Selective motor activation in organelle transport along axons

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Abstract | The active transport of organelles and other cargos along the axon is required to maintain neuronal health and function, but we are just beginning to understand the complex regulatory mechanisms involved. The molecular motors, cytoplasmic dynein and kinesins, transport cargos along microtubules; this transport is tightly regulated by adaptors and effectors. Here we review our current understanding of motor regulation in axonal transport. We discuss the mechanisms by which regulatory proteins induce or repress the activity of dynein or kinesin motors, and explore how this regulation plays out during organelle trafficking in the axon, where motor activity is both cargo specific and dependent on subaxonal location. We survey several well-characterized examples of membranous organelles subject to axonal transport — including autophagosomes, endolysosomes, signalling endosomes, mitochondria and synaptic vesicle precursors — and highlight the specific mechanisms that regulate motor activity to provide localized trafficking within the neuron. Defects in axonal transport have been implicated in conditions ranging from developmental defects in the brain to neurodegenerative disease. Better understanding of the underlying mechanisms will be essential to develop more-effective treatment options.

Signalling endosomes
Membranous organelles formed by internalizing a neurotrophin-bound receptor, which is then trafficked to the soma to trigger downstream effects, including transcriptional changes. Following signalling, the receptor may be degraded or recycled back to the plasma membrane.

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Neurons display highly specialized morphologies befitting their role in information processing and long-distance communication. Signals are received by the dendrites, integrated in the cell body, and then conducted down the axon to signal to target cells, including neurons and muscle fibres[1] (FIG. 1). The nucleus is in the cell body, also known as the soma; therefore, gene transcription, as well as most protein synthesis and organelle biogenesis, is primarily localized to this subcellular compartment[2–4]. The active delivery of new proteins and organelles from the soma (anterograde transport) is required to establish and maintain the signalling regions, known as synapses, in both the dendrites and the axon[5]. Protein and organelle turnover at synapses also requires active transport back towards the soma (retrograde transport) for recycling[6]. Additionally, signalling endosomes containing activated receptors must be transported along the axon towards the soma to regulate transcription and cellular response[7]. Trafficking across the neuron is therefore essential for neuronal function.

The fastest intracellular transport is achieved by molecular motors moving along the microtubule cytoskeleton[8]. Microtubules form polarized tracks in axons: the faster-growing plus ends of the microtubules extend preferentially away from the soma, while the slower-growing minus ends point towards the soma[9] (FIG. 1). By contrast, microtubules in vertebrate dendrites exhibit mixed polarity that can vary with the distance from the soma[10,11] (FIG. 1). On the plus end-out microtubules that predominate in axons, plus end-directed kinesin motors move cargo from the soma to presynapses, while the minus end-directed microtubule motor cytoplasmic dynein 1 exclusively moves cargo from presynapses back to the soma[12–15]. Here we focus on the mechanisms regulating this fast axonal transport of organelles and vesicles. Microtubule motors also mediate slow axonal transport, whereby mostly cytoplasmic components, such as clathrin and tubulin, are trafficked three to four orders of magnitude more slowly along the axon; this transport mechanism was reviewed recently[16] and is not discussed here.

Despite the opposing action of dynein and kinesin, most organelles copurify from brain tissue with both motors bound[17–19]. How then can unidirectional transport be achieved? Further, how are microtubule motors regulated on specific cargos to achieve efficient, directional delivery to, and retrieval from, specific destinations? In this Review we discuss the emerging mechanisms that regulate microtubule motors during organelle transport along axons. We first explore recent advances in our broad understanding of motor coordination, activation and inactivation. We then review the latest developments in the field of axonal transport, using what we know about motor regulation to better

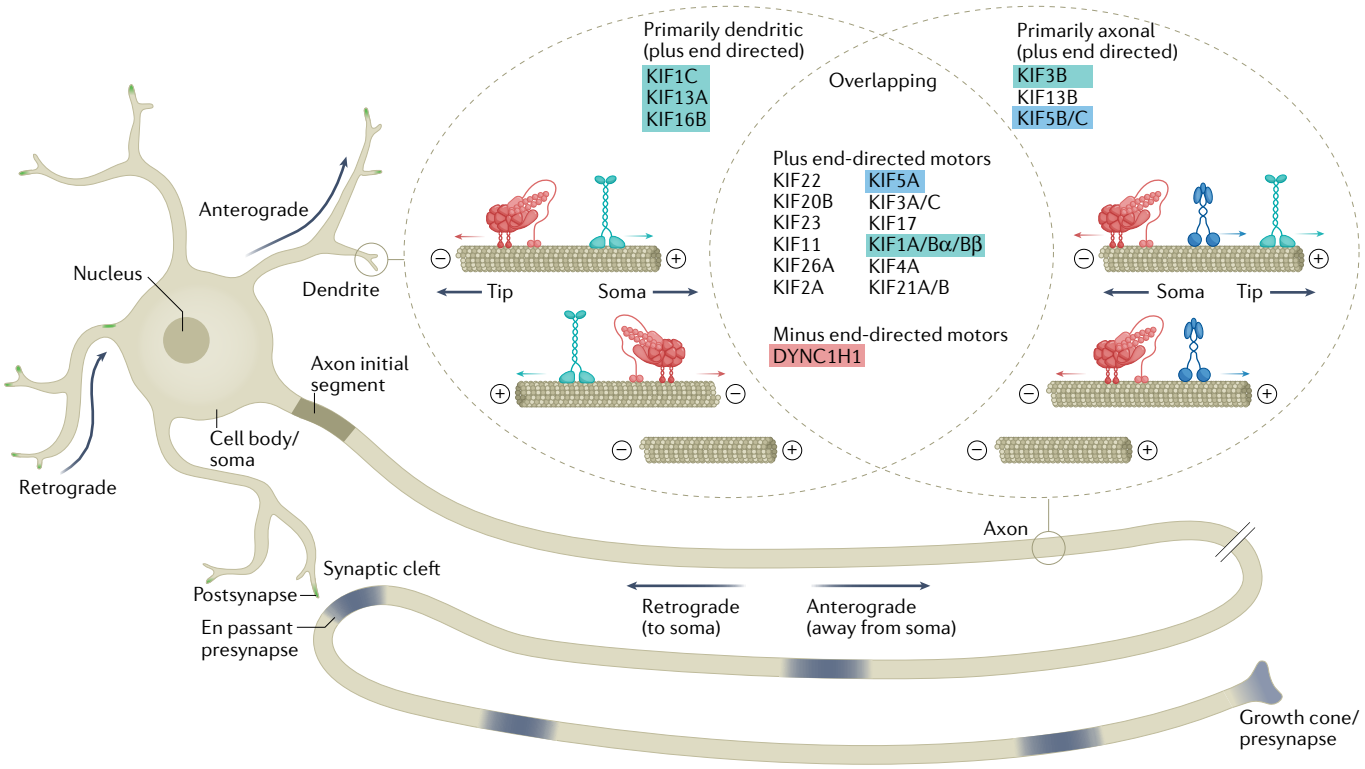


Fig. 1 | **Microtubule and motor organization in neurons.** Neurons can be divided into three functional regions: the soma, where the nucleus is located and most protein translation and organelle biogenesis occur; the long extended axon, which sends signals to other cells at presynapses; and shorter, highly branched dendrites that receive signals at postsynapses. In either the axon or the dendrites, transport away from the soma is anterograde, while transport towards the soma is retrograde. Microtubules in the axon are predominantly plus end out, while microtubules in mammalian dendrites are of mixed polarity (plus and minus ends out). Key motors involved in cargo trafficking in neurons — dynein (red), kinesin 1 family (blue) and kinesin 3 family (cyan) — are highlighted.

understand the trafficking of multiple organelles. Axonal transport is compromised in many neurodegenerative and neurodevelopmental disorders[5,20,21]; thus, a thorough understanding of the underlying regulatory mechanisms is vital for developing new therapeutic approaches for these devastating diseases.

Mechanisms of motor regulation

Kinesins are encoded by a superfamily of 44 distinct genes in humans that can be grouped by similarity into 14 classes[22]. Many of the kinesins are processive motors that move cargo towards microtubule plus ends[23]. The main kinesins driving long-distance transport in neurons are kinesin 1 (both the ubiquitous KIF5B isoform and the neuron-specific KIF5A and KIF5C isoforms), kinesin 3 (KIF1A, KIF1B, KIF13 and KIF16B), kinesin 2 (KIF3 and KIF17) and kinesin 4 (KIF4, KIF21A and KIF21B)[24–32]. Some kinesins localize preferentially to the axon, (for example, KIF5C or KIF13B) or to dendrites (for example, KIF17 or KIF13A) (FIG. 1); additionally, some kinesins are specific to particular cargo organelles[5,33,34]. Axonal localization may be driven by the motor itself, while in the dendrite the attached cargo seems to direct the motor localization[35]. Here we focus primarily on the kinesin 1 and kinesin 3 families, whose cargo and activation in axons are relatively well understood. Kinesin 1 is typically a heterotetramer of two kinesin heavy chains (KHCs; also known as KIF5 proteins) and two kinesin light chains (KLCs)[36]. KHC is composed of a microtubule-binding motor head that can hydrolyse ATP, linked by a coiled-coil (CC) stalk domain that mediates dimerization and interhead coordination to a tail domain[37]. The kinesin 3 family is more diverse, but typically active complexes consist of a homodimer of KIF molecules, which contain a microtubule-binding motor head that can hydrolyse ATP, a CC-containing neck domain that mediates dimerization and a tail domain[38]. Kinesin tail domains differ in length, and may bind to regulatory proteins, including KLCs and cargo-associated proteins[39] (FIG. 2a,b).

In contrast to the specialization seen within the kinesin superfamily, cytoplasmic dynein 1 is a ubiquitous motor driving the vast majority of minus end-directed transport in animal cells[40,41]. Dynein heavy chain (DHC) consists of an ATP-hydrolysing AAA+ ring and a microtubule-binding domain separated by a CC stalk, plus an amino-terminal (N-terminal) tail necessary for dimerization and binding to additional proteins[42]. Dynein light chains, dynein light intermediate chains (DLICs) and dynein intermediate chains (DICs) co-assemble with DHC to form the full 1.4-MDa complex[43]. Dynein assembles with its obligate partner, dynactin, itself a multisubunit 1.1-MDa complex[44]. Additional dynein-activating adaptors are required to

Coiled-coil
(CC). Structural motif in which multiple α-helices are coiled together to form a supercoil. The primary sequence is made up of a series of heptad repeats including both hydrophobic and charged residues. CC domains are often stiff rods that function as molecular spacers.

Dynein-activating adaptors
Dynein effectors that activate dynein motility and link dynein — directly or indirectly — to cargo. Most dynein-activating adaptors contain an extended coiled-coil motif (~38 nm) flanked by conserved motifs for dynein and dynactin binding.

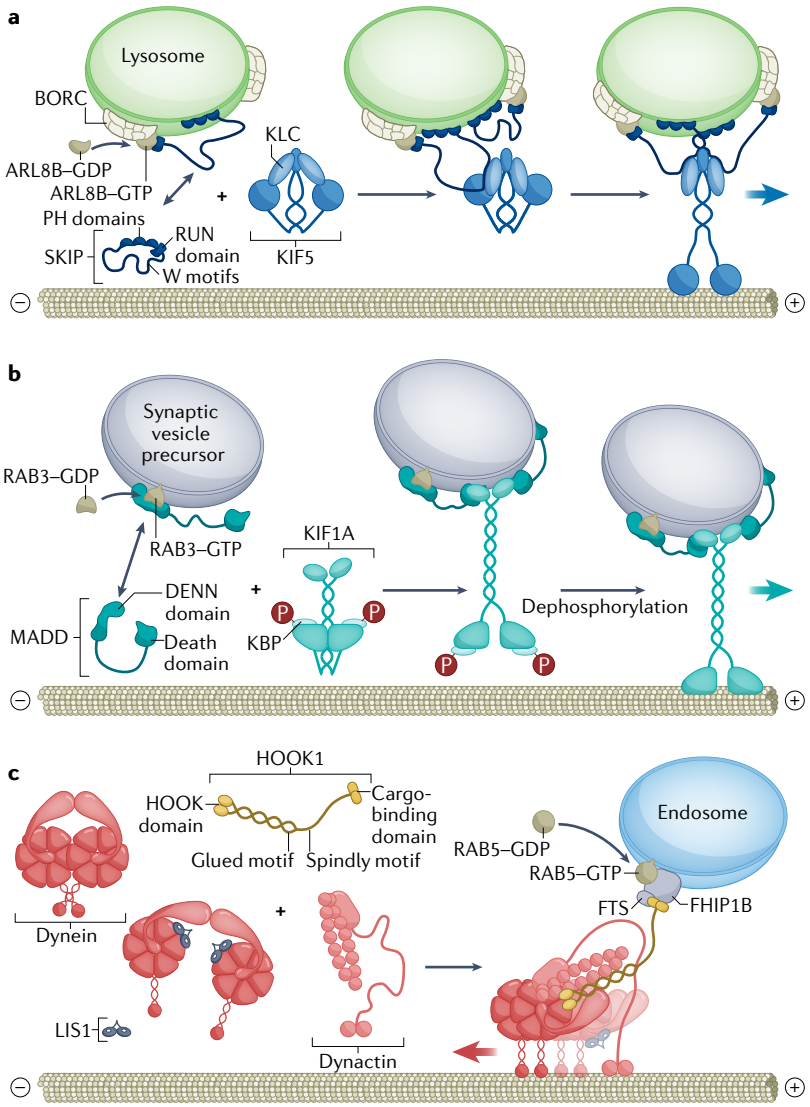


Fig. 2 | **Motor autoinhibition and activation. a** | Kinesin 1 autoinhibition is mediated by a head–tail interaction in the heavy chain (KIF5), which is stabilized by the kinesin light chains (KLCs). Relief from autoinhibition is mediated by effector proteins, such as SKIP, which bind to both KLC and the tail of KIF5 to fully free the motor heads. The effector SKIP is head–tail autoinhibited as well, but binding to GTP–ARL8 on membranes stabilizes its active open conformation; invertebrate BORC (BLOC1-related complex) serves as an ARL8 guanine exchange factor. **b** | Kinesin 3 (KIF1A) is autoinhibited by the binding of stalk coiled-coil domain 1 to the motor heads. Relief from autoinhibition is mediated by effector proteins, such as MADD, which binds to stalk coiled-coil domain 3 and destabilizes the stalk–head interaction, thereby freeing the motor heads. KIF1A is further inhibited by kinesin-binding protein (KBP) sterically blocking motor domain binding to the microtubule, which may be released by dephosphorylation of KBP. The effector MADD is recruited to membranes cooperatively with RAB3 by serving as a RAB3 guanine exchange factor. **c** | Dynein alone oscillates between a closed 'phi' conformation and an open antiparallel conformation that can bind but cannot walk on microtubules. The effector lissencephaly 1 (LIS1) stabilizes the open conformation to facilitate binding with dynein's obligate partner complex dynactin and with an activating adaptor protein (for example, HOOK1). Dynactin induces a conformational change such that the motor heads become parallel, and provides an additional microtubule-binding domain. The activating adaptor stabilizes the dynein–dynactin interaction and mediates contact with cargo. Finally, LIS1 and the activating adaptor can promote the addition of an extra dynein dimer, increasing velocity and processivity. HOOK membrane contact is mediated by an interaction between fused toes protein (FTS), FTS–HOOK-interacting protein 1B (FHIP1B) and RAB5–GTP. Note that the stoichiometries of the interactions displayed here are not all known. PH, pleckstrin homology.

enhance the interaction between dynein and dynactin and to link the megacomplex to cargo[45] (FIG. 2c). Recent reviews detail the biophysical dynamics of microtubule motors[23,37,43,46]; here we discuss the most recent findings regarding motor activation and the coordinated regulation of multiple motors bound to a single cargo.

Common themes in motor regulation. While kinesin and dynein motors differ both structurally and mechanically, there are similarities in their regulatory mechanisms: both are activated by relief from autoinhibition and by forming complexes. Autoinhibition is mediated by diverse intramolecular and intermolecular interactions, but uniformly induces a conformation that is incapable of binding and/or moving along the microtubule[36,38,47–49]. Complex formation includes interaction with ubiquitous obligate partners as well as with various scaffolding proteins, which mediate cargo interactions and/or interact with opposing motors to facilitate unidirectional transport[43,50–53]. In many cases, there are additional regulators involved, such as cargo-specific GTPase effectors or kinases and phosphatases[54–61]. Together, these mechanisms ensure that motors are appropriately activated when bound to cargo, but also ensure that opposing motors can be coordinately regulated to prevent an inefficient tug of war, as discussed later. Additional mechanisms are also critical to the spatiotemporal control of cellular motors in the neuron, including regulation by microtubule-associated proteins and the tubulin code (see BOX 1). These cellular aspects of motor regulation were reviewed recently[62–64]; here we focus on cargo-specific mechanisms.

Activation of kinesin by unfolding. Two primary interactions mediate kinesin 1 autoinhibition: the KHC tail folds onto the KHC motor domain to block interaction with the microtubule, and KLC binding may lead to further separation of the KHC motor domains in the inactive conformation[48] (FIG. 2a). Many kinesin 1-activating proteins bind to the tetratricopeptide repeats (TPRs) or the adjacent leucine–phenylalanine–proline (LFP) motif of KLC; this binding destabilizes the interaction between KLC and the KHC motor heads, partially relieving autoinhibition[49,52,53,65]. Many activators also bind the exposed KHC tail, destabilizing its interaction with the KHC motor domains, and thus freeing the motor domain to bind the microtubule[53,65,66] (FIG. 2a).

Kinesin 3 is autoinhibited when its neck or stalk region binds to its motor domains[38,67] (FIG. 2b). Kinesin 3-activating proteins bind to the heavy chain stalk region, which destabilizes the interaction with the motor domains, freeing them to bind and transit along the microtubule[67,68] (FIG. 2b). Kinesin 3 and several other kinesins are additionally regulated by kinesin-binding protein (KBP; also known as KIF1BP), which binds the motor domain through TPR motifs to sterically block microtubule attachment, akin to KLC[69,70] (FIG. 2b). KBP phosphorylation enhances its interaction with kinesins, so kinesin 3 activation may require local phosphatase activity, but the identity of the phosphatase is unknown[69].

Most kinesins bind their cargo through light chains or an activating protein, although some can directly bind

Box 1 | **Regulation of motor function by microtubule tracks**

In addition to regulation by autoinhibition and complex formation on organelles, motors can also be regulated by microtubule-associated proteins (MAPs) and the microtubules themselves. Motor recruitment to microtubules is regulated dynamically in cells by the 'tubulin code', the 'language' used by cells to differentially and dynamically label microtubule tracks[173–175]. This includes the use of distinct tubulin isoforms, the nucleotide state of the tubulin dimers in the lattice and any post-translational modifications (PTMs) of the microtubule (recently reviewed[63,64]). For example, the differential affinity of kinesin 1 for acetylated and/or detyrosinated microtubules and kinesin 3 for tyrosinated microtubules is thought to bias the trafficking of specific cargos towards either the axon or the dendrite[11,176]. Similarly, tyrosinated microtubules are specifically enriched in the distal tip of the axon; the dynactin subunit p150^{Glued} and the dynein regulator CLIP-170 preferentially bind to tyrosinated tubulin, and thus dynein–dynactin binds more readily to microtubules in the distal tip of the axon to initiate minus end (soma)-directed transport[61,173]. As another example, the kinesin 3 motor KIF1A preferentially binds to GDP–tubulin over GTP–tubulin; newly nucleated microtubule plus ends, which contain GTP–tubulin, are enriched in presynaptic zones, facilitating KIF1A detachment from microtubules in this region[137,142].

The related 'MAP code' refers to the differential binding of MAPs to the microtubule track, which can likewise affect motor binding (recently reviewed[62,138]). In neurons, different subcellular compartments develop distinct sets of MAPs and PTMs to differentially direct motor activity. For instance, MAPs associated with the dendrite (DCX, DCKL1, MAP9 and SEPT9) specifically inhibit kinesin 1 but have no effect on kinesin 3 transport, causing kinesin 1 to preferentially enter the axon as compared with the dendrites, while kinesin 3 localizes to both compartments[35,62,177]. Thus, microtubules and MAPs regulate run initiation and termination to direct specific activity and localization of motors.

Disease-causing mutations can disrupt microtubule organization, PTMs or dynamics, thus affecting axonal transport. Mutations in tubulin itself cause severe brain malformations, although studying their mechanistic roles has proven difficult; at least some are known to directly affect motor binding[178]. Mutations in the microtubule-severing protein spastin cause hereditary spastic paraplegia; many spastin mutations are loss-of-function mutations, and spastin-depleted mice demonstrate longer microtubules with more polyglutamylation, which disrupts KIF5C transport[179,180]. Disease-associated arginine-rich dipeptide repeats generated from amyotrophic lateral sclerosis-causing mutations in the *C9orf72* gene decrease microtubule-based motility by forming 'roadblocks' along the microtubule lattice[181]. In vitro, the positively charged dipeptide repeats bind robustly to the negatively charged carboxy-terminal tails of tubulin along the microtubule lattice, preventing motor binding, which results in reduced transport of a range of cargos inside neurons[181]. Motor proteins are thus self-regulated, regulated by their cargo, and regulated by the tracks along which they travel. Further, disruptions at any of these levels can cause loss of or aberrant axonal transport and severe neurological disorders (see also BOX 2).

cargo membranes through interactions mediated by their Phox homology (PX) or pleckstrin homology (PH) domains in the motor's tail[53,71,72]. Many small GTPases, including RAB proteins, promote recruitment of motor proteins to cargo membranes through interaction with a motor regulatory protein. In addition, some GTPases, such as ARL8 binding to kinesin 3, can directly activate motor proteins by relieving autoinhibition[73–75]. Accordingly, guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins are also involved in motor–cargo interaction[76–79] (FIG. 2).

Activation of dynein by conformational change and complex assembly. Dynein autoinhibition and activation (reviewed recently[43]) are even more complicated. Dynein alone stochastically switches between two inactive conformations: an autoinhibited conformation known as phi, wherein the stalk domains, AAA+ rings and N-terminal linkers interact, and an open conformation, wherein the free motor domains are available for binding to the microtubule but unaligned for processive motility[47,80] (FIG. 2c). Lissencephaly 1 (LIS1) binds to and stabilizes the open conformation, allowing the dynactin complex to bind and orient the dynein motor domains into a parallel conformation that permits more efficient stepping along the microtubule[80–83] (FIG. 2c). The addition of a CC-containing dynein-activating adaptor stabilizes the dynein–dynactin interaction by binding both dynein (via interactions with DLIC) and dynactin (via interactions with the pointed end of the ARP1 filament and/or p150^{Glued}) and promoting a rigid parallel conformation[50,51,84–90]. Dynein-activating adaptors are relatively cargo specific, linking the motor complex either directly or indirectly to the organelle[41]. Some activating adaptors induce the recruitment of a second dynein dimer, forming a macromotor complex that has greater velocity, processivity and force production (FIG. 2c); thus dynein motile behaviour can be tuned for different cargo types[85,86,91,92].

Coordination of opposing motors. The opposing activities of kinesin and dynein motors must be modulated to achieve rapid, processive transport along the axon (FIG. 3). The net directionality of transport is determined by the relationship between plus end-directed and minus end-directed motors on a cargo. This relationship may be competitive. For example, dynein, either alone or in complex with dynactin, is incapable of outcompeting kinesin 1 in a tug of war; however, the addition of activating adaptors that induce the binding of a second dynein dimer allows dynein to 'win'[81,86,93]. These larger complexes exhibit higher forces and slower load-dependent detachment kinetics, indicating that these additional factors are particularly important in determining the outcome of competition[86,93]. In microtubule gliding assays, the number of active kinesin or dynein motors engaging a single microtubule had the largest impact on the net directionality, while ATP concentration, microtubule-associated proteins on the microtubule and stepping kinetics had little impact[94]. Dynein dimers also appear to cluster into 'teams' on membranes; for example, multiple dynein complexes cluster in cholesterol-rich microdomains on the surface of phagosomes, leading to large force production and retrograde transport[95,96].

Although the tug-of-war model is appealing, and straightforward to explain bidirectional transport both in vitro and in cells, this model has important limitations in advancing our understanding of cargo transport in vivo. For example, because of the opposing force generated by the 'loser' motor, the velocities observed in tug-of-war assays are usually lower than those seen for endogenous cargos moving in vivo, suggesting that this model may not satisfactorily explain the rapid organelle transport observed in cells[19] (FIG. 3). Instead, as more studies are performed either in live cell assays or in vitro using motility assays with more physiological motor complexes, accumulating evidence points towards models of selective activation and motor interdependency[19,97,98] (FIG. 3). Efficient unidirectional transport may actually require both motors[99]. At a mechanistic level, many motor adaptor and/or activator

Tetratricopeptide repeats (TPRs). Common structural motifs consisting of 3–16 tandem repeats that form α-helices, which typically fold together to form a leaner solenoid domain. The TPR motif in kinesin light chain is commonly the binding site for kinesin 1-activating proteins.

Leucine–phenylalanine–proline (LFP) motif
A short unstructured motif (also referred to as an 'LFP–acidic motif') involved in protein–protein interactions. The LFP motif in kinesin light chain is commonly the binding site for kinesin 1-activating proteins.

Hereditary spastic paraplegia
A group of rare inherited peripheral nerve disorders characterized by weakness and stiffness of the leg muscles which progress over time.

Amyotrophic lateral sclerosis
Also known as Lou Gehrig disease, a progressive nervous system disease resulting in muscle weakness and other motor symptoms. The causes differ, and onset is typically between 40 years of age and 65 years of age.

Phox homology (PX) or pleckstrin homology (PH) domains
Lipid-binding domains that interact with phosphoinositides, facilitating membrane localization.

ARP1 filament
Dynactin sub-complex consisting of a filament made up of actin-related protein 1 (ARP1), a pointed-end complex (ARP11, p62, p25 and p27) and a barbed-end complex (CapZα and CapZβ).

p150^{Glued}
Dynactin subunit protein containing a microtubule-binding CAP-Gly domain and a globular shoulder domain connected by a flexible coiled-coil-enriched linker domain.

proteins bind both kinesin and dynein (FIG. 4). Some adaptors bind opposing motors simultaneously to coordinate transport (FIG. 4a–c), while others bind opposing motors in a mutually exclusive fashion whereby their motor-binding state can be regulated by upstream effectors[58,68,90,100] (FIG. 4e,f). Our understanding of these diverse motor regulatory mechanisms has substantially expanded in recent years, emphasizing the importance of distinct regulatory mechanisms specific to individual cellular cargos.

Cargo-specific regulation of axonal transport

The efficient delivery of cargo into and out of the axon is a particularly complex task, with human axons reaching up to 1 m in length. Initiation, continuance and cessation of axonal organelle motility must be tightly regulated to develop and sustain activity of presynaptic regions and maintain local energy levels, and to prevent the accumulation of dysfunctional organelles or aggregated proteins. Here we highlight organelle-specific mechanisms of transport — from relief of motor autoinhibition to upstream regulation by kinases — that have been illuminated by recent progress in axonal transport research.

Mitochondrial trafficking. Axons and presynaptic sites have high metabolic demands and require precise regulation of calcium ions, necessitating the highly regulated trafficking of mitochondria[101]. Microtubule motors are scaffolded on mitochondria by interactions between Miro proteins and the motor-binding proteins TRAK proteins and metaxins (MTX proteins)[98,102] (FIG. 5a). Miro proteins, Ca^{2+}-sensitive GTPases embedded in the mitochondrial outer membrane, mediate both long-distance microtubule-based transport of mitochondria and anchoring of mitochondria to the cytoskeleton at specific intracellular locations, where they can generate high local ATP levels[103]. TRAK1 and TRAK2 (Milton in *Drosophila*) are canonical CC-enriched dynein-activating adaptors which bind DLIC helix 1 via a CC1 box motif, dynactin pointed end complex via a Spindly motif and dynactin p150^{Glued} via a Glued motif[51,90,98]. They also bind to kinesin 1 heavy chain using their N-terminal CC domain; the binding to these opposing motors does not appear to be mutually exclusive[98,104]. Both TRAK proteins have been shown to activate kinesin and dynein motility in vitro[90,98,105,106]. TRAK1/2 can form co-complexes with both dynein–dynactin and kinesin; in a minimal recombinant model, TRAK1/2 complexes containing both motors move only in the anterograde direction, but in lysate-based in vitro experiments TRAK2 activation of either kinesin or dynein is dependent on the presence of the opposing motor, indicating cooperativity between the motors[98,106] (FIG. 4a). TRAK1/2 or MTX1/2 knockdown in human neurons led to a loss of mitochondrial motility in both the anterograde direction and the retrograde direction[102]. By contrast, *mtx-1* mutant *Caenorhabditis elegans* displayed mitochondrial motility primarily in the plus-end direction, consistent with the interaction between MTX1 and KLC1 (REF.[102]). Thus, while TRAK1, TRAK2 and MTX2 appear to be implicated in both dynein-directed motility and kinesin-directed motility, it is possible that MTX1 is required only for kinesin-directed mitochondrial motility. More research is necessary to determine whether MTX1 is implicated in unidirectional or bidirectional motion, and whether MTX1 and MTX2 directly activate microtubule motors.

Termination of mitochondrial transport is equally important for local function in presynapses and other locations. Mitochondria are locally anchored through associations with either the microtubule or the actin cytoskeleton[107,108]. For example, high local glucose concentrations induce O-GlcNAcylation of TRAK1/2, which promotes TRAK1 interaction with the actin-binding protein FHL2 and subsequent halting[109]. At presynapses, the microtubule-bound protein syntaphilin can halt mitochondrial transport through association with the mitochondrial outer membrane and/or by sterically

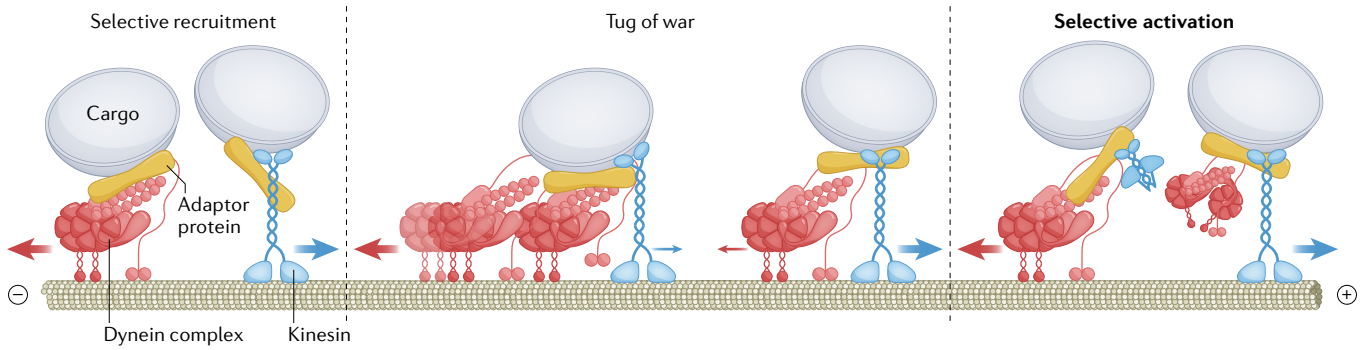


Fig. 3 | **Models of intermotor coordination.** To transport cargo unidirectionally along microtubules, opposing motors must be coordinated. Three main models for this coordination have been proposed. Selective recruitment entails minus end-directed or plus end-directed motors being recruited to a given cargo separately, such that only motors that move in the desired direction are recruited to the cargo. Testing of this model in vitro yields cargos that move at a velocity similar to that seen in cells and in vivo. However, organelles isolated from the brain copurify with both direction motors, suggesting association of one motor type at a time is non-physiological. The tug-of-war model posits that opposing motors bound to a single cargo compete to pull the cargo in their direction of movement. Generally, a single kinesin can outcompete a single dynein dimer, but the formation of a dynein tetramer and/or dynein 'teams' (multiple dynein complexes) allows dynein to 'win'. While this model fits the finding that cargos bind both direction motors simultaneously, the velocities seen in tug-of-war experiments are much lower than what is seen in cells, presumably owing to the competing force produced in the non-motile direction by the opposing motor. The selective activation model, wherein both direction motors are present on cargo but they are differentially activated to induce unidirectional movement, seems to provide the best fit to experimental data.

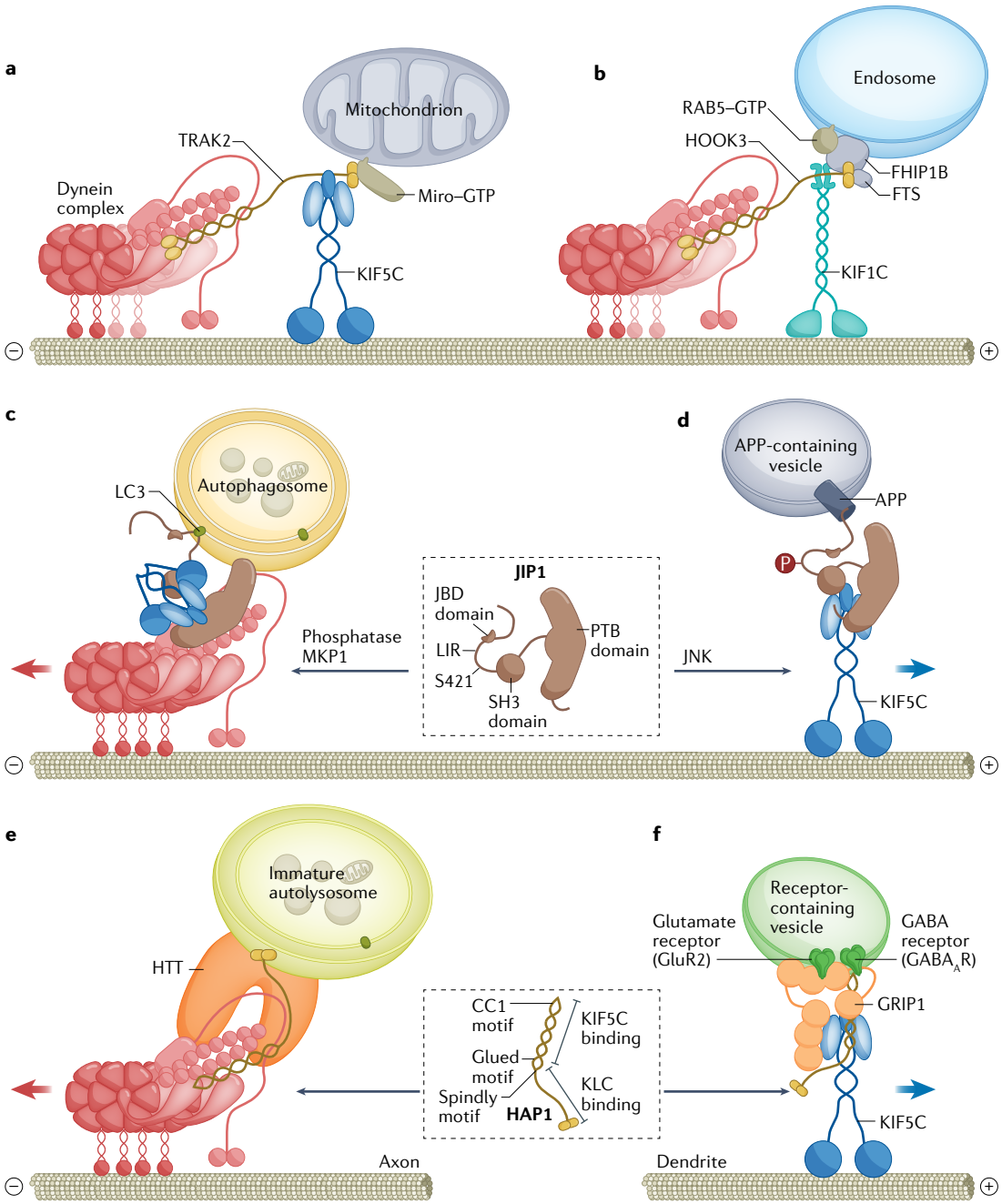


Fig. 4 | **Many motor effectors induce both anterograde and retrograde transport.** Some effectors, for example TRAK2 (part **a**) and HOOK3 (part **b**), can simultaneously form a complex with both dynein–dynactin and with kinesin to coordinate the bidirectional transport of organelles. For TRAK2, processive unidirectional transport requires the formation of these multimotor complexes. Other effectors (for example, JIP1) drive differential transport depending on the phosphorylation status (P) (parts **c**,**d**). **c** | Dephosphorylated JIP1 induces inhibition of KIF5C, facilitating retrograde transport; although JIP1 directly binds dynactin p150^{Glued}, it is unknown whether it activates dynein–dynactin transport. **d** | JIP1 phosphorylation at site S421 by JNK induces activation of KIF5C and anterograde transport of cargo. Some effectors (for example, HAP1) activate dynein or kinesin motility depending on interactions with other proteins (parts **e**,**f**). **e** | HAP1 activates dynein–dynactin motility in coordination with its partner huntingtin (HTT), which binds both HAP1 and dynein intermediate chain. **f** | HAP1 complexes with GRIP1 to relieve KIF5C autoinhibition. Note that the stoichiometries of the interactions displayed here are not all known. APP, amyloid precursor protein; FHIP1B, fused toes protein–HOOK-interacting protein 1B, FTS, fused toes protein; KLC, kinesin light chain.

CC1 box motif
Common dynein-activating adaptor motif that forms a hydrophobic pocket in which the dynein light intermediate chain helix 1 inserts itself.

blocking kinesin binding to the microtubule[106,107]. Miro binding to Ca^{2+} also reduces mitochondrial transport, but there is controversy regarding how this effect is mediated[110]. While the mechanisms underlying both mitochondrial transport and immobilization are still being determined, the dynamics of mitochondrial motility are key to meeting local energy demands in highly polarized neuronal cells.

Spindly motif
Common dynein-activating adaptor motif that mediates interaction with the pointed-end complex of the ARP1 filament of dynactin.

Endosomal motility. Axonal endolysosomes are important for a variety of functions, including degradation of dysfunctional mitochondria and aggregated proteins, protein sorting and even local translation[58,111,112]. The bidirectional transport of endolysosomal vesicles in axons differs depending on the organelle's maturation state. Non-degradative EEA1-labelled and RAB5-labelled early endosomes involved in endocytosis and sorting mature into RAB7-labelled late endosomes, which further mature into highly acidified and degradative lysosomal-associated membrane protein (LAMP)-labelled lysosomes. In both non-neuronal

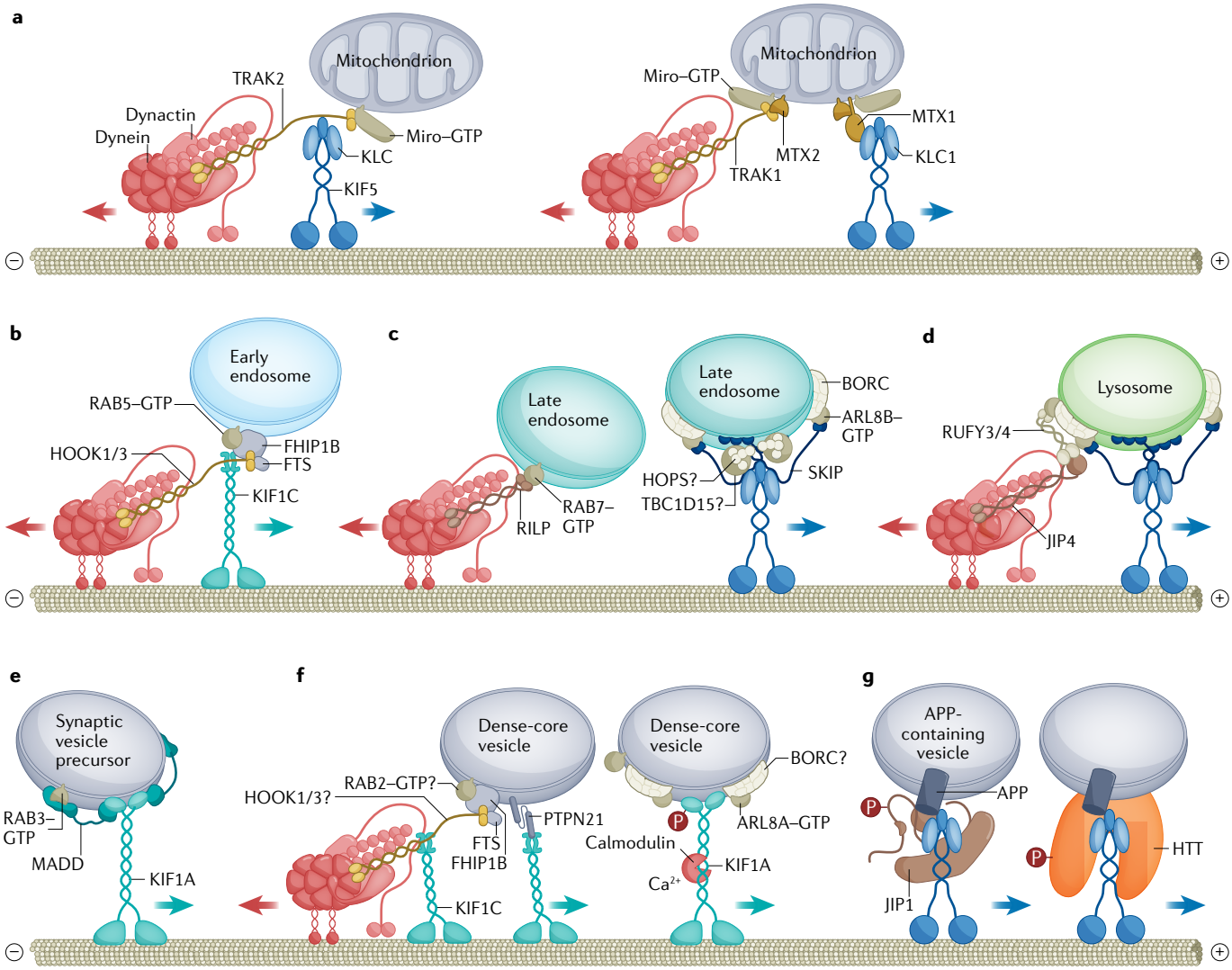


Fig. 5 | **Axonal transport of mitochondria, endolysosomes, and synaptic components. a** | The anterograde and retrograde transport of mitochondria is controlled by interactions between the small GTPase Miro and the motor-binding proteins TRAK1, TRAK2, metaxin 1 (MTX1) and metaxin 2 (MTX2). TRAK1, TRAK2 and MTX2 are likely involved in both retrograde and anterograde axonal transport, but it is unclear whether MTX1 is involved in motility in both directions or only in the anterograde direction. **b**–**f** | Early endosome transport is mediated by the motor activators HOOK1 and HOOK3 binding to dynein–dynactin and kinesin 3 (KIF1C) (part **b**). Late endosome anterograde transport (part **c**) and lysosome anterograde transport (part **d**) are both driven by the KIF5–SKIP–ARL8B–BORC (BLOC1-related complex) complex (see also FIG. 2b). Anterograde-moving RAB7-positive late endosomes are rare, likely because the kinesin activator SKIP recruits the HOPS complex, which in turn recruits the RAB7 GTPase-activating protein TBC1D15, which induces disassociation of RAB7 from the membrane. Late endosome retrograde transport (part **c**) involves RILP but the actual mechanism of dynein activation is not known. Retrograde transport of late endosomes and lysosomes can be driven by the membrane-associated proteins RUFY3 and RUFY4 activating dynein–dynactin through the motor effector JIP4. The anterograde transport of synaptic vesicle precursors (SVPs) and dense-core vesicles are both driven by kinesin 3. The primary regulators of kinesin 3 on SVPs are MADD and RAB3 (part **e**) (see also FIG. 2b), while kinesin 3 on dense-core vesicles (part **f**) is regulated by the Ca^{2+}-binding protein calmodulin, ARL8A, PTPN21 and/or HOOK3. Ca^{2+}-dependent binding of calmodulin to KIF1A activates the motor both by changing the conformation of the tail domains and possibly by inducing Ca^{2+} -dependent and calmodulin-dependent kinase II phosphorylation of the pleckstrin homology domain. HOOK3 or PTPN21 binding to the stalk of KIF1C relieves motor autoinhibition. The retrograde transport of dense-core vesicles is likely scaffolded by the HOOK3–fused toes protein (FTS)–FTS–HOOK-interacting protein 1B (FHIP1B) complex. **g** | Amyloid precursor protein (APP)-containing vesicles are transported in the anterograde direction by KIF5, scaffolded by JNK-phosphorylated JIP1 and/or AKT-phosphorylated huntingtin (HTT) (see also FIG. 2c). Note that multiple motor complexes may be simultaneously bound to a single cargo, and that the interplay between motor complexes is not well understood. KLC, kinesin light chain.

Glued motif
Common dynein-activating adaptor motif that mediates interaction with the second coiled-coil domain of the p150^{Glued} subunit of dynactin.

O-GlcNAcylation
A reversible post-translational modification whereby a monosaccharide (*O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc)) is attached to a serine or threonine residue. O-GlcNAcylation typically occurs in response to changes in nutrient state or stress.

cells and neurons, a gradient can be observed whereby endolysosomes in the perinuclear or somatic region are more degradatively competent than those in the cell periphery or distal processes[113,114].

It is somewhat unclear whether EEA1-positive early endosomes localize to axons, or exclusively to the somatodendritic region. Early endosome transport is controlled by a direct interaction between phosphatidylinositol 3-phosphate, generated by the RAB5 effector VPS34, and the kinesin 3 KIF16B[115]. KIF16B and EEA1 are enriched in the somatodendritic region and are excluded from the axon, likely owing to autoinhibition of KIF16B[25]. However, RAB5-containing early endosomes can be found in axons and are possibly generated directly in the axon by endocytosis[116]. The retrograde transport of RAB5-containing endosomes is regulated by the HOOK1–dynein–dynactin complex[116,117] (FIG. 5b). HOOK proteins are dynein-activating adaptors that bind DLIC through their N-terminal HOOK domain and dynactin through their Spindly and Glued motifs[50,88,90,117] (FIG. 2c). The HOOK proteins are recruited to the endosomal membrane by their carboxy-terminal (C-terminal) cargo-binding domain, likely via interaction with fused toes protein (FTS) and FTS–HOOK-interacting protein 1B (FHIP1B), which in turn bind RAB5–GTP[112,118] (FIG. 5b). RAB5-containing vesicles also move in an anterograde direction towards the distal axon, although the mechanism is not known[116]. In vitro, HOOK3 can form a co-complex with both dynein–dynactin and the kinesin 3 motor KIF1C and can activate transport in both directions (FIG. 4b); thus HOOK3 could potentially activate not only the retrograde motility but also the anterograde motility of endosomes[68,119].

RAB7-positive late endosomes are present in axons and move primarily in the retrograde direction[120,121] (FIG. 5c). Dynein is recruited to RAB7-containing late endosomes via interaction with RILP, which specifically interacts with RAB7–GTP on membranes[77,117,120]. Despite structural similarities with other dynein activators, RILP has not been shown to activate dynein motility in vitro or in cells. In zebrafish axons, retrograde trafficking of a subset of RAB7-containing endosomes is regulated by the transmembrane scaffolding protein vezatin, possibly functioning through a HOOK protein[122].

Late endosomal anterograde motility is mediated by kinesin 1, scaffolded by an effector complex comprising the kinesin 1 adaptor SKIP, the small GTPase ARLA8B and the multisubunit complex BORC (BLOC1-related complex)[77,123]. In *C. elegans*, BORC recruits ARL-8 to lysosomal membranes because its subunit SAM-4 functions as an ARL-8 GEF[78]. The SAM-4 mammalian orthologue is BORCS5 (also known as myrlysin); however, recombinant mammalian BORC was unable to induce ARL8B nucleotide exchange in vitro, and thus the mammalian GEF for ARL8B remains unknown[124]. GTP–ARL8 binds autoinhibited SKIP to relieve its head–tail autoinhibition[71] (FIG. 2a). The conserved acidic tryptophan motif in SKIP then binds the LFP motif of KLC and the tail domain of KHC to relieve kinesin autoinhibition and activate transport[49,53] (FIG. 2a). The number of anterograde-moving late endosomes obtained by quantifying RAB7 vesicles seems limited; however, non-neuronal work has shown that ARL8B–SKIP localization on late endosomal membranes recruits the HOPS complex, which in turn recruits the RAB7 GTPase-activating protein TBC1D15 (FIG. 5c), inducing RAB7 GTP hydrolysis and subsequent release of RAB7–GDP into the cytosol[77]. Thus, there may be an undetected population of RAB7-negative late endosomes in axons moving predominantly in the anterograde direction. Thus, the diverse modes of transit for endosomes continue to expand as we discover even greater diversity in the endosomal population.

Lysosomal trafficking. There is some disagreement as to whether mature degradative lysosomes are present in the axon. In *C. elegans* DA9 neurons, lysosomes are excluded from the axon, although the mechanism is unknown[78]. In mammalian neurons, LAMP1 can be detected in axons, although the identity of LAMP1-positive structures is debated[4,121,123,125]. Some LAMP1-labelled axonal vesicles are unacidified transport carrier vesicles derived from the *trans*-Golgi network; these vesicles move in an anterograde direction in the axon, and fuse with endosomes and autophagosomes to supply maturation factors to these compartments[121]. About half of the LAMP1-positive puncta in the axon have been described as late endosomal structures, as they colocalize with RAB7 and not with lysosomal hydrolases[125]. It is possible that LAMP1 overexpression increases its colocalization with these more immature compartments[4,123,125]. However, a subpopulation of mature degradative lysosomes with low pH and active degradative enzymes has also been observed in mammalian axons[4,123,125].

LAMP1-positive vesicles are delivered into the axon by the same kinesin–ARL8B–SKIP complex (FIGS 2a,5d) that mediates late endosomal transport[4,123,125]. In non-neuronal cells, lysosomal transport can also be driven by kinesin 3–ARL8B–BORC; in this case, ARL8B binds directly to the stalk region of kinesin 3 to relieve its autoinhibition[67,73]. There is some evidence for kinesin 3 activation of lysosomal motility in neurons, although it appears to be dependent on ARL8A rather than ARL8B[31]. BORC and/or the related complex BLOC appears to be involved in not only the anterograde transport but also the retrograde transport of late endosomes and lysosomes[126,127]. The BORC/BLOC subunit SNAPIN binds the middle domain of DIC using a series of nonpolar residues near its C terminus; however, given its size and specific interaction with DIC, it seems likely that SNAPIN serves only as an adaptor rather than an activator of dynein[126,127].

The minus end-directed transport of lysosomes is also driven by the motor regulators JIP3 and JIP4 binding to dynein–dynactin[72,75,128–130]. In zebrafish axons, Jip3/4 function on retrograde lysosomes requires the small GTPase Arf6: specifically, Jip3/4 binding to GTP–Arf6 shifts Jip3/4 from interacting with kinesin to interacting with dynein[75,131]. JIP4 may also be recruited to the lysosomal membrane by the transmembrane lysosomal protein TMEM55B or the ARL8B-binding proteins RUFY3 and RUFY4 (REFS[128,132,133]). RUFY3 and TMEM55B have been shown to recruit JIP4 to lysosomes in non-neuronal cells, and RUFY3 and RUFY4

Active zone
The region of the presynapse where synaptic vesicle fusion and neurotransmitter release occur.

En passant synapses
Presynapses located along the axon shaft.

Axon initial segment
The short region (20–60 μm) of the axon immediately adjacent to the soma which acts as a selective filter to limit axonal transport and initiates action potentials (electrical signalling).

Calmodulin
A secondary messenger protein activated by the binding of Ca^{2+} involved in numerous cell signalling pathways.

JNK
(JUN amino-terminal kinase). A family of mitogen-activated protein kinases that respond to stress stimuli and trigger signalling cascades implicated in inflammation, gene expression, DNA repair, neuronal plasticity, and cell death or senescence.

have both been shown to co-migrate with retrograde lysosomes in axons[128,132,133] (FIG. 5d). JIP3 and JIP4 bind dynein directly through a conserved DLIC-interacting site and interact with the $p150^{Glued}$ subunit of the dynactin complex[72,75,134,135]; however, it is not known whether or how JIP3/4 directly activates dynein motility. More work is necessary to not only reconcile the debate over the identity of LAMP1-containing structures in the axon but also to better understand how they are transported.

Synaptic vesicle precursor trafficking. Most synaptic proteins and vesicles are produced in the soma and transported into the axon by either slow or fast microtubule-based axonal transport[136,137]. There are three partially overlapping classes of organelles transported from the soma to presynapses: (1) synaptic vesicle precursors (SVPs), which mature into the neurotransmitter-containing synaptic vesicles involved in canonical fast synaptic signalling; (2) dense-core vesicles (DCVs), which release neuropeptides and hormones from synapses to exert temporally and spatially wider signals; and (3) organelles containing the active zone proteins that form the infrastructure of the presynapse[138]. Proteins may also be translated locally for use at synapses, which requires the anterograde transport of mRNA and ribosomes from the soma to synapses; we do not discuss this transport here as it was recently reviewed elsewhere[139].

SVPs move predominantly in an anterograde direction, as evidenced by their predominant accumulation on the somal (proximal) side of a dissected nerve[140]. The anterograde transport of SVPs is facilitated primarily by kinesin 3 (KIF1A and KIF1Bβ)[79,141] (FIG. 5e). KIF1A facilitates the transport of SVPs into axons and specifically delivers them to presynaptic sites by sensing the nucleotide state of the microtubule array[137]. Newly nucleated microtubules and microtubule plus ends, which contain GTP–tubulin, are enriched in both the axon tip and en passant synapses[137,142] (FIG. 1). In vitro, KIF1A binds more weakly to GTP–tubulin-containing lattices than to GDP–tubulin-containing lattices, facilitating microtubule detachment[137]. Thus, KIF1A moves along the axonal microtubule array until it reaches a presynapse where GTP–tubulin is enriched; there, the motor detaches from the microtubule, allowing its SVP cargo to be retained at the presynaptic site[137,142].

In *C. elegans*, kinesin 3 (UNC-104) can directly bind to the SVP membrane through its PH domain[143]. Kinesin 3 can also be scaffolded on SVPs by the ARL-8–BORC complex, the same complex that moves lysosomes into axons in mammalian cells[78]. This led to the hypothesis that SVPs may in fact be transported with and/or as part of lysosomes in axons. In *C. elegans* and *Drosophila*, most SVPs and active zone proteins are co-transported with lysosomal markers in an ALR-8/Arl8-dependent fashion[144,145]. However, in mammalian cells this co-transport is contested: as much as 85% and as little as 20% co-migration has been observed between lysosomal and synaptic structures in the same mammalian cell type[74,144]. Furthermore, in mammalian axons, SVP transport does not seem to rely on ARL8 scaffolding[74]. The GEF MADD (also known as DENN) activates the small GTPase RAB3 on SVP membranes and also binds the KIF1A CC3 domain[31,79] (FIG. 2b). Additionally, the bidirectional motor scaffold JIP3 could play a role, as *C. elegans* null mutants for its orthologue (UNC-16) show disruption in the anterograde transport of SVPs[146]. While JIP3 activation of kinesin 3 (KIF1A) has not been shown, JIP3 is known to activate kinesin 1 by binding both the TPR domain of KLC and the tail domain of KIF5C to relieve autoinhibition[52,65,66,72,147]. Further studies, especially in mammalian neurons, are necessary to settle the many debates over SVP transport.

Dense-core vesicle transport. DCVs exhibit an unusual mode of transport known as circulation and sporadic capture. Rather than being delivered by kinesins directly from the soma to the numerous presynapses, DCVs are delivered initially all the way to the axon tip, with some stopping to localize at en passant synapses on the way; at the tip, excess DCVs switch to retrograde transport, returning to the axon initial segment, where they again switch to anterograde transport[148,149]. This phenomenon has been best studied in *Drosophila* but appears to be conserved in mammalian neurons[148,149]. DCV anterograde transport is driven by KIF1A, which is activated by ARL8A binding to its CC3 domain and/or Ca^{2+}-dependent binding of calmodulin to the region between its CC2 and CC3 domains[31,150] (FIG. 5f). Ca^{2+}-dependent binding of calmodulin to KIF1A activates the motor both by changing the conformation of the tail domains and possibly by inducing Ca^{2+}- and calmodulin-dependent kinase II phosphorylation of the PH domain, which increases KIF1A affinity for DCV and SVP membranes[31,150]. It is not known whether ARL8 and calmodulin can bind KIF1A simultaneously.

Kinesin 3 KIF1C has also been shown to drive anterograde transport of DCVs[26], although the regulation is wholly different. KIF1C is autoinhibited by interactions between its stalk region and its microtubule-binding domain, and autoinhibition is relieved upon binding to the phosphatase PTPN21 or the motor adaptor HOOK3 (REFS[68,119]) (FIG. 5f). The N terminus of PTPN21 or the C terminus of HOOK3 binds the stalk region of KIF1C, which frees the motor domain for activity[119]. Because HOOK3 is also known to activate dynein–dynactin motility, as described earlier herein, it is possibly responsible for retrograde transport of DCVs in axons, although the actual mechanism of DCV retrograde transport is unknown[68,84]. Rab2 loss in *Drosophila* causes a loss of bidirectional DCV transport, with a particularly strong phenotype in the retrograde direction; thus, perhaps Rab2 is involved in recruiting dynein to the DCV membrane[76] (FIG. 5f).

Importantly, there are several methods by which transport is halted at synapses to facilitate the retention of DCVs at these sites. In addition to the reduced affinity of KIF1A for GTP-enriched microtubule plus ends at presynapses, KIF1A-dependent capture of DCVs can also be regulated by neuronal activity through a phosphorylation-dependent event[149]. Neuronal activity increases JNK activity in synapses, leading JNK to phosphorylate the active zone protein synaptotagmin 4 (SYT4), which undergoes co-trafficking with DCVs[149].

Piccolo–Bassoon transport vesicles
trans-Golgi-derived vesicles that transport Piccolo and Bassoon, two large scaffolding proteins that help form the active zone, from the soma to the presynapses.

Amyloid precursor protein
(APP). A transmembrane protein enriched at synapses believed to be important for synaptic formation and plasticity. APP can be differentially cleaved, and the cleavage product, β-amyloid, accumulates in neurodegenerative diseases, including Alzheimer disease.

Huntingtin
(HTT). A large scaffolding protein (~350 kDa) involved in multiple pathways, including axonal transport and transcription. Expansion of the polyglutamine repeat region in the amino terminus of the protein results in Huntington disease.

AKT
Also known as protein kinase B, a family of serine/threonine kinases involved in cell survival, proliferation and metabolism.

ATG8
A family of ubiquitin-like proteins localized primarily to the autophagosomal membrane and necessary for both selective and bulk autophagy, autophagosome biogenesis and autophagosome–lysosome fusion. LC3B is a well-characterized member of this family commonly used as a marker for autophagosomes in mammalian cells.

Activity-dependent phosphorylation of SYT4 at site S135 disrupts the direct interaction between SYT4 and KIF1A, halting the transport of DCVs at sites of neuronal activity[149]. It is worth noting that the direct interaction between SYT4 and KIF1A can be modulated by Ca^{2+}-dependent binding of calmodulin to KIF1A[150]. DCV capture at synapses may additionally rely on the direct binding between KIF1A and the active zone protein liprin-α; however whether this mechanism depends on liprin-α activation of KIF1A or on liprin-α anchoring KIF1A to actin to stop motility is unclear[150,151]. In conclusion, motor inactivation may be as important as activation to facilitate delivery to presynaptic sites.

Delivery of other synaptic components. Many active zone proteins appear to be transported in concert with SVPs or DCVs. However, they can also be transported independently, for example as Piccolo–Bassoon transport vesicles[32]. As mentioned earlier, at least one active zone protein, liprin-α, interacts directly with and may activate KIF1A (via binding to the region between the CC2 domain and the CC3 domain of KIF1A)[150,151]. The active zone protein syntaxin 1 indirectly interacts with KIF5B through the kinesin adaptors FEZ1 and/or syntabulin, although neither adaptor has been shown to independently activate kinesin motility[32,152,153]. Additionally, the microtubule-associated protein Par-1 (also known as MAPK) and the small GTPase Rab2 may play a role in the axonal transport of active zone proteins in *Drosophila*, although the specific mechanisms are unknown[76,154]. More studies in mammalian cells are necessary to understand which synaptic components are co-trafficked and whether that co-transport changes across neurodevelopment.

Amyloid precursor protein (APP) is also necessary for presynaptic homeostasis and is transported with an anterograde bias in axons[54,57]. APP transport relies on the phosphorylation state of two motor scaffolding proteins, JIP1 and huntingtin (HTT). JIP1 phosphorylation at S421 by JNK induces preferential binding to kinesin 1 over dynein, and HTT phosphorylation at S421 by the kinase AKT kinase promotes binding to kinesin 1; both of these events favour the anterograde transport of APP-containing vesicles[54,57] (FIG. 4c). Phospho-JIP1 activates kinesin 1 motility by relieving both KLC TPR-mediated autoinhibition and head–tail autoinhibition[57,65], but the mechanism by which phospho-HTT affects kinesin motility is unclear[55]. Moving forwards, understanding the regulation of these upstream phosphorylation events is crucial.

Transport of autophagic vacuoles. Because neurons are postmitotic and long-lived, protein and organelle turnover and recycling are essential to maintain their homeostasis and proper function throughout an individual's lifetime. One degradative pathway occurring continuously in axons is autophagy; autophagosomes engulf proteins and organelles in the distal axon and fuse with late endosomes and lysosomes during transport to the soma to degrade their cargo[18]. Although autophagic vacuoles traffic almost uniformly in the retrograde direction, distinct dynein regulatory proteins control their motility depending on subaxonal location, likely because fusion with endolysosomes during transit changes the composition of their membrane and membrane-associated proteins[90] (FIG. 6a).

Early autophagosomes associate with motor regulator JIP1 via the ATG8 family protein LC3 (REF.[155]). JIP1 specifically inactivates kinesin 1 when bound to LC3 (FIG. 4c), thus allowing the initiation of retrograde transport from the distal axon[90,155] (FIG. 6a). Shortly after autophagosome formation, LC3 and other ATG8 orthologues are cleaved from the external surface; thus, the function of JIP1 in autophagosomal transport is limited primarily to the distal axon[90,155]. Subsequently, dynein scaffolding on autophagosomes in the mid axon is controlled by the scaffolding protein HTT and its interacting protein HAP1 (REF.[90]) (FIG. 6a). HAP1 is a dynein-activating adaptor that binds DLIC using its CC1 box motif and dynactin via the Spindly and Glued motifs to induce the formation of a motile complex[87,90]. HTT links the dynein–dynactin–HAP1 complex to the autophagosomal membrane, presumably through putative interactions with lysosomal membrane proteins (for example, VPS35 and multiple vATPase subunits)[156,157] (FIG. 4e). Interestingly, HAP1 in complex with a different scaffold protein, GRIP1, can activate kinesin 1 motility; this is required for the transport of neurotransmitter receptors into dendrites[100,158] (FIG. 4f).

Finally, following autophagosome–lysosome fusion, mature degradative autolysosomes in the mid and proximal axon require the bidirectional motor scaffold JIP3 to continue motility near the soma[90] (FIG. 6a). JIP3 is required for autophagic vacuole transport in both *C. elegans* neurons and mammalian neurons, and can bind both kinesin and dynein–dynactin; however, the mechanism by which it drives retrograde transport is not yet known[52,72,90,159]. It may interact with the autolysosome membrane via its binding partner and small GTPase ARF6, which is important for autophagosomal trafficking in zebrafish[75,160]. The function of JIP3 on autolysosomes and the association of JIP1 and HAP1 with the autophagic vacuole membrane is dependent on the organelle's maturation state[90]. Specifically, JIP1 and HAP1 appear to disassociate from mature autolysosomes, especially those that have broken down their inner autophagosomal membrane; in contrast, JIP3 associates with the majority of autophagosomes regardless of the maturation state, but is active only on those that have broken down their inner membrane[90,161]. Further, the function of all three effectors is directly or indirectly regulated by phosphorylation: JIP1 dephosphorylation by the phosphatase MKP1 induces it to inactivate kinesin[57,155] (FIG. 4c); the calcineurin-mediated dephosphorylation of the HAP1 effector HTT leads to decreased affinity for kinesin[55,56]; and JIP3 may be downstream of LRRK2-mediated phosphorylation of RAB GTPases on the autolysosomal membrane[58]. Additional proteins, including SNAPIN and RILP, have been suggested to regulate dynein on axonal autophagic vacuoles, but their specific roles in transport are poorly understood[120,162]. The tightly regulated handover between motor effector proteins on a single cargo during unidirectional transport has so far been observed only

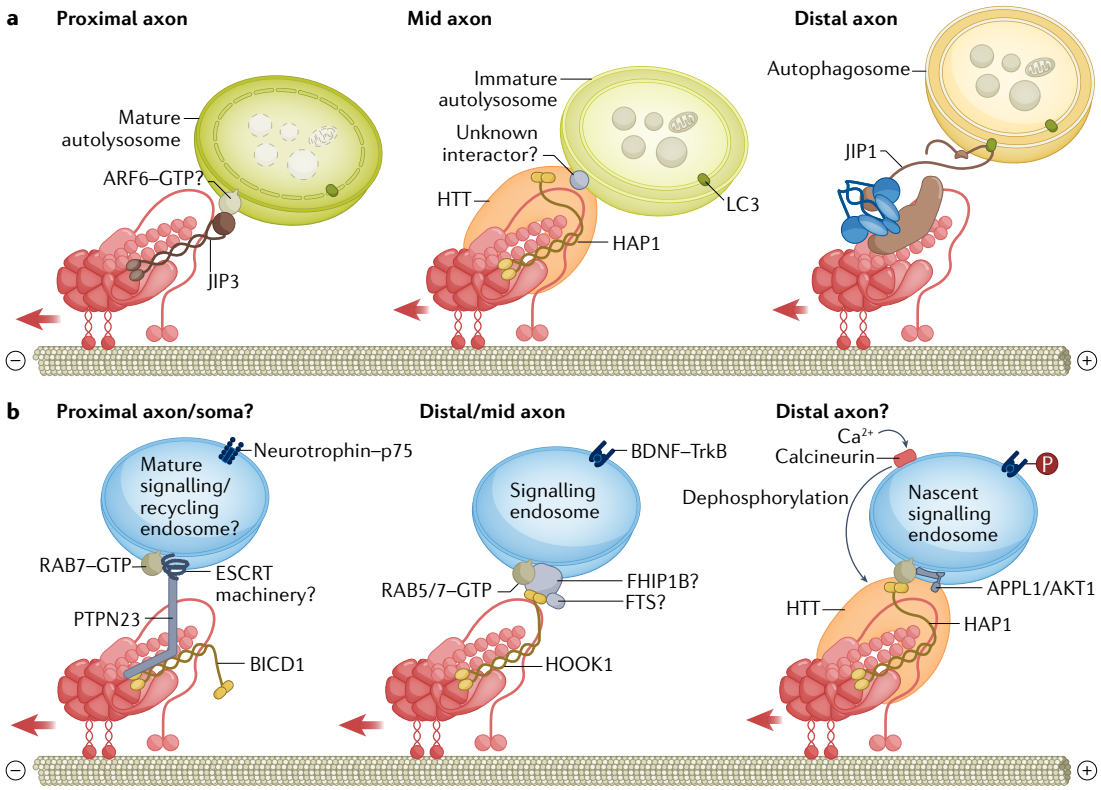


Fig. 6 | **Axonal transport of autophagic vacuoles and signalling endosomes.** Autophagic vacuoles and signalling endosomes both move in a retrograde direction through the axon, maturing during transit through either fusion with endolysosomes or RAB conversion. During this maturation, it is known (part **a**) or proposed (part **b**) that multiple dynein effectors sequentially drive transport. **a** | Autophagic vacuole transport is initiated by JIP1, which inactivates kinesin. Transport along the mid axon is driven by dynein in complex with the activating adaptor HAP1 and its binding partner HTT. Dynein is scaffolded on mature autolysosomes by JIP3, although whether and how JIP3 activates dynein are unknown. It may be recruited to the autophagosomal membrane by the small GTPase ARF6. **b** | Nascent signalling endosomes are formed at presynaptic sites following neurotrophin (for example, BDNF) binding to a receptor (for example, TrkB or p75). These endosomes must then be transported in a retrograde direction to the soma to induce signalling, after which they may be recycled back to the membrane or degraded. Neurotrophin–receptor binding in the distal axon induces local Ca^{2+} signalling and BDNF phosphorylation, which may induce calcineurin to dephosphorylate the motor scaffold huntingtin (HTT). Dephosphorylated HTT binds kinesin less efficiently, potentially helping initiate retrograde transport. The dynein activator HAP1 has also been detected colocalizing with signalling endosomes; thus, it may activate their retrograde transport. HOOK1 activates dynein on signalling endosomes in the distal axon and mid axon, which may involve the HOOK-interacting proteins fused toes protein (FTS) and FTS–HOOK-interacting protein 1B (FHIP1B). The dynein-activating adaptor BICD1 and the ESCRT-interacting protein PTPN23 also appear to regulate the transit of signalling endosomes, possibly during sorting for recycling or degradation. Note that multiple motor complexes may be simultaneously bound to a single cargo, and that the interplay between motor complexes is not well understood.

for autophagic vacuoles. However, it may be relevant for other cargos that mature or traverse long distances.

Signalling endosome transport. Nascent RAB5-positive signalling endosomes form in the distal axon by endocytosing activated neurotrophin receptors and their bound ligands, which are then transported to the soma to modulate transcription. Like autophagosomes, signalling endosomes mature in the axon by acquiring RAB7, and this maturation is important for their retrograde transport[163]. Multiple dynein regulatory proteins have been implicated in the transport of signalling endosomes; thus, we predict they may use multiple sequential motor effectors to scaffold their long unidirectional transport. However, the different motor effectors may instead be specific for different types of signalling endosomes, for example TrkB receptor-containing signalling endosomes versus p75 receptor-containing signalling endosomes, although recent work suggests these neurotrophin receptors are internalized and possibly trafficked together[164].

The earliest step in retrograde signalling endosome transport may be initiated by local Ca^{2+}signalling triggered by neurotrophins binding to receptors at the presynapse[56]. This local increase in Ca^{2+} concentration triggers the activity of the phosphatase calcineurin, which was shown to dephosphorylate S421 of HTT, decreasing the HTT–kinesin interaction and promoting retrograde transport by binding to dynein[56] (FIG. 6b). The HTT interactor HAP1 also localizes to neurotrophin receptors upon neurotrophin binding, and appears to be required for their signalling to the

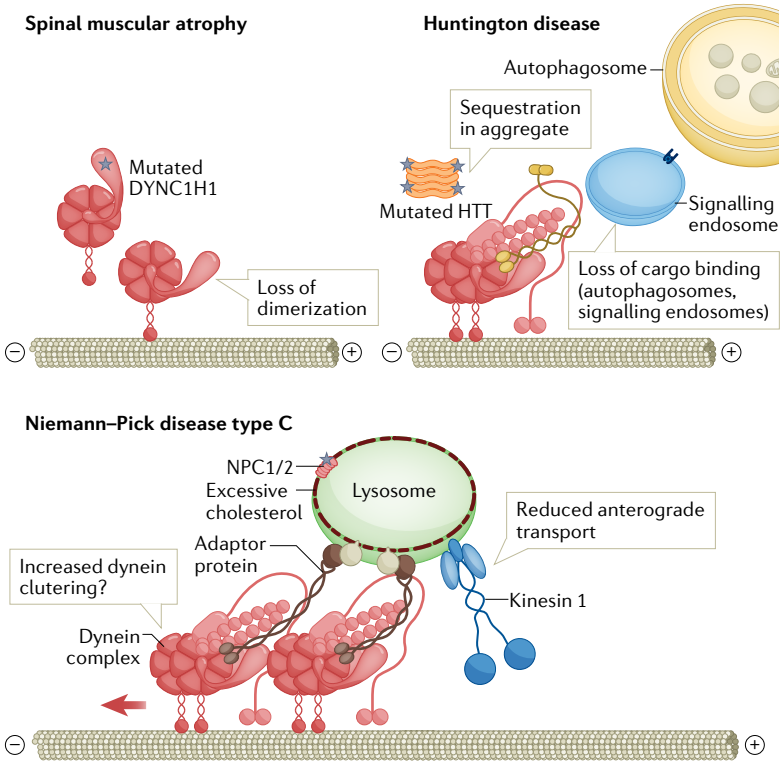
Neurotrophin
Extracellular-signalling factor (typically a small protein or peptide) that triggers cascades in neurons, including to survival, development/growth and function.

Box 2 | **Dysfunction of axonal trafficking**

Perturbations of axonal trafficking have considerable pathological consequences; here we highlight just a few examples. Mutations in motor proteins themselves or their regulators can result in disease. Mutations in the gene encoding cytoplasmic dynein 1 heavy chain 1 (DYNC1H1) lead to malformations of the brain cortex, muscle weakness and/or neurodegeneration[182,183]. Few disease-causing mutations are seen in the AAA+ motor domain, likely because they would be lethal early in development, due to essential roles for cytoplasmic dynein in cell division and cell polarity[183]. Neurodegeneration-causing mutations, such as those seen in spinal muscular atrophy with lower-extremity predominance, are primarily located in the tail domain; these mutations are predicted to affect dimerization and/or complex formation with other dynein subunits, dynactin or cargo adaptors[182,183] (see the figure). Lissencephaly, a specific form of cortical malformation, can be caused by mutations in the dynein regulatory protein lissencephaly 1 (LIS1), which is important for relieving dynein autoinhibition[80].

Disease-causing mutations in the kinesin 1 KIF5A are mostly in the motor domain, where they primarily affect ATPase activity and microtubule binding[184]. A newly identified group of mutations in the cargo-binding domain result in a truncated protein unable to bind cargo and cause amyotrophic lateral sclerosis[185,186]. Mutations in the kinesin 3 KIF1A result in progressive juvenile neurodegeneration[187]. The specific symptoms differ on the basis of the location of the mutation; most mutations are clustered in the motor domain, although some have been identified in the carboxy terminus of the protein [187]. Many KIF1A mutations are loss-of-function mutations, inhibiting ATPase activity, microtubule interactions or cargo binding[188,189]. Others can be classified as gain-of-function mutations, leading to increased microtubule binding or motor hyperactivity[137,187,190]. Rare missense mutations in the kinesin 4 motor KIF21B are also gain-of-function mutations, and seem to disrupt normal autoinhibition, leading to hyperactive KIF21B and subsequent neuronal migration and neurodevelopmental phenotypes, including microcephaly[191].

Mutations in cargo adaptor proteins and their upstream regulatory proteins may lead to more-specific disruptions in the axonal transport of particular organelles. Mutations in the scaffolding protein huntingtin (HTT), which interacts with the dynein-activating adaptor HAP1, disrupt the axonal transport of autophagosomes (see the figure); this inhibits protein and organelle turnover and possibly contributes to the development of Huntington disease[90,157]. HTT mutation may also disrupt the transport of amyloid precursor protein (APP)-containing vesicles and signalling endosomes[54,56] (see the figure). Autophagosome motility is also disrupted by Parkinson disease-causing mutations in the kinase LRRK2; LRRK2 mutants are hyperactive, leading to increased phosphorylation of RAB proteins, including RAB35 and RAB10, increasing their recruitment to the autophagosomal membrane[58,192]. The RAB proteins then recruit the putative kinesin activator JIP4 to the autophagosomal membrane, which may be responsible for the decreased retrograde autophagosome motility[58]. Mutations in the lysosomal membrane proteins NPC1 and NPC2 lead to a childhood neurodegenerative disorder called 'Niemann–Pick disease type C', a lysosomal storage disorder[193]. Loss of NPC1/2 leads to excess lipids, especially cholesterol, accumulating on the lysosomal membrane, which impairs axonal lysosome transport either owing to increased dynein clustering driving lysosomes to remain at the microtubule minus ends in the soma and/or reduced interaction of kinesin 1 with its effector proteins on the lysosomal membrane[95,96,193] (see the figure). Although there are many other disease-causing mutations that affect axonal transport, these examples demonstrate the variety of mechanisms by which mutations in motor proteins and their regulators can induce neurodevelopmental and neurodegenerative disease.



nucleus[165]. Thus, HAP1 may activate dynein on signalling endosomes[90]. HOOK1 has been shown to drive the retrograde motility of both RAB5-positive and RAB7-positive signalling endosomes in the distal and mid axon, where it likely binds via FTS–FHIP1B–RAB5 (REFS[112,116,118]) (FIG. 6b). Similarly to transport of endolysosomes, vezatin and vezatin-like protein (Vezl) have been implicated in signalling endosome transport in zebrafish and *Drosophila* neurons, especially along the mid axon[122,166].

Additionally, BICD1, a dynein-activating adaptor that binds dynein–dynactin through a CC1 box motif and a Spindly motif, was shown to be involved in the axonal transport of p75-containing signalling endosomes, although the subaxonal specificity was not examined[87,88,167]. BICD1 is recruited to signalling endosomal compartments via interaction with PTPN23, an ESCRT-interacting protein[167]. Interestingly, loss of PTPN23 led to a build-up of neurotrophin receptors in vacuole-like compartments, where they became highly ubiquitylated. This suggests that BICD1 has a role at later stages of signalling endosome processing, when the neurotrophins and their receptors are being sorted for recycling or degradation[167,168]. The scaffold SNAPIN may also help recruit dynein to signalling endosomes[169]. Thus, signalling endosomes engage multiple means of transport, and may use a motor effector handover scheme as in autophagosomal transport. Many questions remain, including whether different neurotrophin receptors are co-transported, how signalling endosomes mature along the axon and how the various motor regulators involved in signalling endosome transport interrelate.

Spinal muscular atrophy with lower-extremity predominance
An inherited neuromuscular disorder characterized by muscle weakness and wasting in the lower limbs, which primarily appears in childhood.

Lissencephaly
A neurodevelopmental disorder characterized by a 'smooth brain' without normal cortical folds.

Microcephaly
A birth defect wherein a baby's head is smaller than normal owing to abnormal brain development.

Huntington disease
A neurodegenerative disease resulting from a polyglutamine expansion in the gene encoding huntingtin (HTT). Symptoms typically appear in early adulthood, include both motor and cognitive problems, and worsen over time.

Parkinson disease
A progressive neurodegenerative disease resulting from the degradation of the dopaminergic neurons in the substantia nigra, part of the midbrain. Symptoms include tremors, stiffness and difficulty moving, especially controlling or initiating movement.

Lysosomal storage disorder
A class of inherited metabolic disorders wherein lysosomal degradation is defective. They can affect a range of tissues, including the brain, eyes, muscles and kidneys. Most patients develop symptoms during childhood, and these worsen over time.

ESCRT
Endosomal sorting complexes required for transport (0–III) made up of cytosolic proteins that facilitate membrane remodelling including, multivesicular body formation and membrane abscission during cytokinesis. They can also be involved in protein–protein interactions, especially to recruit other proteins to the endosomal membrane.

Conclusions and perspective

The tight regulation of microtubule motors via autoinhibition and via cargo interaction is key to organizing the cytoplasm, especially in cells as complex as neurons. Properly orchestrated organellar transport in axons is integral for neuronal development and homeostasis, and dysfunction at any level can result in neurodevelopmental or neurodegenerative disease (see BOX 2). Owing to high-resolution structural studies of purified complexes and in vitro reconstituted motility, massive strides have been made in recent years in our understanding of microtubule-based motility. The emerging picture is that distinct but analogous motor activation schemes are used for different cargo, whereby each cargo can be transported with specificity to its subcellular destination to serve its function.

With this greater mechanistic knowledge of dynein-based and kinesin-based transport, the next stage of understanding axonal trafficking may begin. We list here several open questions, which we deem will be essential to provide a thorough mechanistic description of cargo trafficking and its organization in highly polarized neurons. Which cargos are co-transported, and are they co-transported as a single membrane-bound vesicle? For example, it remains to be seen what proportion of active zone proteins are co-transported in mammalian cells with SVPs or DCVs[32,140,154]. How many motor complexes localize to a single cargo? How many of these are active simultaneously? The number of motor complexes likely depends on organelle size and copy number of binding partners present on a given organelle; further, the number of active complexes may be dependent on membrane curvature[170,171]. What is the interplay between the multiple different motor-containing complexes implicated in the transport of any given cargo? For example, can a KIF5–ARL8B complex[71,77,172] and a KIF1C–HOOK3 complex[68,119] be active simultaneously to induce anterograde transport of a late endosome? Or do different motor complexes localize to distinct organelles?

How is competition between kinesin and dynein prevented to achieve the velocities observed in cells? This is especially complicated when one is considering motor effectors such as TRAK1 and TRAK2 that co-complex with dynein and kinesin and are capable of activating both motors[98,106]. What is the interplay between motor activators (for example, dynein-activating adaptor proteins) and scaffolding proteins (for example, HTT or SNAPIN)? Are scaffolds required only to recruit motors and facilitate complex formation, or do they remain associated with motile complexes? Organelles are dynamic in the axon, changing through fission and fusion (for example, mitochondria or autophagic vacuoles) and/or RAB conversion (for example, endosomes). How is motor regulation affected by these changes[90]? Upstream regulation of motile complexes by GTPase-activating proteins/GEFs and kinases/phosphatases is likely important for most cargos[56,78,124]. What are the relevant upstream regulators for each motor–cargo pair, and how are they maintained and activated in specific subaxonal compartments and/or on relevant cargo?

Accumulating evidence indicates that axonal transport is affected in both neurodevelopmental diseases and neurodegenerative diseases (BOX 2). While in some cases it may be possible to design targeted therapies to correct defects in specific motors, such as KIF1A mutations linked to KIF1A-associated neurological disorder, other molecular motors, such as dynein, are structurally complex and/or play pleiotropic roles throughout our lifetime, making direct targeting by gene replacement difficult to envision. Thus, it is essential that we develop a more comprehensive understanding of the regulatory mechanisms involved in essential homeostatic pathways such as autophagy or mitochondrial trafficking, or essential signalling pathways such as the trafficking of neurotrophic factors from the synapse to the soma. A full molecular understanding will provide more opportunities for therapeutic approaches such as tuning motor activation or motor recruitment via selective modulation of kinases, such as the recent demonstration that pharmacological inhibition of hyperactive LRRK2 enhances the processive retrograde motility of axonal autophagosomes[58]. The cargo-specific mechanisms detailed here suggest that it may be possible to selectively activate defective biological pathways without generally perturbing function, making future therapeutic approaches more specific.

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