that break due to animal movements [58]. Careful mechanobiological studies later showed that spectrin induces constitutive tension along axons [59], and that together with microtubules, the MPS plays a key role in protecting axons against mechanical stress [27]. Recently, unfolding of spectrin repeats within the MPS tetramers was proposed as a mechanism to release longitudinal stress along stretched axons [60•].

A molecular view of how the MPS can deform and contract itself is also starting to emerge. Simulation have explored how the MPS could provide rigidity to the axonal plasma membrane [61]. Actomyosin contractility at the MPS participates in both longitudinal and radial tension along the axon [62], suggesting that myosins could associate with actin rings. Recent studies have located pMLC and NMII heads apposed to the rings at the nanoscale, while heavy chains are preferentially located in-between rings [19•,34•,35•]. The majority of myosin dimers may thus crosslink neighboring rings, a finding confirmed at the ultrastructural level by the localization of pMLC and myosin rods using immunogold PREM and correlative SMLM/PREM [21••]. How such crosslinking myosins - or a minor population of within-ring associated myosins – can pull along the braided filaments that form rings to generate both radial and axial contractility remains to be clarified [63,64].

Another widely held hypothesis is that the MPS, by periodically arranging anchored ion channels such as sodium and potassium channels at the AIS, could influence the way the action potential is generated and propagated along the axon [7] – but no experimental results have confirmed this so far. It was nevertheless shown that surface diffusion of membrane components is compartmented by the submembrane actin rings along the proximal axon [65]. Moreover, the MPS is able to organize signaling from axonal membrane proteins such as CB1, L1-CAM and FGFR1/TrkB by transiently recruiting activated receptors and intracellular effectors at the center of spectrin tetramers (see above) [18••], and live-cell imaging evidenced activated CB1 receptors immobilization at this location [36]. Beyond receptor signaling, the dense mesh of the MPS as seen on PREM images is likely to prevent endocytosis from the plasma membrane. Indeed, activated CB1 endocytosis is enhanced in ß2-spectrin depleted neurons [18••]. Whether the MPS has a more general role in regulating endo/exocytosis along the axon shaft is an exciting question for future research.

Conclusions

One the few truly novel cellular structures discovered by super-resolution microscopy so far, the MPS is now probed using a variety of complementary approaches to understand its architecture, dynamics, and functions. Yet a lot remains unknown, and new advances will undoubtedly be stimulated by progress in technologies: better spatial and temporal resolution, better molecular identification capabilities. At the structural level, it will be possible to go beyond the longitudinal periodicity to zoom on the architecture of single rings, using more elaborate strategies such as SMLM-based single-particle averaging [66]. This *in situ* structural biology will be complemented by multiplexed approaches such as automated sequential DNA-PAINT [67] to obtain the molecular arrangement of multiple MPS components at once. Deep-learning will be instrumental to understand the heterogeneity of patterning between various axonal compartments, quantifying the amount of periodicity and the presence of other actin-based structures [15••]. New perturbation strategies with better molecular, spatial and temporal specificity such as optically-driven inhibitors will allow to dissect the role of the MPS in axonal physiological processes, before zooming out to assess the consequences on neuronal connectivity and information processing. Finally, potential dysfunctions of the MPS in neuropsychiatric diseases, from spectrinopathies [68,69] to Alzheimer's disease, remain to be explored.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Marner L, Nyengaard JR, Tang Y, Pakkenberg B: **Marked loss of myelinated nerve fibers in the human brain with age**. *J Comp Neurol* 2003, **462**:144-152.

2. Leterrier C, Dubey P, Roy S: **The nano-architecture of the axonal cytoskeleton**. *Nat Rev Neurosci* 2017, **18**:713-726.

3. Tas RP, Kapitein LC: **Exploring cytoskeletal diversity in neurons**. *Science* 2018, **361**:231-232.

4. Leterrier C: **A pictorial history of the neuronal cytoskeleton**. *J Neurosci* 2021, **41**:11-27.

5. Papandréou M-J, Leterrier C: **The functional architecture of axonal actin**. *Mol Cell Neurosci* 2018, **91**:151-159.

6. Xu K, Zhong G, Zhuang X: **Actin, spectrin, and associated proteins form a periodic cytoskeletal structure in axons**. *Science* 2013, **339**:452-456.

7. Unsain N, Stefani FD, Cáceres A: **The actin/spectrin membrane-associated periodic skeleton in neurons**. *Front Synaptic Neurosci* 2018, **10**:10.

8. Gokhin DS, Fowler VM: **Feisty filaments: actin dynamics in the red blood cell membrane skeleton**. *Curr Opin Hematol* 2016, **23**:206-214.

9. D'Este E, Kamin D, Velte C, Göttfert F, Simons M, Hell SW: **Subcortical cytoskeleton periodicity throughout the nervous system**. *Sci Rep* 2016, **6**:22741.

10. He J, Zhou R, Wu Z, Carrasco MA, Kurshan PT, Farley JE, Simon DJ, Wang G, Han B, Hao J *et al.*: **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* 2016, **113**:6029-6034.

11. D'Este E, Kamin D, Balzarotti F, Hell SW: **Ultrastructural anatomy of nodes of Ranvier in the peripheral nervous system as revealed by STED microscopy**. *Proc Natl Acad Sci U S A* 2016, **114**:E191-E199.

12. Huang CY-M, Zhang C, Zollinger DR, Leterrier C, Rasband MN: **An αII spectrin-based cytoskeleton protects large-diameter myelinated axons from degeneration**. *J Neurosci* 2017, **37**:11323-11334.

13. Zhong G, He J, Zhou R, Lorenzo D, Babcock HP, Bennett V, Zhuang X: **Developmental mechanism of the periodic membrane skeleton in axons**. *eLife* 2014, **3**:194.

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

15. •• Lavoie-Cardinal F, Bilodeau A, Lemieux M, Gardner M-A, Wiesner T, Laramée G, Gagné C, Koninck PD: **Neuronal activity remodels the F-actin based submembrane lattice in dendrites but not axons of hippocampal neurons**. *Sci Rep* 2020, **10**:11960

This work applies deep-learning to segment areas of periodic actin organization along dendrites and axons, highlighting that the axonal MPS is insensitive to activity variation, but periodic segments along dendrites are.

16. Jacquemet G, Carisey AF, Hamidi H, Henriques R, Leterrier C: **The cell biologist's guide to super-resolution microscopy**. *J Cell Sci* 2020, **133** jcs240713.

17. Leterrier C, Potier J, Caillol G, Debarnot C, Rueda Boroni F, Dargent B: **Nanoscale architecture of the axon initial segment reveals an organized and robust scaffold**. *Cell Rep* 2015, **13**:2781-2793.

18. •• Zhou R, Han B, Xia C, Zhuang X: **Membrane-associated periodic skeleton is a signaling platform for RTK transactivation in neurons**. *Science* 2019, **365**:929-934

The authors reveal a novel role for the MPS in axonal signaling: membrane receptors reversibly cluster in-between actin rings upon activation, generating a transient signaling pulse.

19. • Costa AR, Sousa SC, Pinto-Costa R, Mateus JC, Lopes CD, Costa AC, Rosa D, Machado D, Pajuelo L, Wang X *et al.*: **The membrane periodic skeleton is an actomyosin network that regulates axonal diameter and conduction**. *eLife* 2020, **9**: e55471

Together with [21], [34] and [35], this work establishes the MPS as an actomyosin assembly. The authors show that ring-associated myosins can regulate the axonal diameter and action potential conduction.

20. Huang CY-M, Zhang C, Ho TS-Y, Oses-Prieto J, Burlingame AL, Lalonde J, Noebels JL, Leterrier C, Rasband MN: **αII spectrin forms a periodic cytoskeleton at the axon initial segment and is required for nervous system function**. *J Neurosci* 2017, **37**:11311-11322.

21. •• Vassilopoulos S, Gibaud S, Jimenez A, Caillol G, Leterrier C: **Ultrastructure of the axonal periodic scaffold reveals a braid-like organization of actin rings**. *Nat Commun* 2019, **10**:5803

The first visualization of the MPS by electron microscopy, revealing that actin rings are made of braids of long actin filaments embedded in a mesh of spectrins. Also visualizes pMLC and putative myosins crosslinking actin rings at the molecular level.

22. Qu Y, Hahn I, Webb SED, Pearce SP, Prokop A: *Periodic actin structures in neuronal axons are required to maintain microtubules*. 2017:296-308.

23. • Abouelezz A, Stefen H, Segerstråle M, Micinski D, Minkeviciene R, Lahti L, Hardeman EC, Gunning PW, Hoogenraad CC, Taira T *et al.*: **Tropomyosin Tpm3.1 is required to maintain the structure and function of the axon initial segment**. *Iscience* 2020, **23** 101053

This work identifies tropomyosin (Tpm3.1) as a partner of actin rings and intracellular actin clusters at the AIS of neurons, and their role in AIS structural integrity.

24. Pielage J, Cheng L, Fetter RD, Carlton PM, Sedat JW, Davis GW: **A presynaptic giant Ankyrin stabilizes the NMJ through regulation of presynaptic microtubules and transsynaptic cell adhesion**. *Neuron* 2008, **58**:195-209.

25. D'Este E, Kamin D, Göttfert F, El-Hady A, Hell SW: **STED nanoscopy reveals the ubiquity of subcortical cytoskeleton periodicity in living neurons**. *Cell Rep* 2015, **10**:1246-1251.

26. Leite SC, Sampaio P, Sousa VF, Nogueira-Rodrigues J, Pinto-Costa R, Peters LL, Brites P, Sousa MM: **The actin-binding protein α-adducin is required for maintaining axon diameter**. *Cell Rep* 2016, **15**:490-498.

27. Krieg M, Stühmer J, Cueva JG, Fetter R, Spilker K, Cremers D, Shen K, Dunn AR, Goodman MB: **Genetic defects in β-spectrin and tau sensitize *C. elegans* axons to movement-induced damage via torque-tension coupling**. *eLife* 2017, **6**:e20172.

28. Barabas FM, Masullo LA, Bordenave MD, Giusti SA, Unsain N, Refojo D, Cáceres A, Stefani FD: **Automated quantification of protein periodic nanostructures in fluorescence nanoscopy images: abundance and regularity of neuronal spectrin membrane-associated skeleton**. *Sci Rep* 2017, **7** 16029.

29. Martínez GF, Gazal NG, Quassollo G, Szalai AM, Cid-Pellitero ED, Durcan TM, Fon EA, Bisbal M, Stefani FD, Unsain N: **Quantitative expansion microscopy for the characterization of the spectrin periodic skeleton of axons using fluorescence microscopy**. *Sci Rep* 2020, **10**:2917.

30. Wen G, Vanheusden M, Acke A, Valli D, Neely RK, Leen V, Hofkens J: **Evaluation of direct grafting strategies via trivalent anchoring for enabling lipid membrane and cytoskeleton staining in expansion microscopy**. *ACS Nano* 2020, **14**:7860-7867.

31. Lorenzo DN: **Cargo hold and delivery: ankyrins, spectrins, and their functional patterning of neurons**. *Cytoskeleton* 2020, **77**:129-148.

32. Wang Y, Ji T, Nelson AD, Glanowska K, Murphy GG, Jenkins PM, Parent JM: **Critical roles of αII spectrin in brain development and epileptic encephalopathy**. *J Clin Invest* 2018 http://dx.doi.org/10.1172/jci95743.

33. Jones SL, Korobova F, Svitkina T: **Axon initial segment cytoskeleton comprises a multiprotein submembranous coat containing sparse actin filaments**. *J Cell Biol* 2014, **205**:67-81.

34. • Berger SL, Leo-Macias A, Yuen S, Khatri L, Pfennig S, Zhang Y, Agullo-Pascual E, Caillol G, Zhu M-S, Rothenberg E *et al.*: **Localized myosin II activity regulates assembly and plasticity of the axon initial segment**. *Neuron* 2018, **97**:555-570.e6

Together with [19], [21] and [35], this work establishes the MPS as an actomyosin assembly. The authors show that pMLC concentrates at the AIS in an activity-dependent manner.

35. • Wang T, Li W, Martin S, Papadopulos A, Joensuu M, Liu C, Jiang A, Shamsollahi G, Amor R, Lanoue V *et al.*: **Radial contractility of actomyosin rings facilitates axonal trafficking and structural stability**. *J Cell Biol* 2020, **219**

Together with [19], [21] and [34], this work establishes the MPS as an actomyosin assembly. The authors reveal a fast, myosin-dependent

plasticity of actin rings that can open to accommodate the passage of large axonal cargoes.

36. Li H, Yang J, Tian C, Diao M, Wang Q, Zhao S, Li S, Tan F, Hua T, Qin Y *et al.*: **Organized cannabinoid receptor distribution in neurons revealed by super-resolution fluorescence imaging**. *Nat Commun* 2020, **11**:5699.

37. Hamdan H, Lim BC, Torii T, Joshi A, Konning M, Smith C, Palmer DJ, Ng P, Leterrier C, Oses-Prieto JA *et al.*: **Mapping axon initial segment structure and function by multiplexed proximity biotinylation**. *Nat Commun* 2020, **11**:100.

38. Zhanghao K, Chen X, Liu W, Li M, Liu Y, Wang Y, Luo S, Wang X, Shan C, Xie H *et al.*: **Super-resolution imaging of fluorescent dipoles via polarized structured illumination microscopy**. *Nat Commun* 2019, **10**:4694.

39. Schrod N, Vanhecke D, Laugks U, Stein V, Fukuda Y, Schaffer M, Baumeister W, Lucic V: **Pleomorphic linkers as ubiquitous structural organizers of vesicles in axons**. *PLoS One* 2018, **13**: e0197886.

40. Hoffmann PC, Giandomenico SL, Ganeva I, Wozny MR, Sutcliffe M, Kukulski W, Lancaster MA: **The supramolecular landscape of growing human axons**. *Biorxiv* 2020 http://dx.doi.org/10.1101/2020.07.23.216622.

41. Kiesel P, Viar GA, Tsoy N, Maraspini R, Gorilak P, Varga V, Honigmann A, Pigino G: **The molecular structure of mammalian primary cilia revealed by cryo-electron tomography**. *Nat Struct Mol Biol* 2020 http://dx.doi.org/10.1038/s41594-020-0507-4.

42. Palani S, Balasubramanian MK, Köster DV: **Calponin-Homology Domain mediated bending of membrane associated actin filaments**. *Biorxiv* 2020 http://dx.doi.org/10.1101/2020.07.10.197616.

43. Glenney JR, Glenney P, Weber K: **F-actin-binding and cross-linking properties of porcine brain fodrin, a spectrin-related molecule**. *J Biol Chem* 1982, **257**:9781-9787.

44. Gardner K, Bennett V: **Modulation of spectrin-actin assembly by erythrocyte adducin**. *Nature* 1987, **328**:359-362.

45. Ganguly A, Tang Y, Wang L, Ladt K, Loi J, Dargent B, Leterrier C, Roy S: **A dynamic formin-dependent deep F-actin network in axons**. *J Cell Biol* 2015, **210**:401-417.

46. Chakrabarty N, Dubey P, Tang Y, Ganguly A, Ladt K, Leterrier C, Jung P, Roy S: **Processive flow by biased polymerization mediates the slow axonal transport of actin**. *J Cell Biol* 2019, **218**:112-124.

47. Sidenstein SC, D'Este E, Böhm MJ, Danzl JG, Belov VN, Hell SW: **Multicolour multilevel STED nanoscopy of actin/spectrin organization at synapses**. *Sci Rep* 2016, **6**:26725.

48. Stiess M, Maghelli N, Kapitein LC, Gomis-Rüth S, Wilsch-Bräuninger M, Hoogenraad CC, Nørrelykke IMT, Bradke F: **Axon extension occurs independently of centrosomal microtubule nucleation**. *Science* 2010, **327**:704-707.

49. Qu X, Kumar A, Blockus H, Waites C, Bartolini F: **Activity-dependent nucleation of dynamic microtubules at presynaptic boutons controls neurotransmission**. *Curr Biol* 2019, **29**:4231-4240.e5.

50. Guedes-Dias P, Nirschl JJ, Abreu N, Tokito MK, Janke C, Magiera MM, Holzbaur ELF: **Kinesin-3 responds to local microtubule dynamics to target synaptic cargo delivery to the presynapse**. *Curr Biol* 2019, **29**:1-41.

51. Hellal F, Hurtado A, Ruschel J, Flynn KC, Laskowski CJ, Umlauf M, Kapitein LC, Strikis D, Lemmon V, Bixby J *et al.*: **Microtubule stabilization reduces scarring and causes axon regeneration after spinal cord injury**. *Science* 2011, **331**:928-931.

52. Ruschel J, Hellal F, Flynn KC, Dupraz S, Elliott DA, Tedeschi A, Bates M, Sliwinski C, Brook G, Dobrindt K *et al.*: **Axonal regeneration. Systemic administration of epothilone B promotes axon regeneration after spinal cord injury**. *Science* 2015, **348**:347-352.

53. Dörrbaum AR, Kochen L, Langer JD, Schuman EM: **Local and global influences on protein turnover in neurons and glia**. *eLife* 2018, **7**:e34202.

54. Fornasiero EF, Mandad S, Wildhagen H, Alevra M, Rammner B, Keihani S, Opazo F, Urban I, Ischebeck T, Sakib MS *et al.*: **Precisely measured protein lifetimes in the mouse brain reveal differences across tissues and subcellular fractions**. *Nat Commun* 2018, **9** 71–17.

55. Abouelezz A, Micinski D, Lipponen A, Hotulainen P: **Sub-membranous actin rings in the axon initial segment are resistant to the action of latrunculin**. *Biol Chem* 2019, **400**:1141-1146.

56. Unsain N, Bordenave MD, Martinez GF, Jalil S, von Bilderling C, Barabas FM, Masullo LA, Johnstone AD, Barker PA, Bisbal M *et al.*: **Remodeling of the actin/spectrin membrane-associated periodic skeleton, growth cone collapse and F-actin decrease during axonal degeneration**. *Sci Rep* 2018, **8**:3007.

57. Wang G, Simon DJ, Wu Z, Belsky DM, Heller E, O'Rourke MK, Hertz NT, Molina H, Zhong G, Tessier-Lavigne M *et al.*: **Structural plasticity of actin-spectrin membrane skeleton and functional role of actin and spectrin in axon degeneration**. *eLife* 2019, **8**: e38730.

58. Hammarlund M, Jorgensen EM, Bastiani MJ: **Axons break in animals lacking beta-spectrin**. *J Cell Biol* 2007, **176**:269-275.

59. Krieg M, Dunn AR, Goodman MB: **Mechanical control of the sense of touch by β-spectrin**. *Nat Cell Biol* 2014, **16**:224-233.

60. • Dubey S, Bhembre N, Bodas S, Veer S, Ghose A, Callan-Jones A, Pullarkat P: **The axonal actin-spectrin lattice acts as a tension buffering shock absorber**. *eLife* 2020, **9**:e51772
This article examines the role of the MPS elasticity in the mechanical properties of the axon, showing that spectrin unfolding can buffer longitudinal stress.

61. Zhang Y, Abiraman K, Li H, Pierce DM, Tzingounis AV, Lykotrafitis G: **Modeling of the axon membrane skeleton structure and implications for its mechanical properties**. *PLoS Comput Biol* 2017, **13**:e1005407.

62. Fan A, Tofangchi A, Kandel M, Popescu G, Saif T: **Coupled circumferential and axial tension driven by actin and myosin influences in vivo axon diameter**. *Sci Rep* 2017, **7**:14188.

63. Costa AR, Sousa MM: **Non-muscle myosin II in axonal cell biology: from the growth cone to the axon initial segment**. *Cells* 2020, **9**:1961.

64. Mikhaylova M, Rentsch J, Ewers H: **Actomyosin contractility in the generation and plasticity of axons and dendritic spines**. *Cells* 2020, **9**:2006.

65. Albrecht D, Winterflood CM, Sadeghi M, Tschager T, Noé F, Ewers H: **Nanoscopic compartmentalization of membrane protein motion at the axon initial segment**. *J Cell Biol* 2016, **215**:37-46.

66. Sieben C, Douglass KM, Guichard P, Manley S: **Super-resolution microscopy to decipher multi-molecular assemblies**. *Curr Opin Struc Biol* 2018, **49**:169-176.

67. Guo S-M, Veneziano R, Gordonov S, Li L, Danielson E, de Arce KP, Park D, Kulesa AB, Wamhoff E-C, Blainey PC *et al.*: **Multiplexed and high-throughput neuronal fluorescence imaging with diffusible probes**. *Nat Commun* 2019, **10**:4377.

68. Liu C-H, Rasband MN: **Axonal spectrins: nanoscale organization, functional domains and spectrinopathies**. *Front Cell Neurosci* 2019, **13**:234.

69. Cousin MA, Breau KA, Creighton BA, Spillman RC, Torti E, Dontu S, Tripathi S, Ajit D, Harper KM, Stankewich MC *et al.*: **Pathogenic SPTBN1 variants cause a novel autosomal dominant neurodevelopmental syndrome**. *medRxiv* 2020 http://dx.doi.org/10.1101/2020.08.31.20184481.