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Organelle distribution in neurons: Logistics behind polarized transport

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Abstract

Highly polarized neurons need to carefully regulate the distribution of organelles and other cargoes into their two morphologically and functionally distinct domains, the somatodendritic and axonal compartments, to maintain proper neuron homeostasis. An outstanding question in the field is how organelles reach their correct destination. Long-range transport along microtubules, driven by motors, ensures a fast and controlled availability of organelles in axons and dendrites, but it remains largely unclear what rules govern their transport into the correct compartment. Here, we review the emerging concepts of polarized cargo trafficking in neurons, highlighting the role of microtubule organization, microtubule-associated proteins, and motor proteins and discuss compartment-specific inclusion and exclusion mechanisms as well as the regulation of correct coupling of cargoes to motor proteins.

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Neurons, Polarized transport, MT organization, MAPs, Motors, Motor adaptors, Organelles, Proximal axon, AIS, PAEZ, Organelle contacts.

Introduction

Neurons are highly polarized and compartmentalized cells with two morphologically distinguished subdomains, the somatodendritic and the axonal domain. It is well known that organelles have a specific distribution in neurons. For example, somatodendritic vesicles, rough endoplasmic reticulum (ER), Golgi, and Golgi outposts are present in the somatodendritic domain and not found in the axon, whereas lysosomes, mitochondria, and smooth ER are present in both somatodendritic and axonal domains, and presynaptic precursor vesicles are mainly distributed along the axon [1]. The transport of these organelles and other cellular cargoes into specific neuronal domains must be finely regulated to fulfill local demands for proper neuronal function. Alternatively, dysregulation of this process may contribute to neurological disease pathogenesis [2].

However, it remains unclear how organelles are transported into dendrites or the axon. What are the mechanisms that regulate the entrance of somatodendritic and axonal organelles into the correct domain? In this review, we will mainly discuss recent studies that show complementary mechanisms are in place to ensure the highly organized and compartmentalized distribution of organelles in neurons. These mechanisms include (1) a differential microtubule (MT) array, MT stability, and association to different MT-associated proteins (MAPs), which allow molecular motors to bind MTs with different affinities; (2) exclusion and inclusion mechanisms of organelles into the axon and dendrites; and (3) the regulation of motor–cargo recognition by adaptor proteins and interorganelle interactions.

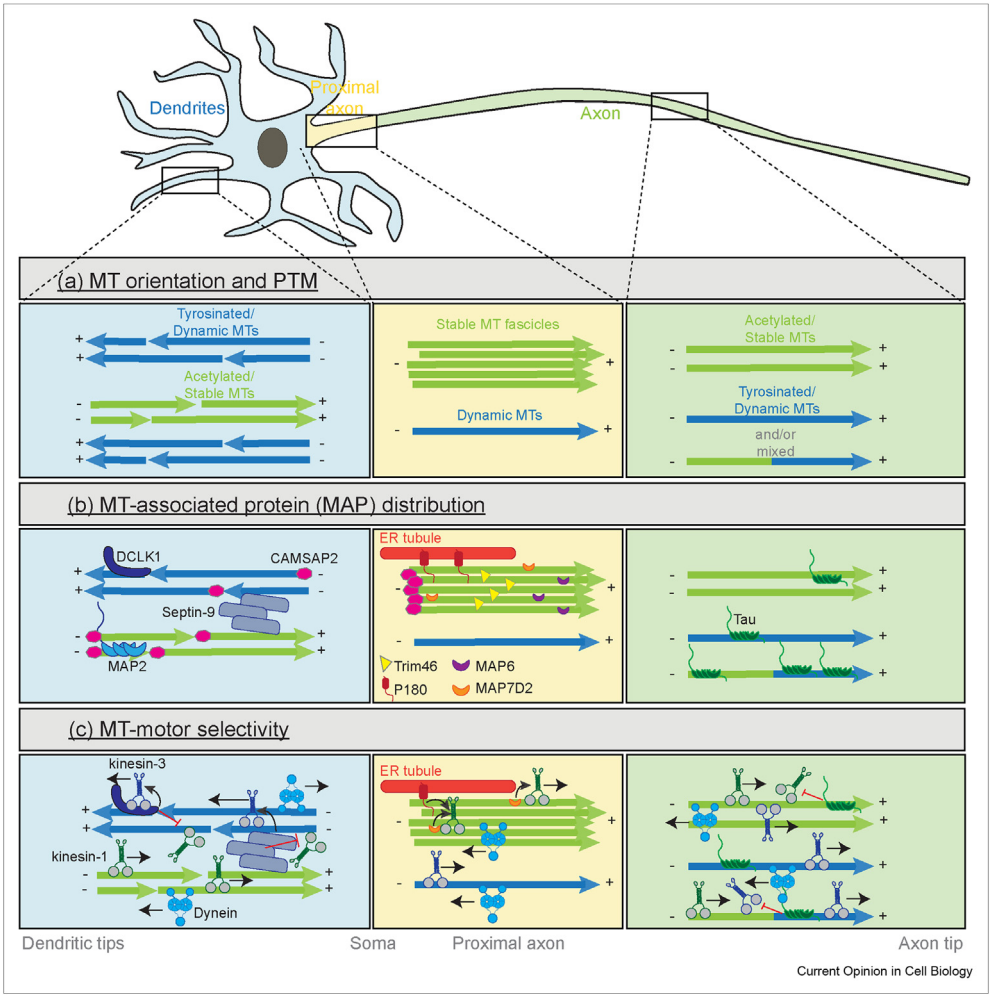
How motors navigate MT diversity for polarized cargo transport

From lower organisms to highly complex mammalian neurons, it has been well documented that the orientation of MTs differs between the axon and dendrites. In mammalian neurons, axonal MTs are oriented with the growing plus-end-out, whereas dendritic MTs have an equally mixed orientation, with both plus-end and minus-end out MTs [3,4]. In addition to this differential MT orientation, MTs in these compartments have different post-translational modifications, which are associated with more stable or dynamic MT arrays. The axon is featured by the presence of more acetylated/detyrosinated MTs (stable MTs), whereas dendrites contain two different pools of MTs—acetylated/detyrosinated MTs and tyrosinated (dynamic) MTs [3,4]. How do the many different kinesin and cytoplasmic dynein motors present in neuronal cells coordinate cargo trafficking and navigate along these complex arrays of MTs? [5]. Initial studies using motor translocation assays have shown that most kinesin motor domains

accumulate in axon tips, whereas only a few of them, including kinesin-3 members, also accumulate in dendritic tips [6].

An outstanding question is how the MT array, diversity of MTs, and MT motor selection contribute to the polarized transport of organelles. Recent studies have revealed how motors navigate the diverse MTs in neurons. Tas et al. developed a novel motor–based nanoscopy technique termed motor-PAINT, in which the incorporation of purified and fluorescently labeled *Drosophila* kinesin heavy chain plus-end motor proteins in neurons, after extraction and fixation, allowed the study of motor navigation in relation to MT orientation, modifications, and stability [3]. This revealed an unanticipated dendritic MT array, spatially segregated into bundles by orientation and modifications, in which minus-end-out–oriented MT bundles are more stable and acetylated, whereas the plus-end-out MT bundles are more dynamic and tyrosinated. The use of kinesin-1 and kinesin-3 rigor mutants, which can bind to MTs but not dissociate from nor walk along them, revealed how kinesin-1 highly prefers more stable MT bundles with a minus-end-out orientation, whereas kinesin-3 binds more to dynamic MT bundles with a plus-end-out orientation [3]. These observations could explain how kinesin-1 selectively transports cargoes into the axon and why kinesin-3 can transport cargoes into both

Figure 1



Regulation of polarized transport by MTs, MAPs, and motor proteins. Polarized transport of organelles/cargoes into dendrites and axons is determined by several mechanisms. (**a**) Diversity in MT orientation, post-translational modifications (PTMs) and stability in dendrites, the proximal axon, and the distal axon. The exact array of dynamic and stable MTs is less clear in the axon. (**b**) Various microtubule-associated proteins (MAPs) have a specific distribution in each neuronal subdomain. This figure panel shows examples of conventional and unconventional MAPs with a highly polarized distribution. (**c**) The landing of motor proteins to specific MTs and their processivity can be coordinated by MAPs. Both DCLK1 and Septin-9 have been shown to promote kinesin-3 transport into dendrites while they inhibit kinesin-1 loading, possibly preventing kinesin-1 from landing on dynamic MTs. Dynein likely binds and travels along both stable and dynamic MTs in both dendrites and axons. MAP7D2 and the ER-resident protein P180 are able to bind to both MTs and kinesin-1 and thereby possibly promote landing of kinesin on stable MTs in a preaxonal region. In more distal axons, Tau can bind both stable and dynamic microtubules and affect MT landing of both kinesin-1 and kinesin-3.

dendrites and the axon. Moreover, although initial binding of dynein to MTs has been shown to be facilitated by MT tyrosination *in vitro* and along axonal MTs [7,8], they can likely also bind to and travel along stable MTs. Together with the finding of spatially segregated MT arrays with a mixed orientation in dendrites, this could explain why cargoes that bind to both kinesin-1 and dynein do not accumulate in the tips of dendrites after kinesin-1 disruption in mammalian neurons (Figure 1).

Considering that axonal MTs are mostly plus-end-out-oriented, highly acetylated, and stable, it remains unknown whether mixed properties or spatially segregated bundles account for the navigation of kinesin-1 and kinesin-3 motors into the axon. Mapping how the ±15–20 identified kinesins navigate dendritic and axonal MTs with high spatiotemporal resolution will help to understand how MT motor recognition guides the transport of specific cargoes into the different neuronal domains.

MAPs: adding clues to MT-driven motors for polarized cargo transport

In addition to the intrinsic properties of MTs, MT diversity is further expanded by extrinsic factors that can associate with specific sets of MTs, called MAPs. Diverse canonical and noncanonical MAPs decorate MTs and show domain-specific spatiotemporal expression and distribution. In polarized neurons, the MAPs, MAP2 and Tau, are classically used as markers for dendrites and the axon, respectively. Recent studies have started to uncover the distribution of several other MAPs, showing that they can have a preferential distribution along dendrites or the proximal and distal axon (Figure 1).

Although initial work has described MAPs as MT-stabilizing factors, recent studies have proven that they can have additional functions. For instance, MAPs act as cytoskeletal crosslinkers, organize specialized MT arrays, connect MTs and membranes, and regulate motor binding, activity, and processivity on MTs [9]. The currently emerging concept is that a "MAP code" has the capacity to bias directed transport along specific sets of MTs [9,10]. Supporting this model, recent findings have revealed important roles for MAPs in loading or preventing the landing of motor proteins onto specific sets of MTs in different neuronal domains (Figure 1). For instance, doublecortin-like kinesin-1 (DCLK1) guides kinesin-3-mediated cargo transport in dendrites along tyrosinated MT bundles and was also found to inhibit kinesin-1 binding *in vitro* [10,11]. In addition, Septin-9, which binds MTs at proximal dendrites, selectively enhances the motility of the kinesin-3 member KIF1A while preventing kinesin-1 from entering dendrites. This is probably achieved by preventing its interaction with dynamic plus-end-out-oriented MTs, as in intact dendrites, kinesin-1 prefers stable minus-end-out-oriented MTs [3,4,12]. The MAP7 member MAP7D2 locally promotes kinesin-1-mediated cargo transport into the axon by inducing the recruitment of kinesin-1 to MTs at the proximal axon [13]. MAP6, also enriched in the proximal axon, mediates MT stabilization, allowing the efficient trafficking of organelles into the axon [14]. Although most of the identified MAPs are cytosolic, the association of MAPs to membranous compartments has also been observed. MAP6 shuttles between its association to Golgi-derived vesicles and axonal MTs [14], and the ER-resident and MT-associated protein P180 binds to and stabilizes MT at the proximal axon [15].

It remains unknown how different MAPs localize to the distinct neuronal compartments and how the local distribution contributes to motor-driven polarized organelle transport. Some studies have shown that MAP phosphorylation controls dissociation from MTs, MAP relocation within the cell, or can alter MAP function [16]. In addition, it was shown that palmitoylation of MAP6 controls its shuttling between vesicles and local recruitment onto MTs at the proximal axon [14]. Other studies have proposed that competition between MAPs determines the correct distribution and balance of motor activity [17].

Thus, both the distribution of specific MAPs in each neuronal compartment and the relative abundance of MAPs can contribute to polarized transport by regulating motor protein binding to MTs.

Regulation of MT motor selectivity by cargo binding

Although the motor domain of kinesins and their binding to specific set of MTs is essential for selective organelle translocation, the current 'smart motor' model, in which the motor domain defines translocation selectivity, has recently been revised in light of new findings. For instance, the tail domain of the kinesin-3 member KIF13A was shown to bind transferrin receptor (TfR) carrier vesicles, which are restricted to the somatodendritic domain [18]. Full-length KIF13A expression displays a somatodendritic distribution, whereas its motor domain alone is translocated mainly into the axon, and its tail domain is preferentially distributed in dendrites [6,18,19]. Another example is the kinesin-2 member KIF17, which is distributed in the somatodendritic domain and binds somatodendritic vesicles containing N-methyl-D-aspartate (NMDA) receptors, but its motor domain has a preferential axonal translocation [18,20,21]. These examples suggest a 'cargo-steering' model, in which selective motor translocation into the correct neuronal compartment is not solely defined by the properties of its motor domain but can be modified by cargo regulation.

It remains unknown how cargo–tail interactions might influence the efficiency of motor proteins to interact with MTs and translocate. The development of novel techniques to track only activated motor–cargo navigation and compare it with motor domain translocation with high spatiotemporal precision is required to advance our knowledge of how motor–cargo interactions regulate polarized trafficking of different sets of vesicles and organelles. It will be particularly interesting to elucidate the trafficking rules for a single cargo binding to different motors, as well as for different cargoes recognized by same motor.

Mechanisms of organelle exclusion and inclusion at the proximal axon

Given that dendrite-entering kinesins can also enter the axon, additional mechanisms must be in place to prevent axonal entry of dendritic cargoes. This could include cargo regulation, as more than one motor protein is able to interact with a single cargo. In addition to this, the axon initial segment (AIS) is known to act as a surface diffusion barrier and an intracellular traffic filter that prevents the entrance of somatodendritic proteins into the axon [22,23]. Several models have been proposed, in which actin filaments at the AIS halt somatodendritic vesicles and serve as tracks for retrograde myosin-driven cargo transport [24,25]. However, although myosin-V accumulated in actin-rich hotspots at the AIS has been shown to arrest kinesin-driven cargoes, it does not transport them back to the soma [26]. Instead, after the arrest of cargoes, local activation of the minus-end–directed motor dynein could then retrieve cargoes into the soma aided by the dynein regulator NDEL1 through its recruitment and localization to the AIS by the AIS master regulator Ankyrin-G [27]. Accordantly, KIF17 relieves autoinhibition after cargo binding and targets cargoes into both dendrites and the axon, but in the AIS, they are halted and retrogradely transported to the soma by dynein to relocate cargo into dendrites [21]. Additional mechanisms have been observed that ensure the retrieval of somatodendritic vesicles from more distal axons [28].

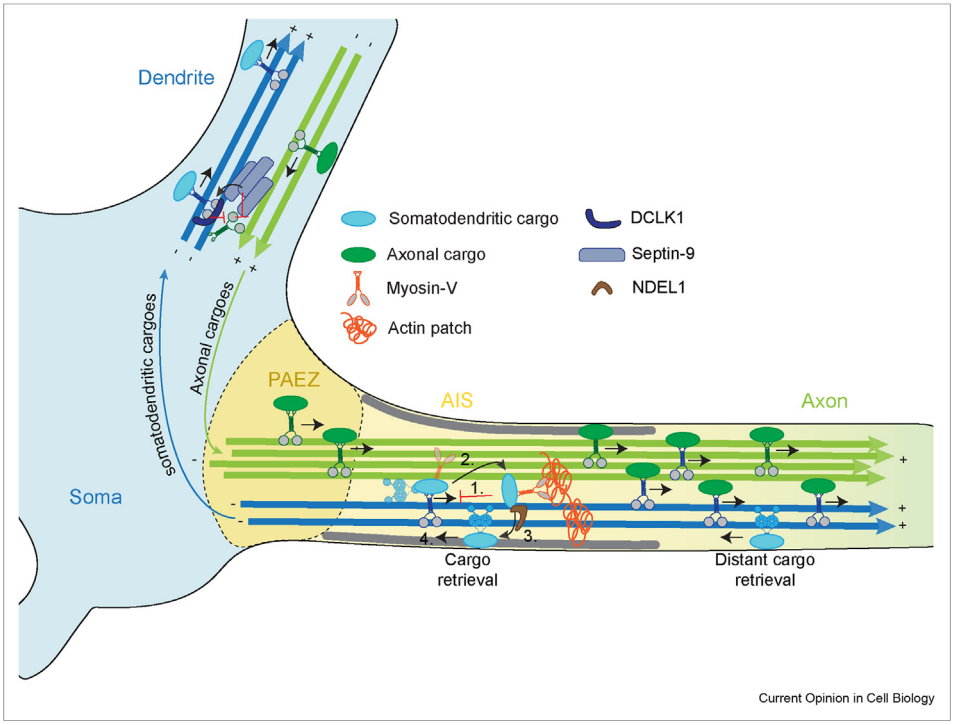
If the main barrier for somatodendritic vesicles to access the axon is located in the AIS and there is an equal targeting of motor-coupled cargoes into dendrites and the axon, it would be expected that somatodendritic cargoes can actively enter the AIS at the same rate as they enter proximal dendrites. However, most somatodendritic vesicles, as well as the Golgi and the rough ER, are already excluded from the axon at a region preceding the AIS, called the preaxonal exclusion zone (PAEZ) or axon hillock [1,29,30]. A comparative analysis of the sorting of somatodendritic TfR-containing vesicles showed that approximately 84.5% of these vesicles are transported directly into dendrites, and only 15.5% of the vesicles reach the proximal axon, where they can either return to the soma or travel on more distally [30]. The PAEZ, devoid of somatodendritic organelles, is the region in which bundles of closely and regularly spaced MTs, termed MT fascicles, are organized and enter the AIS [31,32]. It is characterized by the presence of the acentrosomal minus-end-bundling protein CAMSAP2, as well as by the plus-end MT-bundling protein TRIM46, which organizes parallel MT fascicles and is also present in the AIS [33,34]. TRIM46-dependent fasciculation of parallel MTs could regulate cargo inclusion into the axon, as TRIM46 disruption has been shown to reduce cargo transport into the axon [33,34]. Kinesin-1 binds with high affinity to these MT fascicles in the proximal axon, as shown by the preferential binding site of the kinesin-1 rigor mutant [30]. Coupling of a somatodendritic cargo to a kinesin-1-binding protein is sufficient to drive the transport of a somatodendritic vesicle containing many cargoes through the proximal axon toward the distal axon [30]. Together, this suggests that the initial sorting of organelles/cargoes into the axon starts at the proximal axon, where MT fascicles could serve as specialized tracks for the inclusion and transport of organelles into the axon.

We propose that many different exclusion and inclusion mechanisms are in place to ensure the correct distribution of organelles into the axon, in which somatodendritic cargoes that escape sorting at the proximal axon can be halted and retrieved from the AIS back into the somatodendritic domain. The presence of a unique MT array at the proximal axon can promote efficient axonal motor-dependent organelle translocation into the axon (Figure 2). In dendrites, a physical barrier has not been identified; however, organelle exclusion and inclusion models have also been proposed, including the previously mentioned preferred binding of kinesin-1 to minus-end-out-oriented MTs and the role of MAPs in promoting or preventing motor landing in dendritic MTs (Figures 1 and 2).

Mechanisms of motor–cargo recognition for polarized cargo transport

Adaptor proteins together with GTPases link cargoes to the tail domain of motor proteins for cargo transport. Although several adaptor proteins and GTPases have been identified, most of these studies have focused on the transport of a specific cargo-containing vesicle [5,35]. For organelles that are actively transported by different motors to both dendrites and the axon, such as mitochondria and lysosomes, the role of adaptor proteins is crucial to regulate cargo–motor selectivity (Figure 3). The GTPase Miro and the adaptor protein TRAK (TRAK 1/2) link kinesin-1 and dynein motors to the mitochondrial membrane, thereby regulating mitochondrial transport. TRAK2 preferentially associates to dynein and is more abundant in dendrites, whereas TRAK1 is preferentially distributed in the axon and binds kinesin-1 for

Figure 2

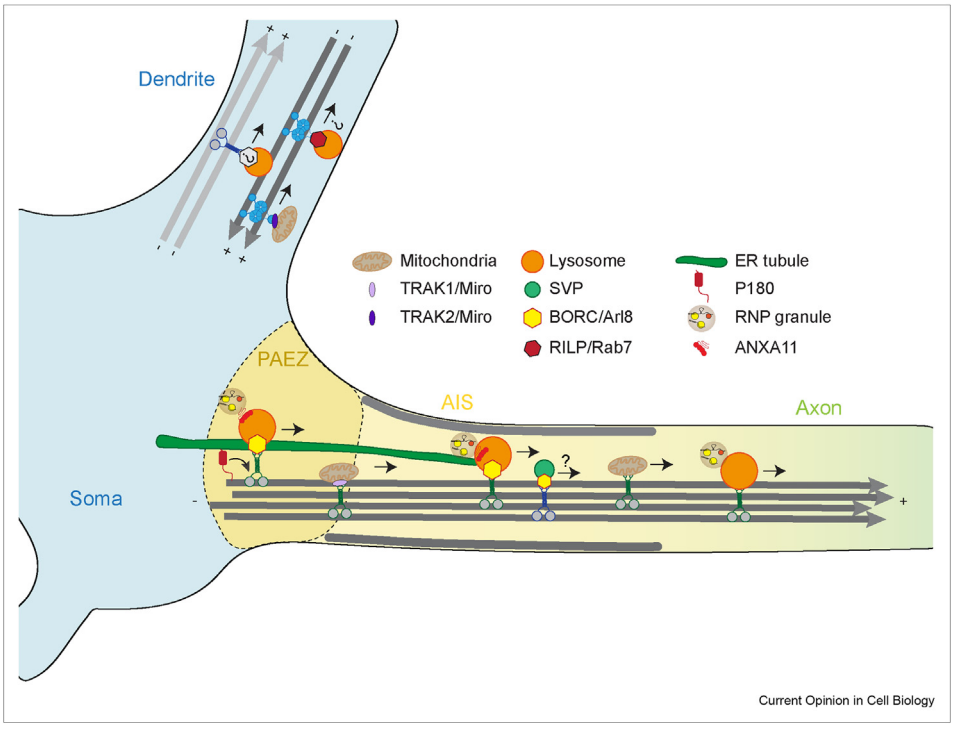


Inclusion and exclusions mechanisms regulating neuronal cargo distribution. Several inclusion and exclusion mechanisms exist in both dendrites and axons that ensure the proper distribution of organelles/cargoes in neurons. In dendrites, exclusion of kinesin-1-bound axonal cargoes is achieved by the minus-end-out orientation of stable MTs and via inhibition of MT landing by MAPs such as DCLK1 and Septin-9. Conversely, there is an inclusion of kinesin-3-dependent cargoes into dendrites mediated by MT organization and MAPs. Kinesin-1-dependent axonal cargoes preferentially bind to stable MT bundles formed at the preaxonal exclusion zone (PAEZ). Somatodendritic cargoes are sorted at the PAEZ, in which cargoes lacking the binding to an axonal motor are prevented from entering the axon. Somatodendritic cargoes that escape this sorting are halted at actin patches (1) in the AIS via their association with Myosin-V (2). NDEL1 subsequently activates local dynein (3) which returns escaped somatodendritic cargoes back to the somatodendritic domain (4). Somatodendritic cargoes can also be retrieved from more distal axonal regions and can be transported back toward the soma by dynein.

anterograde transport into the axon. TRAK1 has been shown to enhance efficient transport even in crowded environments through a direct interaction with MTs [36,37]. For lysosomes, the adaptor protein complex BORC and the GTPase Arl8 ensure the polarized transport of lysosomes into the axon by linking lysosomes to the kinesin-binding protein SKIP, which binds to the kinesin light chain—kinesin-1 motor [38,39]. The regulation of lysosome transport into dendrites is less clear, but they could be transported along stable minus-end-out—oriented MTs by dynein, mediated by the recruitment of the adaptor protein RILP and the GTPase Rab7 to the lysosomal membrane. Alternatively, kinesin-3 could transport lysosomes along dynamic MTs oriented plus-end-out, mediated by recruitment of BORC and Arl8 [40,41], although this is unlikely because BORC and Arl8 disruption mainly affect axonal transport of lysosomes [38]. Interestingly, BORC and Arl8 have also been found to play a role in the axonal transport of the kinesin-3-dependent synaptic vesicle precursors (SVPs) in *C. elegans* neurons [42], which raises the question if two different kinesins, kinesin-1 and kinesin-3, can use the same adaptor complex to couple lysosomes and SVPs, respectively, or whether they are transported together in the same compartment. Studies in mouse and rat hippocampal neurons showed that SVPs are transported into the axon in lysosome-related compartments, in which 25%—85% of lysosome and SVPs markers are co-transported [39,43]. However, neurons from a BORC KO mouse model showed that lysosomes but not SVPs required BORC for anterograde transport into the axon [39], suggesting other adaptor proteins could compensate or play a more prominent role in the transport of SVPs into the axons of mammalian neurons. From these two studies, it remains unknown if the 25%—85% co-transported markers correspond to a single organelle with mixed protein composition and whether or not they are binding to both kinesin-1 and kinesin-3 motors using the same adaptor proteins. The tracking of endogenous markers using gene-editing technology combined with super-resolution imaging would help to clarify the nature of these co-transported cargoes, including which motors and adaptors are involved [44].

An often-overlooked type of motor—cargo regulation is the role of interorganelle interactions in organelle

Figure 3



Adaptor proteins and interorganelle interactions regulate polarized transport into dendrites and the axon. Various organelle—adaptor protein—motor protein complexes have been identified in neurons. This figure highlights mechanisms of polarized organelle-motor coupling that enable their entrance into dendrites or the axon. The ER, mitochondria, lysosomes, and RNP granules are distributed in both neuronal domains, suggesting a fine regulation is in place to meet local demands. For instance, mitochondria are transported into dendrites via the TRAK2/Milton complex and dynein, whereas TRAK1/Milton couples mitochondria to kinesin-1 for their anterograde transport into the axon. Lysosomes are transported into axons, but not into dendrites, via coupling to the BORC/Arl8/SKIP complex and kinesin-1. The mechanism of lysosome transport into dendrites is unclear but this could be mediated via dynein and RILP/Rab7 or via kinesin-3 and an unknown adaptor. Both RNP granules (via ANXA11) and ER tubules use hitchhiking on lysosomes for their axonal translocation. Synaptic vesicle precursors possibly use kinesin-3 and the BORC/Arl8 complex for axonal transport in mammalian neurons.

transport (Figure 3). In different cell types, including neurons, several organelles frequently form dynamic contacts with each other, without fusing their membranes [45–47]. Emerging evidence has shown that these interorganelle contacts play key roles in organelle transport [48,49]. For instance, the ER-resident protein protrudin acts as a receptor for kinesin-1 and transfers this motor onto lysosomes for proper lysosome translocation [50]. Another study recently showed that ER tubules promote translocation of lysosomes from the soma into the axon mediated by an interaction between P180, kinesin-1, and MT fascicles at the proximal axon [51]. ER tubules themselves are transported into the axon by kinesin-1 [15], but adaptor proteins coupling the ER to motor proteins have not been identified. Interestingly, recent studies have also shown that the ER can 'hitchhike' on endosomes or lysosomes using their motors and adaptors, in fungi, mammalian cell lines, and neurons [48,52,53]. This 'hitchhiking' on endolysosomes using specific adaptor proteins was also shown to drive the transport of RNA granules [54,55] and miRNAs [56] into axons.

Future perspectives

As outlined here, various mechanisms and a large number of molecular players operate together to ensure proper polarized transport of organelles and cargoes into the different neuronal domains. However, our knowledge on selective organelle/cargo transport in neurons remains fragmented, with only a few known examples that highlight the complexity of polarized organelle transport. The development of novel techniques to visualize active motors at single-molecule level on intact neuronal MTs and their correlation with MT modifications and distribution of MAPs will be required to uncover the trafficking rules for all neuronal motors. More studies are necessary to identify which motors, adaptor proteins, and GTPases are bound to specific organelles in the different neuronal compartments. This could be achieved using recently developed proximity labeling

techniques such as APEX2 and BioID and their derivatives, possibly in combination with (microfluidic) devices to separate neuronal compartments [57–59]. In addition, it has become clear that organelles should no longer be studied in isolation because a fine coordination and interplay between them plays an essential role in polarized transport important for neuronal development and function.

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Conflict of interest statement

Nothing declared.

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