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Axon and dendritic trafficking

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Neuronal trafficking is crucial to the formation and dynamics of presynaptic and postsynaptic structures and the development and maintenance of axonal and dendritic processes. The mechanism for delivering specific organelles and synaptic molecules in axons and dendrites primarily depends on molecular motor proteins that move along the cytoskeleton. Adaptor proteins, regulatory molecules and local signaling pathways provide additional layers of specificity and control over bidirectional movement, polarized transport and cargo delivery. Here we review recent advances and emerging concepts related to the transport machinery of crucial neuronal components, such as mitochondria and presynaptic cargoes, and the mechanisms that modulate their polarized axo-dendritic sorting and synaptic delivery.

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Introduction

More than a century ago Spanish histologist Ramon y Cajal pointed out that neurons are highly polarized cells, with several dendrites and a single long axon. The dendrites are short and highly branched and receive information from other neurons, while the axon delivers information and typically extends long distances to contact other neurons or muscle cells. Since axons and dendrites are functionally completely different, they require different sets of specific building blocks and cellular organelles, such as postsynaptic receptors in dendrites and synaptic vesicle precursors (SVPs) in the axon. Nowadays there is good evidence that neurons employ active transport driven by motor proteins to sort cargoes between axons and dendrites and to deliver them to synaptic sites [1,2].

The molecular mechanism of cargo trafficking in neurons is quite complex and not fully understood. The basic transport processes are clear: microtubule-based transport mainly facilitates the long-range transport into distal axons and dendrites, whereas actin-based transport is important for short-range trafficking and local delivery of cargoes to synapses and growth cones. The actin cytoskeleton facilitates motility of motor proteins of the myosin family, whereas microtubules serve as tracks for two families of motor proteins, the kinesins and dyneins, which move toward the microtubule plus-end or minus-end, respectively [3–5]. Recent studies demonstrated that the inherent microtubule polarity provides the fundamental sorting routes for polarized cargoes in neurons. Axonal targeting of cargoes is governed by the uniformly oriented plus-end distal microtubules allowing kinesin-based transport, whereas dynein-dependent cargo sorting to dendrites is facilitated by the minus-end distal-oriented microtubules exclusively present in dendrites [6]. In dendrites of mammalian neurons, the microtubules coalesce into bundles of mixed polarity and a single motor type could mediate bidirectional cargo transport by switching opposite polarity microtubules, which raises interesting models with regard to neuronal trafficking rules [1]. The mechanisms that define the microtubule organization in axons and dendrites [7,8•,9] and how microtubule structure, stability and dynamics affect neuronal development are emerging fields of research [10–13].

Here, we will review the regulatory mechanisms for controlling axonal and dendritic trafficking. We will focus on distinct classes of neuronal transport cargoes that are crucial for synapse formation and neuronal homeostasis but yet distinct in their trafficking mechanisms.

Transport and regulation of presynaptic cargoes

Presynapses in the axon are the major communication site between neurons. They are characterized by the accumulation of hundreds of synaptic vesicles (SVs), filled with neurotransmitter, as well as dense core vesicles (DCVs) containing neuropeptides. Another major constituent of presynapses is the active zone (AZ) cytomatrix, a protein network facilitating rapid vesicle exocytosis and endocytosis. Presynaptic proteins and vesicle membranes are synthesized and assembled in the cell body; however, they need to be delivered specifically into the axon and often to subcellular domains within the axon. Hence, proper regulation of axonal transport is absolutely crucial for accurate presynapse assembly, maintenance and neuronal function. How do SVPs, DCVs and AZs specifically enrich in the axon? Are they exclusively delivered into the axon or are they trafficked throughout the entire

neuron and only captured and stabilized at axonal presynaptic sites?

A recent study focusing on the trafficking behavior of SVPs in *Caenorhabditis elegans* revealed that these organelles are not directly and exclusively transported into the axon, but rather trafficked throughout the entire neuron including dendrites and distal axonal domains devoid of presynapses [14]. The authors concluded that polarized transport of SVPs into the axon together with SVP capturing at presynaptic release sites are necessary for proper presynapse formation at the right location. Furthermore, studies focusing on the mobility of mature SVs in the axon of vertebrate neurons demonstrated that SVs are highly motile organelles, interchangeable between many presynapses instead of being restricted to only a specific release site [15–17]. SVs form so-called superpools of vesicles spanning many presynaptic sites, which might provide a neuron with a versatile mechanism to rapidly tune single synapse function to changing needs.

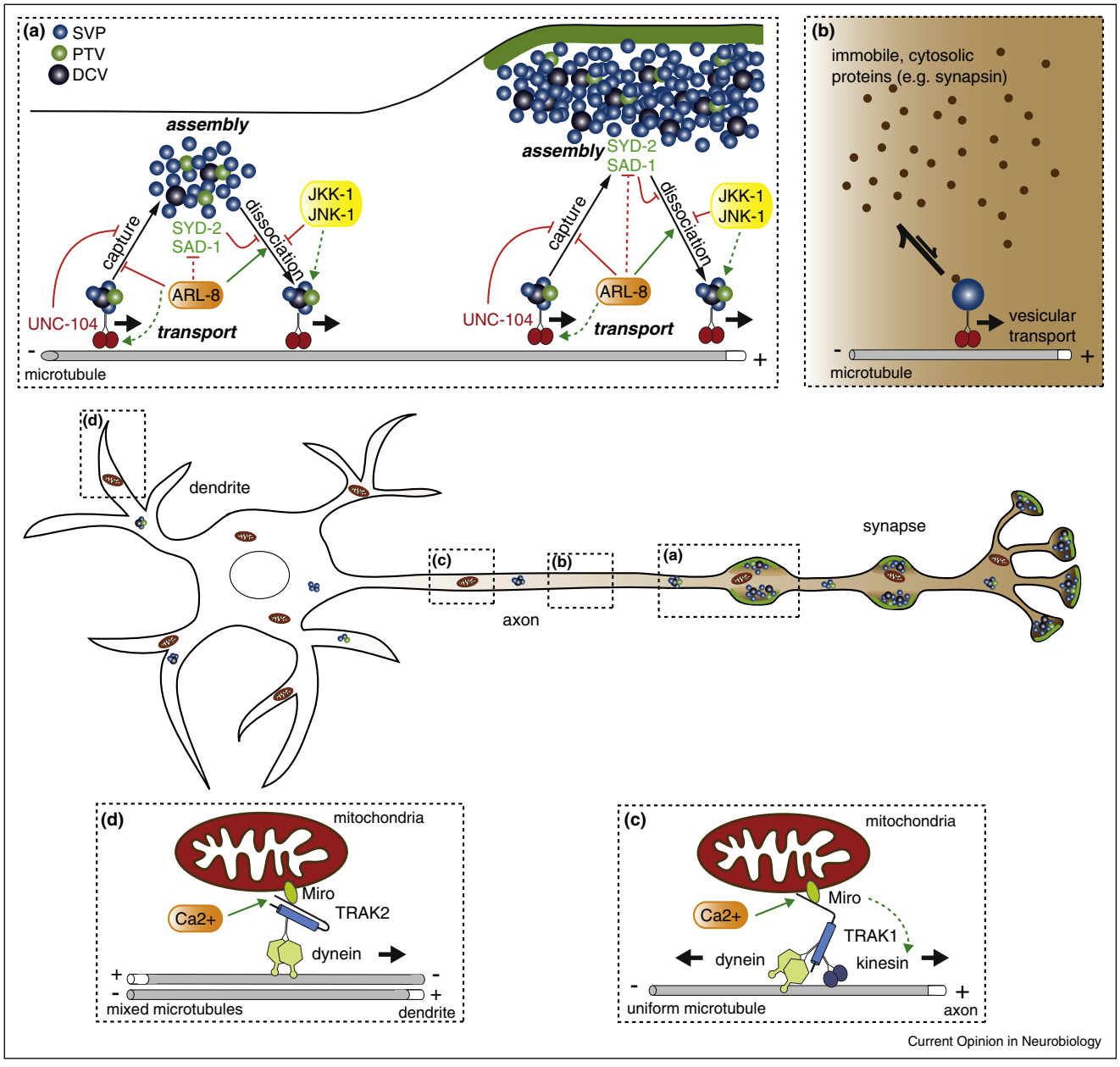
Previous work has demonstrated that a number of AZ molecules including Piccolo, Bassoon and ELKS-2/CAST, are carried as preassembled complexes to presynaptic sites by Piccolo-Bassoon transport vesicles (PTVs) [18,19]. However, other constituents of the AZ, such as Munc-18, as well as SV proteins are packaged into different types of transport vesicles [20]. Nevertheless several recent studies revealed that AZ and SV proteins are co-trafficked [21••,22], most likely as heterogeneous transport packets consisting of both one or two dense core PTVs and a few clear core SVPs [23].

A great challenge for a neuron is to evenly distribute synaptic material among neighboring synapses, so called *en-passant* synapses. An elegant study in *Drosophila* followed the movement of single DCVs between *en-passant* boutons [24•]. The authors found that rather than one-way anterograde transport of DCVs to nerve terminals, DCVs constantly circulate between the proximal axon and the synaptic boutons. The combination of inefficient capture at presynaptic sites and the forth-and-back movements facilitates uniform distribution of DCVs at *en-passant* synapses. The key to this model is two fold. First, the direction of movement of similar cargoes must be precisely regulated so that they can circulate between the proximal axon and the distal synaptic bouton. Second, the capture of mobile vesicles by the synapses must be inefficient to prevent excessive aggregation at any given synapse. What are the molecular mechanisms that regulate these two aspects of axonal trafficking?

Recently, several studies in *C. elegans* have identified two postmitotic cyclin-dependent protein kinases as negative regulators of the retrograde motor dynein [25,26]. Single mutant animals for either *cdk-5* or *pct-1* (Pctaire-kinase) displayed mislocalized AZs, SVPs and DCVs into the dendrite. In double mutants, SVs and AZs are completely mislocalized to the dendrite leaving the axon devoid of any presynaptic specializations. Interestingly these double mutant animals do not show a lack of SVP transport into the axon, rather they display an imbalance in anterograde and retrograde trafficking eventually resulting in mistargeted presynaptic material into the dendrite. In zebrafish, Cdk5 has also been shown to affect the transport of synapsin, a presynaptic constituent, which is trafficked independently of SVPs and AZs to synapses [27]. Interestingly cdk5 has not only been implicated in long-range trafficking of presynaptic components, but also in local SV mobility at presynaptic terminals [28,29]. Pharmacological or genetic ablation of Cdk5 activity increased the readily releasable pool of SVs docked at the AZ by recruiting vesicles from the resting pool, ultimately resulting in increased synaptic function.

Interactions between trafficking cargoes and stable cargoes likely regulate 'cargo capture', which ultimately determines the size of the stable packet. Studies on SV and AZ trafficking in *C. elegans* have shed light on the molecular regulation of this process (Figure 1a). The isolation of mutants with excessive and inefficient aggregation of SVs suggests that specific molecular programs regulate SV clustering. Loss of ARL-8, a SV localized small arf-like GTPase, leads to excessive presynaptic cargo aggregation in the proximal axon, suggesting that the SV cargoes bring their own aggregation regulator to antagonize the aggregation reaction [30]. Interestingly, the aggregation of SVs is mediated by known AZ proteins including SYD-2/liprin, SYD-1, and SAD-1 even during the trafficking process. High sensitivity imaging of *in vivo* axons revealed that trafficking SV packets encounter numerous 'mini' presynapses along the axon shaft, which contain both SVs and AZs. These 'mini' presynaptic sites frequently stop transport packets and initiate their aggregation process. Similar 'hotspots' for stopping transport packets were also reported in vertebrate axons [22]. ARL-8 controls the aggregation and dissociation between the transport packets and the 'minisynapses' by inhibiting aggregation and promoting dissociation [21••]. In other words, presynaptic cargoes make many stops along their way to the synaptic terminals. While the movements of these transport packets are fast (1.5–2.5 μm/s), they are interspersed by long pauses at the 'minisynapses'. Interestingly, the same mode of 'stop and go' trafficking was observed for cytosolic proteins such as synapsin and CaMKII, which undergo slow axonal transport [31••] (Figure 1b). These results suggest that during these modes of axonal transport, trafficking cargoes interact with stationary sites and the kinetics of the interaction play important roles in the overall rate of transport as well as the distribution of synaptic cargoes.

Figure 1



Transport and regulation of neuronal cargoes to axons and dendrites. **(a)** The balance between transport and assembly is regulated by a molecular network consisting of the small G-protein ARL-8, the active zone molecules the kinesin motor UNC-104 and the JNK MAP kinase pathway. **(b)** Slow axonal transport of cytosolic proteins is facilitated by their stochastic and transient association with fast moving vesicles. **(c and d)** Mitochondria employ different transport machinery for their delivery either to the axon or the dendrite. **(c)** TRAK-1 steers mitochondria into axons through its ability to bind to both kinesins and dyneins. **(d)** Adaptor protein TRAK2 binds preferentially to dynein and mediates dendritic targeting of mitochondria.

Transport and regulation of mitochondria

One of the most studied transport cargoes in axons and dendrites are mitochondria [32,33]. The majority of mitochondria are stationary for long periods of time (~70%), but some mitochondria move large distances in both anterograde and retrograde directions (~30%) [34]. Docking and pausing in between movements and abrupt changes in direction indicate that mitochondria are coupled to kinesins, dyneins, and anchoring machineries whose actions can compete or oppose one another. Positioning mitochondria at areas with high-energy requirements is critical for neuronal development and synaptic function. For example, synaptic transmission is regulated by local mitochondria immobilization at presynaptic

terminals [35]. In addition, recent work demonstrated that mitochondria anchoring is required for axonal branching [36•,37].

Elucidating the machinery of mitochondria trafficking in neurons has begun to yield basic insights into how neuronal cargo movement is regulated. In the last several years the following fundamental questions have been addressed. How are mitochondria sorted in axons and dendrites? How do mitochondria regulate opposing motor activity? How do mitochondria put a brake on their movement? It has become increasingly clear that motor-adaptor interactions play an important role in the regulation of cargo trafficking [38,39]. Several adaptor proteins have been identified that interact with the mitochondrial outer surface and are potential candidates for regulating mitochondrial distributions throughout the neuron [32]. The core of this conserved adaptor complex consists of mitochondrial Rho GTPase Miro/RhoT and milton/TRAK and is required for microtubule-based transport of mitochondria in *Drosophila* neurons. Miro has two EF-hand Ca^{2+} binding domains and acts as Ca^{2+} sensor for activity-dependent regulation of mitochondrial transport [40,41], while TRAK links Miro at the mitochondria to microtubule-based motor proteins. More recent finding demonstrated that mammalian adaptor proteins TRAK1 and TRAK2 utilize different transport machineries to steer mitochondria into axons and dendrites [42••]. Adaptor protein TRAK1 binds to both kinesin-1 and dynein and steers mitochondria into axons (Figure 1c), whereas TRAK2 predominantly interacts with dynein/dynactin and mediates dendritic targeting (Figure 1d). The functional differences between TRAK1 and TRAK2 are explained by conformational differences; the backfolding of TRAK2 affects its interaction with kinesin-1 and allows transport of the TRAK2–dynein complex into dendrites. It is tempting to speculate that conformational switching of adaptor proteins is a general regulatory mechanism that coordinates bidirectional transport and influences polarized trafficking.

Once the mitochondria have reached their proper destination they need to stop their bidirectional motility. How does neuronal cargo puts a brake on its movement? Three mechanisms have been proposed; the mitochondria stops by dissociating from the microtubule track, statically anchors to the microtubules or links to other cytoskeleton filaments, such as actin filaments [32]. One model that has been proposed involves syntaphilin, a mitochondria specific 'anchor protein' that acts as molecular brake for mitochondria by docking them to the microtubule cytoskeleton. A recent study demonstrated that syntaphilin mediates the immobilization of mitochondria by inhibiting the kinesin-1 motor ATPase activity [43]. A similar stop-and-go mechanism is proposed for lysosomal trafficking in dendrites [44]. Myosin motors have also been shown to oppose microtubule-based transport and to facilitate docking of cargo to actin filaments. The immediate stalling of kinesin-driven cargo observed upon increased myosin V activity reveals an effective arrest mechanism [45]. In cultured *Drosophila* neurons, depletion of myosin V and myosin VI increased speed and length of microtubule-based runs [46]. The function of the myosins in these cells may be to remove mitochondria from microtubules and potentially tether them to the actin cytoskeleton to create a stationary pool.

Cytosolic Ca^{2+} is one of the best-studied regulators of mitochondrial movement. It is well known that elevation of cytosolic Ca^{2+} stops mitochondria motility in neurons, but other mechanisms have also been uncovered to arrest mitochondria [32]. For instance, a recent study has identified the LKB1–NUAK1 pathway in controlling mitochondria immobilization in axons [36•]. Further evidence suggests that the parkin ubiquitin ligase and its regulatory kinase PINK1, often mutated in familial early-onset Parkinson's disease, have a central role in arresting mitochondria trafficking [47,48]. Parkin and PINK1 have been found to act in a common pathway to promote the autophagic degradation of damaged mitochondria. In this pathway the PINK1 senses mitochondrial fidelity and recruits Parkin selectively to mitochondria that lose membrane potential. Parkin subsequently ubiquitinates Miro, prevents mitochondria movement and induces autophagic elimination. By mitochondria immobilization, the PINK1/Parkin pathway may quarantine damaged mitochondria prior to their clearance. Recent data suggest that Parkin dramatically alters the ubiquitylation status of many more outer mitochondrial membrane proteins [49]. Parkin and PINK1 mutations can lead to abnormal mitochondria accumulations and may eventually cause Parkinson's disease

Conclusions

Accurate transport is indispensible for neuronal function, starting at development when axons and dendrites are specified and synapses are built, and continuing throughout a neuron's life to maintain its function and to provide rapid means for neuronal plasticity. Over the last few decades, the field has uncovered the main framework of neuronal transport, such as motor proteins and cytoskeletons, however, much less is known about how these building blocks interplay with each other and how they are regulated to give rise to a functional neuron. For example how does motor-cargo recognition work? How many and what type of motors bind simultaneously to a cargo and how are they coordinated to yield appropriate directional transport? How is cargo pick-up and drop-off regulated at specific locations? And what determines speed, processivity and quantity of transport *in vivo*?

For instance, recent advances in imaging technology permitted the revisiting of the difference of slow cytosolic and fast vesicular axonal transport. While previously

postulated as cytoplasmic diffusion, slow axonal transport has now been shown to consist of sparse and transient associations of higher-order assemblies of cytosolic proteins with vesicles, which are moved by fast axonal transport [31••,50] (Figure 1b). Hence slow axonal transport represents yet a modulation of fast axonal transport, employing the same transport principles just with different dynamic parameters.

Defects in both axonal and dendritic trafficking is implicated in human neurological disorders and neurodegenerative diseases such as amytrophic lateral sclerosis, Alzeimer's and Huntington's disease [51]. In the course of these diseases, ectopic accumulations of proteins and organelles become apparent, which may highlight early and causative damage to the neurons. Impairment of neuronal trafficking in these diseases manifest at many different levels, for example at the level of motor proteins, the cytoskeletal tracks and the cargoes. Gaining a better understanding in the regulatory mechanisms underlying polarized transport in healthy neurons will for sure advance our insight into neurodegenerative diseases and may lead to novel therapeutic treatments.

Conflict of interest

The authors declare no conflict interests.

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