Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Neurobiology

Local mechanisms regulating selective cargo entry and long-range trafficking in axons

Laura F Gumy[1] and Casper C Hoogenraad[2]

The polarized long-distance transport of neuronal cargoes depends on the presence of functional and structural axonal subcompartments. Given the heterogeneity of neuronal cargoes, selective sorting and entry occurs in the proximal axon where multiple subcellular specializations such as the axon initial segment, the pre-axonal exclusion zone, the MAP2 pre-axonal filtering zone and the Tau diffusion barrier provide different levels of regulation. Cargoes allowed to pass through the proximal axon spread into the more distal parts. Recent findings show that diverse cargo distributions along the axon depend on the compartmentalized organization of the cytoskeleton and the local regulation of multiple motor proteins by microtubule associated proteins. In this review, we focus on the local mechanisms that control cargo motility and discuss how they play a role in the overall circulation of axonal cargoes.

Addresses
[1] Department of Anatomy, University of Otago, Dunedin, New Zealand
[2] Cell Biology, Department of Biology, Faculty of Science, Utrecht University, Utrecht, The Netherlands

Corresponding authors: Gumy, Laura F (laura.gumy@otago.ac.nz), Hoogenraad, Casper C (c.hoogenraad@uu.nl)

Current Opinion in Neurobiology 2018, **51**:23–28

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

Edited by **Juan Burrone** and **Erika Holzbaur**

For a complete overview see the Issue and the Editorial

Available online 3rd March 2018

https://doi.org/10.1016/j.conb.2018.02.007

Introduction

Neurons are structurally and functionally asymmetric cells, which typically consist of a soma, a very long and thin axon and branched dendrites that receive the electrical input from other neurons. Consequently, the axon transmits action potentials from the cell body to the synaptic sites of other neurons. The survival, maintenance, growth and regeneration of axons fundamentally depend on active transport mechanisms to ensure accurate distribution of various cellular cargoes, including organelles, proteins and RNA. Axonal transport depends on molecular motors that move cargoes directionally along either of two types of cytoskeletal structures: microtubules and actin filaments. Microtubules serve as tracks for two families of motor proteins, kinesin and dynein, which facilitate long-range transport within axons. Kinesin family members drive anterograde axonal transport whereas dynein drives retrograde transport. Actin facilitates the motility of motor proteins of the myosin family, which mostly mediate local delivery of cargoes, for example to distal axonal terminals. Defects in axonal transport components have been linked to axonal damage, neurodevelopmental or neurodegenerative diseases, highlighting the importance of active axonal intracellular transport for healthy neuronal functioning [1–5]. In this review, we provide an up to date overview of how, in recent years, high-resolution imaging methods have provided a more profound understanding of the complexity of molecular mechanisms that regulate the selective entry of cargoes from the soma into the axon and their subsequent long-distance transport.

Cargo transport is not uniform along the axon

Morphological and functional studies show that the axon is organized into different compartments. The axon initial segment (AIS), perhaps the most studied axonal substructure, stretches 20–50 μm along the proximal axon. The AIS initiates action potentials but also acts as an intracellular barrier to prevent somatodendritic proteins from entering the axon [6]. In addition to the AIS, new research shows that selective axonal cargo filtering also occurs in the Tau diffusion barrier located within the AIS, in the pre-axonal exclusion zone and in the microtubule associated protein 2 (MAP2)-defined region between the soma and the AIS [7•,8••,9•]. These recent studies indicate that several complementary mechanisms act in parallel in the proximal axon to achieve organelle entry. Distally to the AIS starts the main axon shaft. The so-called mid-axon has generally been considered a morphological uniform region in comparison to other neuronal compartments. It would be expected, therefore, to display equal axonal distribution patterns for different cargoes. Axons, however, can extend to more than a meter in length and many different cargoes are specifically targeted to varied distant axonal locations. Under conditions of uniformity how can this be achieved? New studies are beginning to reveal local differences in the regulation of cargo motility along the mid-axon. The distal axonal terminals innervate target cells or tissues, such as muscles and skin. Here is where the supply of anterograde cellular material from the soma is finally delivered or recycled. Collective ideas that emerge from these imaging studies point to synergy between the compartmentalized organization of the cytoskeleton in the axon and the local regulation of multiple motor teams attached to one type of cargo. However, how changes in individual cargo motility properties, defined

by molecular motors, translate into cargo distributions in axons has not been extensively characterized. Recent work suggests that the minimal basic unit required for regulated cargo transport between neuronal compartments can be achieved by a single compartmentalized MAP and tuneable kinesin motor activities, where the MAP regulates the activity of different kinesin motor teams bound to the same cargo [8••]. Thus, a new layer of axonal trafficking regulation is emerging in which spatially constricted classical microtubule-associated proteins form a 'MAP code' that ensures the distribution of cargoes to appropriate axonal destinations.

Mechanisms regulating selective cargo entry from the soma into the proximal axon

The proximal axon comprises a region that spans from the transition area at the base of the soma to the end of the AIS. Traditionally, this proximal region was viewed as a gatekeeper to restrict the entry of somatodendritic cargoes into the axon [10–13]. However, new studies indicate that axonal cargo entry does not occur by default, rather, cargoes are also actively filtered into the axon [7•,8••,9•]. Structurally, the area between the soma and the AIS is defined by the localized enrichment of MAP2 and TRIM46 [8••,14]. Live-imaging studies have shown that the proximal axon can be further divided into functionally distinct regions. For example, within the AIS a Tau diffusion barrier has been described and inside the MAP2/TRIM46-defined proximal region stands the pre-axonal exclusion zone [7•]. Multiple lines of evidence indicate that the proximal axon is a critical region for regulating cargo traffic and its role in selective axonal cargo sorting and filtering are only now emerging (Figure 1).

The axon initial segment (AIS)

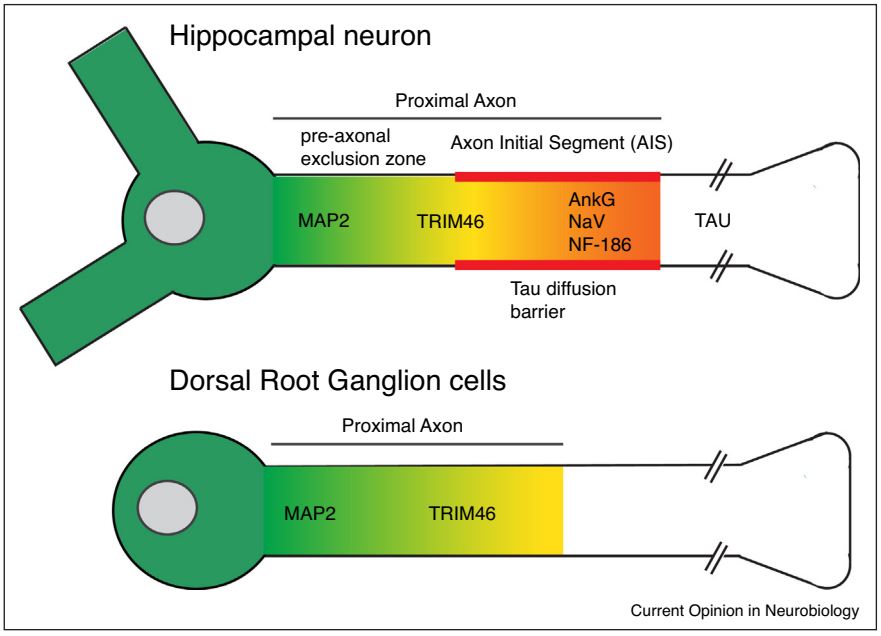
The best-studied structure in the proximal axon is the AIS. The AIS is enriched in ion-channels, scaffolding proteins, cellular adhesion molecules and cytoskeletal proteins. It is supported by a highly specialized protein network where the scaffolding protein Ankyrin-G (AnkG) is the structural hallmark of the AIS linking transmembrane proteins and βIV-spectrin to the actin and microtubule cytoskeleton [15–20]. Numerous actin structures have been described in the AIS. For example, cytosolic actin has been shown to form actin patches, actin rings and actin trails [21–25]. Microtubules play a major organizational role in the AIS and are connected to AnkG via the microtubule plus-end binding proteins EB1 and EB3 [26,27]. Microtubule bundling and lattice stabilization at the AIS is also achieved through the microtubule crosslinking factor 1 (MTCL1) [28]. The Tau diffusion barrier, located within the AIS, controls retrograde (axon-to-soma) and anterograde (soma-to-axon) traffic of Tau [9•,29]. Originally discovered for its function in retaining Tau in the axon, it now appears that the Tau diffusion barrier functions anterogradely by discriminating which Tau isoforms can enter the axon. The mechanism relies on the structural integrity of the AIS and on local microtubule dynamics within the AIS, however the precise molecular details remain unclear.

At the AIS, scaffolding and cytoskeletal proteins set up a dense meshwork underneath the plasma membrane that is essential for structuring and maintaining a functional diffusion barrier for membrane components and an intracellular filter to exclude somatodendritic cargoes from entering the axon [30]. Several models have been proposed on how microtubules, actin and associated motor proteins cooperate in filtering cargo in the proximal axon. The common theme is that kinesin motors transport cargoes into the distal axon [31]. Myosin-V can arrest kinesin-driven cargo in the AIS accumulating in distinct actin-rich hotspots [32]. Subsequently, dynein activation redirects cargoes back into the soma. Kinesin-2 family protein KIF17 is an interesting example of motor regulation at the AIS. Live-cell imaging studies showed that dendritic KIF17 autonomously enters the axon [33]. However, an interaction between KIF17-bound cargoes and the local actin cytoskeleton at the AIS prevents KIF17 vesicles from moving further into the axon. Here, activation of dynein-based motor activity deflects KIF17-coupled cargoes back into dendrites. Specific dynein activation at the AIS is potentially aided by CDK5 phosphorylation of NDEL1 that localizes to the AIS via interaction with AnkG [34,35]. Depletion of NDEL1 or LIS1 results in the axonal entry of somatodendritic cargoes. Enhanced dynein motor activity in the AIS is observed through the interaction of the small GTPase Rab5 with Fused Toes (FTS)-Hook-FTS and Hook-interacting protein (FHF) complex, which interacts with dynein and its activator dynactin to maintain the axonal retrieval of transferrin receptor and glutamate receptor types towards the soma [36]. Dynein-dependent axonal retrieval is also observed for integrins through specific localisation of EFA6 at the AIS and a pathway that involves ARF6 and Rab11 [37–39].

Pre-axonal exclusion zone

The pre-axonal exclusion zone lies within the MAP2/TRIM46-defined axonal region and was identified by live-imaging as an area where somatodendritic cargoes were re-routed to the soma before reaching the AIS [7•]. The rationale for investigating alternative axonal sorting mechanisms to the AIS was based on findings showing that during neuronal development in culture, polarized transport of somatodendritic and axonal vesicles was established before the appearance of the AIS [40]. Live-imaging screening of several different organelle markers showed that somatodendritic AMPA-type glutamate receptor GluR1, the Golgi matrix protein GM130, the *trans*-Golgi network (TGN) protein TGN38, and the endoplasmic reticulum protein CLIMP-63 were all excluded from entering the pre-axonal exclusion zone. From previous studies it has

Figure 1



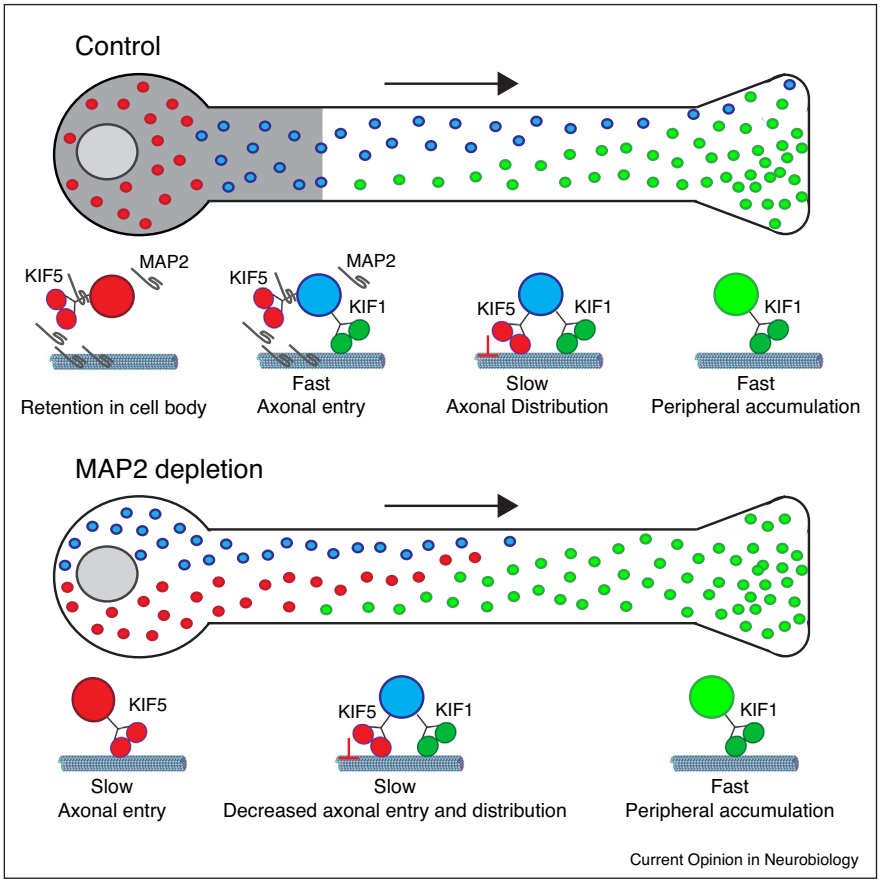
The proximal axon is a critical region for regulating cargo trafficking. The proximal axon consists of functionally and or/structurally defined subcompartments. In hippocampal neurons, the axon initial segment (AIS) generates axon potentials and is a diffusion barrier for membrane components and an intracellular filter to exclude somatodendritic cargoes from entering the axon. The pre-axonal exclusion zone lies within the MAP2/TRIM46-defined axonal region and can re-route cargos to the soma before reaching the AIS. The Tau diffusion barrier is involved in the somatodendritic sorting of different Tau isoforms. Adult dorsal root ganglia neurons lack a classical AIS but have a MAP2/TRIM46-defined axonal region that selectively filters cargoes into the axon.

become clear that the combined activity of different motors determines transport destination and selectivity. Indeed, recruiting KIF5 to these cargoes re-routed them to the axon and tubulin acetylation appeared to contribute to the specificity of KIF5. Therefore, some motor combinations are capable of moving into the axon, whereas others are not. Different individual motor proteins can have distinct preferences for the underlying microtubule cytoskeleton that might determine their selective transport to either the axon or dendrites.

MAP2-defined pre-axonal filtering zone

What motor combinations allow cargo to enter the axon? Work from adult dorsal root ganglia (DRG) neurons, that lack a classical AIS, showed that cargoes such as lysosomes are mostly retained in the soma whereas secretory vesicles or synaptic vesicles are trafficked to the axon [8••]. This selective regulation of transport depends on the localized restriction of MAP2 to the proximal axon by TRIM46 [8••,14]. Loss of MAP2 perturbs the trafficking of multiple cargoes, with some normally enriched in the axon now accumulating in the soma and vice versa. Secretory vesicles, are trafficked by the cooperative action of KIF5 and KIF1 motors in both mammalian and *Drosophila* axons [8••,41]. Analysis of their motility led to the finding that MAP2 directly inhibits KIF5 motor activity and that axonal cargo entry and distribution depends on the balanced activities between KIF5 and KIF1 bound to the same cargo [8••] (Figure 2). Thus, an axonal transport model emerges in which cargoes bound to the dominant motor KIF5 are unable to enter the axon. Cargoes bound to motors that are not influenced by MAP2 are able to enter the axon and move to the distal terminals. However, cargoes bound to KIF5 and to different motor proteins not affected by MAP2 will enter the axon but their axonal distribution will depend on the reactivation of KIF5 past the proximal axon as the inhibition by MAP2 wears off. The identification of a pre-axonal MAP2 filtering zone that is distinct from the AIS and pre-axonal exclusion zone adds to the cellular repertoire for regulating the trafficking of various types of cargo bound for different destinations in the cell. Given the many different kinesins and cargo types in neurons, it seems likely that in the proximal axon additional regulatory mechanisms exist. Other compartmentalized classical MAPs have been shown to play a major role in steering motor transport in neurons. Doublecortin (DCX) and doublecortin-like kinase (DCLK1) specifically label a subset of dendritic microtubules and are required for KIF1-dependent cargo trafficking into dendrites [31]. MAP6, for example, stabilizes microtubules in the proximal axon and promotes KIF5-dependent organelle trafficking [42].

Figure 2



Axonal cargo entry and distribution in sensory neurons. In control neurons MAP2, enriched in the proximal axon, selectively inhibits KIF5 motor activity by preventing its landing on microtubules. Cargoes bound to only KIF5 are unable to enter the axon (red). Cargoes bound to motors that are not regulated by MAP2 enter the axon and move fast to the distal terminals (green). However, cargoes bound to KIF5 and to different motor proteins, not affected by MAP2, enter the axon and are axonally distributed upon gradual reactivation of KIF5 (blue). Following knockdown of MAP2, KIF5 is active in the proximal axon and cargoes are redistributed. Cargoes that were initially retained in the soma and proximal axon, move into the axon whereas cargoes that spread along the axon become slower and are retained in the soma and the proximal axon, leading to cargo depletion distally. Thus, the presence of two distinct kinesin motors on a single vesicle and selective regulation of kinesin activity by MAP2 in the proximal axon regulates axon entry and cargo distribution.

Regulation of long-distance anterograde cargo distribution in axons

Insights into how local changes in cargo motility properties translate to full-length axonal distributions are only now coming to light. Several local microtubule and kinesin-based trafficking mechanisms in axons have been elucidated, although their significance for global cargo distribution patterns is unclear [43–46]. Likewise, the long-range 'sushi belt' circulation of dense core vesicles in Drosophila and hippocampal axons [47,48•] and mitochondria in hippocampal neurons [49] have been described, although the precise mechanism underlying their axonal distributions remains unclear. Analysis of anterogradely moving mitochondria showed that the velocity of anterograde movement varies along the length of the axon suggesting that the location within the axon can play a significant role in the distribution of axonal cargo [49]. This implies that the activity of motors is differentially regulated along the axon to ensure correct cargo transport and delivery [50].

Individual types of cargoes transported by one anterograde kinesin type are the best studied aspect of axonal transport. Recent work using live cell imaging at different spatiotemporal scales showed that the retrograde motor dynein is directly transported from the cell body to the distal terminal by kinesin-1 in a non-vesicular-dependent manner [51]. Many transport processes, however, in different cell types and organisms, are driven by multiple kinesins bound to the same cargo. In mammalian neurons, kinesin-1 and kinesin-2 are required for the transport of Adenomatous Polyposis Coli (APC) to the growth cone and kinesin-1 and kinesin-3 cooperate to distribute dense core vesicles along the full axonal length [8••,52]. Why are

multiple kinesin motor teams necessary for axonal transport since one motor alone would seem enough to transport cargo down the axon? One explanation comes from analyzing dense core vesicle motility in full-length DRG axons where it was shown that the balance between kinesin-3 'fast' and kinesin-1 'slow' velocities promotes the proper spreading of the vesicles into the distal axon. Indeed, interference with either kinesin led to aberrant vesicle distributions [8••]. Considering the length of the axon and that dense core vesicles are secretory/exocytotic organelles, docking in the wrong axonal location could have deleterious systemic effects.

Future perspectives

The incredible length of axons poses a unique cargo transport challenge to the neuron. New high and super-resolution imaging tools are allowing the identification of multiple anatomical subdomains. A recent study highlights the close interplay between the local organization of the actin and microtubule cytoskeleton and motor-driven transport in achieving axonal cargo distribution [53•]. Furthermore, live-imaging studies have revealed that transport regulation is not uniform along the whole axonal length and that the need for different cargo delivery patterns requires the sophisticated interplay between the local cytoskeleton, molecular motors and regulatory proteins. Although this could argue for increased focus on subdomain mechanisms, new studies are beginning to unravel how local changes in cargo motility impact on net axonal distributions. Nevertheless, there are still several challenges ahead. Most current studies focus on cargo trafficking at discrete time points and within varying axonal regions, giving only snapshots of transport events *in vitro*. In the future, studies applying a more systematic transport analysis approach in full length axons coupled to *in vivo* high spatiotemporal resolution imaging will provide a deeper understanding of trafficking pathways in healthy and diseased neurons.

Conflict of interest statement

Nothing declared.

Acknowledgements

This work was supported by the Netherlands Organization for Scientific Research (NWO-ALW-VICI) and the European Research Council (ERC) (ERC-consolidator).

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. Adalbert R, Coleman MP: **Review: axon pathology in age-related neurodegenerative disorders**. *Neuropathol Appl Neurobiol* 2013, **39**:90-108.

2. Lipka J, Kuijpers M, Jaworski J, Hoogenraad CC: **Mutations in cytoplasmic dynein and its regulators cause malformations of cortical development and neurodegenerative diseases**. *Biochem Soc Trans* 2013, **41**:1605-1612.

3. Millecamps S, Julien J-P: **Axonal transport deficits and neurodegenerative diseases**. *Nat Rev Neurosci* 2013, **14**:161-176.

4. Maday S, Twelvetrees AE, Moughamian AJ, Holzbaur ELF: **Axonal transport: cargo-specific mechanisms of motility and regulation**. *Neuron* 2014, **84**:292-309.

5. De Vos KJ, Hafezparast M: **Neurobiology of axonal transport defects in motor neuron diseases: opportunities for translational research?** *Neurobiol Dis* 2017, **105**:283-299.

6. Leterrier C: **The axon initial segment, 50 years later**. *Curr Topics Membr* 2016:185-233.

7. • Farías GG, Guardia CM, Britt DJ, Guo X, Bonifacino JS: **Sorting of dendritic and axonal vesicles at the pre-axonal exclusion zone**. *Cell Rep* 2015, **13**:1221-1232.
This paper established the presence of an alternative axo-dendritic cargo sorting compartment in the proximal axon termed the pre-axonal exclusion zone. Live-imaging of a large number of neuronal cargoes found that the majority was re-directed back to the soma before reaching the axon initial segment, revealing the existence of additional filtering mechanisms to the AIS.

8. •• Gumy LF, Katrukha EA, Grigoriev I, Jaarsma D, Kapitein LC, Akhmanova A, Hoogenraad CC: **MAP2 defines a pre-axonal filtering zone to regulate KIF1- versus KIF5-dependent cargo transport in sensory neurons**. *Neuron* 2017, **94**:347-362 e7.
This paper demonstrates the existence of a MAP2-dependent axonal cargo filtering region in the proximal axon that is functional even in the absence of a classical structured AIS. Live-imaging of secretory vesicles in full-length axons revealed that MAP2 regulates the activities of different kinesin motor teams bound to the same cargo to achieve axonal entry and long-range distribution.

9. • Zempel H, Dennissen FJA, Kumar Y, Luedtke J, Biernat J, Mandelkow E-M, Mandelkow E: **Axodendritic sorting and pathological missorting of Tau are isoform-specific and determined by axon initial segment architecture**. *J Biol Chem* 2017, **292**:12192-12207.
This paper describes the presence of a Tau diffusion barrier located within the AIS that is involved in the somatodendritic sorting of different Tau isoforms. Using superresolution nanoscopy and live-cell imaging it was observed that the Tau diffusion barrier depends on highly dynamic microtubules and on the integrity of the AIS. This study adds to the repertoire of cargo sorting mechanisms in the proximal axon.

10. Song A, Wang D, Chen G, Li Y, Luo J, Duan S, Poo M: **A selective filter for cytoplasmic transport at the axon initial segment**. *Cell* 2009, **136**:1148-1160.

11. Leterrier C, Dargent B: **No Pasaran! Role of the axon initial segment in the regulation of protein transport and the maintenance of axonal identity**. *Semin Cell Dev Biol* 2014, **27**:44-51.

12. Nirschl JJ, Ghiretti AE, Holzbaur ELF: **The impact of cytoskeletal organization on the local regulation of neuronal transport**. *Nat Rev Neurosci* 2017, **18**:585-597.

13. Jones SL, Svitkina TM: **Axon initial segment cytoskeleton: architecture, development, and role in neuron polarity**. *Neural Plast* 2016, **2016**:6808293.

14. van Beuningen SFB, Will L, Harterink M, Chazeau A, van Battum EY, Frias CP, Franker MAM, Katrukha EA, Stucchi R, Vocking K *et al.*: **TRIM46 controls neuronal polarity and axon specification by driving the formation of parallel microtubule arrays**. *Neuron* 2015, **88**:1208-1226.

15. Grubb MS, Burrone J: **Activity-dependent relocation of the axon initial segment fine-tunes neuronal excitability**. *Nature* 2010, **465**:1070-1074.

16. Rasband MN: **The axon initial segment and the maintenance of neuronal polarity**. *Nat Rev Neurosci* 2010, **11**:552-562.

17. Bennett V, Lorenzo DN: **Spectrin- and ankyrin-based membrane domains and the evolution of vertebrates**. *Curr Top Membr* 2013:1-37.

18. 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.

19. Jenkins PM, Kim N, Jones SL, Tseng WC, Svitkina TM, Yin HH, Bennett V: **Giant ankyrin-G: a critical innovation in vertebrate evolution of fast and integrated neuronal signaling**. *Proc Natl Acad Sci U S A* 2015, **112**:957-964.

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

21. Watanabe K, Al-Bassam S, Miyazaki Y, Wandless TJ, Webster P, Arnold DB: **Networks of polarized actin filaments in the axon initial segment provide a mechanism for sorting axonal and dendritic proteins**. *Cell Rep* 2012, **2**:1546-1553.

22. Xu K, Zhong G, Zhuang X: **Actin, spectrin, and associated proteins form a periodic cytoskeletal structure in axons**. *Science (80-)* 2013, **339**:452-456.

23. 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.

24. 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.

25. Qu Y, Hahn I, Webb SED, Pearce SP, Prokop A: **Periodic actin structures in neuronal axons are required to maintain microtubules**. *Mol Biol Cell* 2017, **28**:296-308.

26. Leterrier C, Vacher H, Fache M-P, d'Ortoli SA, Castets F, Autillo-Touati A, Dargent B: **End-binding proteins EB3 and EB1 link microtubules to ankyrin G in the axon initial segment**. *Proc Natl Acad Sci* 2011, **108**:8826-8831.

27. Freal A, Fassier C, Le Bras B, Bullier E, De Gois S, Hazan J, Hoogenraad CC, Couraud F: **Cooperative interactions between 480 kDa ankyrin-G and EB proteins assemble the axon initial segment**. *J Neurosci* 2016, **36**:4421-4433.

28. Satake T, Yamashita K, Hayashi K, Miyatake S, Tamura-Nakano M, Doi H, Furuta Y, Shioi G, Miura E, Takeo YH *et al.*: **MTCL1 plays an essential role in maintaining Purkinje neuron axon initial segment**. *EMBO J* 2017, **36**:1227-1242.

29. Li X, Kumar Y, Zempel H, Mandelkow E-M, Biernat J, Mandelkow E: **Novel diffusion barrier for axonal retention of Tau in neurons and its failure in neurodegeneration**. *EMBO J* 2011, **30**:4825-4837.

30. 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.

31. Lipka J, Kapitein LC, Jaworski J, Hoogenraad CC: **Microtubule-binding protein doublecortin-like kinase 1 (DCLK1) guides kinesin-3-mediated cargo transport to dendrites**. *EMBO J* 2016, **35**:302-318.

32. Janssen AFJ, Tas RP, van Bergeijk P, Oost R, Hoogenraad CC, Kapitein LC: **Myosin-V induces cargo immobilization and clustering at the axon initial segment**. *Front Cell Neurosci* 2017, **11**:260.

33. Franker MA, Esteves da Silva M, Tas RP, Tortosa E, Cao Y, Frias CP, Janssen AFJ, Wulf PS, Kapitein LC, Hoogenraad CC: **Three-step model for polarized sorting of KIF17 into dendrites**. *Curr Biol* 2016, **26**:1705-1712.

34. Klinman E, Tokito M, Holzbaur ELF: **CDK5-dependent activation of dynein in the axon initial segment regulates polarized cargo transport in neurons**. *Traffic* 2017, **18**:808-824.

35. Kuijpers M, van de Willige D, Freal A, Chazeau A, Franker MA, Hofenk J, Rodrigues RJC, Kapitein LC, Akhmanova A, Jaarsma D *et al.*: **Dynein regulator NDEL1 controls polarized cargo transport at the axon initial segment**. *Neuron* 2016, **89**:461-471.

36. Guo X, Farías GG, Mattera R, Bonifacino JS: **Rab5 and its effector FHF contribute to neuronal polarity through dynein-dependent retrieval of somatodendritic proteins from the axon**. *Proc Natl Acad Sci* 2016, **113**:E5318-E5327.

37. Franssen EHP, Zhao R-R, Koseki H, Kanamarlapudi V, Hoogenraad CC, Eva R, Fawcett JW: **Exclusion of integrins from CNS axons is regulated by Arf6 activation and the AIS**. *J Neurosci* 2015, **35**:8359-8375.

38. Koseki H, Donegá M, Lam BY, Petrova V, van Erp S, Yeo GS, Kwok JC, Ffrench-Constant C, Eva R, Fawcett JW: **Selective rab11 transport and the intrinsic regenerative ability of CNS axons**. *Elife* 2017:6.

39. Eva R, Koseki H, Kanamarlapudi V, Fawcett JW: **EFA6 regulates selective polarised transport and axon regeneration from the axon initial segment**. *J Cell Sci* 2017, **130**:3663-3675.

40. Petersen JD, Kaech S, Banker G: **Selective microtubule-based transport of dendritic membrane proteins arises in concert with axon specification**. *J Neurosci* 2014, **34**:4135-4147.

41. Lim A, Rechtsteiner A, Saxton WM: **Two kinesins drive anterograde neuropeptide transport**. *Mol Biol Cell* 2017, **28**:3542-3553.

42. Tortosa E, Adolfs Y, Fukata M, Pasterkamp RJ, Kapitein LC, Hoogenraad CC: **Dynamic palmitoylation targets MAP6 to the axon to promote microtubule stabilization during neuronal polarization**. *Neuron* 2017, **94**:809-825 e7.

43. Kevenaar JT, Bianchi S, van Spronsen M, Olieric N, Lipka J, Frias CP, Mikhaylova M, Harterink M, Keijzer N, Wulf PS *et al.*: **Kinesin-binding protein controls microtubule dynamics and cargo trafficking by regulating kinesin motor activity**. *Curr Biol* 2016, **26**:849-861.

44. Tanaka Y, Niwa S, Dong M, Farkhondeh A, Wang L, Zhou R, Hirokawa N: **The molecular motor KIF1A transports the TrkA neurotrophin receptor and is essential for sensory neuron survival and function**. *Neuron* 2016, **90**:1215-1229.

45. Dey S, Banker G, Ray K: **Anterograde transport of Rab4-associated vesicles regulates synapse organization in drosophila**. *Cell Rep* 2017, **18**:2452-2463.

46. Farías GG, Guardia CM, De Pace R, Britt DJ, Bonifacino JS: **BORC/kinesin-1 ensemble drives polarized transport of lysosomes into the axon**. *Proc Natl Acad Sci U S A* 2017, **114**: E2955-E2964.

47. Wong MY, Zhou C, Shakiryanova D, Lloyd TE, Deitcher DL, Levitan ES: **Neuropeptide delivery to synapses by long-range vesicle circulation and sporadic capture**. *Cell* 2012, **148**:1029-1038.

48. • Bharat V, Siebrecht M, Burk K, Ahmed S, Reissner C, Kohansal-Nodehi M, Steubler V, Zweckstetter M, Ting JT, Dean C: **Capture of dense core vesicles at synapses by JNK-dependent phosphorylation of synaptotagmin-4**. *Cell Rep* 2017, **21**:2118-2133.

This paper shows long-range circular trafficking pattern for dense core vesicles in hippocampal axons and describes a synaptotagmin-4-dependent mechanism by which these vesicles are captured at synaptic sites by switching from microtubule-dependent transport to capture at en passant presynaptic boutons by actin.

49. Niescier RF, Kwak SK, Joo SH, Chang KT, Min K-T: **Dynamics of mitochondrial transport in axons**. *Front Cell Neurosci* 2016, **10**:123.

50. Bressloff PC, Levien E: **Synaptic democracy and vesicular transport in axons**. *Phys Rev Lett* 2015, **114**:168101.

51. Twelvetrees AE, Pernigo S, Sanger A, Guedes-Dias P, Schiavo G, Steiner RA, Dodding MP, Holzbaur ELF: **The dynamic localization of cytoplasmic dynein in neurons is driven by kinesin-1**. *Neuron* 2016, **90**:1000-1015.

52. Ruane PT, Gumy LF, Bola B, Anderson B, Wozniak MJ, Hoogenraad CC, Allan VJ: **Tumour suppressor adenomatous polyposis coli (APC) localisation is regulated by both kinesin-1 and kinesin-2**. *Sci Rep* 2016, **6**:27456.

53. • Yogev S, Cooper R, Fetter R, Horowitz M, Shen K: **Microtubule organization determines axonal transport dynamics**. *Neuron* 2016, **92**:449-460.

This paper uses live-imaging of cargo transport and microtubule dynamcs in *C. elegance* motor axons to determine how microtubule organization impacts on axonal trafficking.