

Axonal transport and neurodegenerative disease: vesicle-motor complex formation and their regulation

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Abstract: The process of axonal transport serves to move components over very long distances on microtubule tracks in order to maintain neuronal viability. Molecular motors – kinesin and dynein – are essential for the movement of neuronal cargoes along these tracks; defects in this pathway have been implicated in the initiation or progression of some neurodegenerative diseases, suggesting that this process may be a key contributor in neuronal dysfunction. Recent work has led to the identification of some of the motor-cargo complexes, adaptor proteins, and their regulatory elements in the context of disease proteins. In this review, we focus on the assembly of the amyloid precursor protein, huntingtin, mitochondria, and the RNA-motor complexes and discuss how these may be regulated during long-distance transport in the context of neurodegenerative disease. As knowledge of these motor-cargo complexes and their involvement in axonal transport expands, insight into how defects in this pathway contribute to the development of neurodegenerative diseases becomes evident. Therefore, a better understanding of how this pathway normally functions has important implications for early diagnosis and treatment of diseases before the onset of disease pathology or behavior.

Keywords: kinesin, dynein, amyloid precursor protein, huntingtin, microtubules

Introduction

Axonal transport within neurons is essential for cell viability, supplying the synapse with important components for survival. Unlike other cell types, neurons are polarized and can extend long distances (up to 1 m in humans). Many proteins that are newly made in the cell body are actively transported down axons along microtubule tracks by molecular motors. Microtubules (MTs) are long, hollow cylinders approximately 25 nm in diameter that are comprised of α - and β -tubulin dimers.¹ Therefore, all of the transported components synthesized in the cell body must be assembled, associated within complexes, attached to a particular engine or motor, and efficiently transported down the axon. As such, all of these steps must be highly regulated and any misregulation could result in transport deficits leading to neuronal dysfunction.

Experiments on transport kinetics classified two types of axonal transport, as fast and slow.² Fast axonal transport moves components at rates of 100–400 mm day⁻¹ (1–5 $\mu\text{m s}^{-1}$), while slow transport moves components considerably slower, 0.2–5 mm day⁻¹ (0.01–0.001 $\mu\text{m s}^{-1}$). Fast axonal transport primarily transports vesicular cargoes and membrane-bound organelles, while slow axonal transport transports cytoskeletal components. Current knowledge indicates that the same molecular motors mediate both fast and slow axonal transport.^{3–5}

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In this review, we focus on fast axonal transport and its motor-cargo complexes. Given the complexity of axonal transport machinery, deficits in axonal transport can occur by several means, including complications with motor function, problems with cargo complex assembly or cargo attachment to motors, as well as malfunctions in regulatory elements. Recent studies have shown that deficits in these processes can either cause or exacerbate neurodegeneration.^{6,7} Some proteins involved in neurodegenerative diseases have also been implicated in this pathway with human disease mutations in these proteins causing defects in axonal transport.⁸⁻¹² Importantly, mutations in motor proteins have also been reported to cause neurodegeneration.¹³ Furthermore, many of these disease proteins normally form complexes that have functional roles in axonal transport. Alterations in the assembly or defects in the regulation of these protein complexes may result in transport deficits instigating the disease pathology and behavioral defects seen in disease states. Thus, elucidating the mechanisms of axonal transport has important implications for potential early diagnosis of neuronal disease and for the development of therapeutic interventions before the onset of neuronal death.

Motor proteins and regulatory models

Conventional kinesin motor

The kinesin-1 motor protein, also called “conventional kinesin”, is part of a subfamily of motor proteins associated with MTs and move toward their plus ends (Figure 1).¹⁴ An important feature of kinesin-1 is its ability to take 8 nm steps as measured using in vitro experiments, performing processive movement.¹⁵ Kinesin-1 was first identified in squid axoplasm^{16,17} and is highly conserved in many species.¹⁸⁻²² Biochemical analyses of kinesin-1 from bovine brains revealed that it is a tetramer consisting of two heavy chains (120 kd) and two light chains (62 kd).^{23,24}

In invertebrates, such as *Drosophila*, a single gene is responsible for each subunit while, in mammals, kinesin-1 is more complex and requires three genes for kinesin heavy chains (*KIF5A*, *KIF5B*, *KIF5C*) and three for kinesin light chains (*KLC1*, *KLC2*, *KLC3*).^{20,21}

Sequence analysis, expression studies,²² and electron microscopy^{25,26} revealed that the kinesin heavy chain (KHC) subunit contains three domains: a motor domain; a stalk domain; and a tail domain. The globular motor domain at the N-terminus of KHC contains sites for MT and adenosine triphosphate (ATP) binding. The α -helical coiled-coil stalk domain contains sites where two heavy chains can

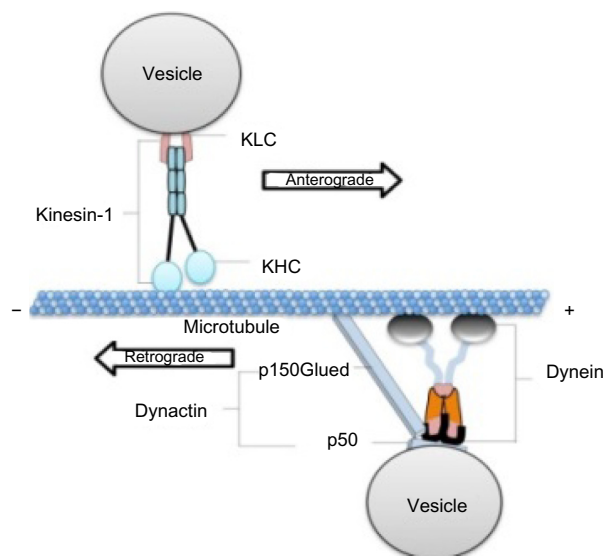


Figure 1 Conventional kinesin (kinesin-1) and cytoplasmic dynein motor complex on a MT for axonal transport.

Notes: Kinesin-1 through the KHC head motor domains binds MTs. Vesicles associate with kinesin-1 via associations with the KLC or via adaptor scaffolding proteins. Cargo complexes attached to kinesin-1 are transported anterogradely (toward + end of MTs; arrow). Cytoplasmic dynein associates with dynactin for retrograde movement. Dynactin consists of multiple proteins, but only p150Glued and p50 are shown for simplicity. Dynein interacts with dynactin via associations between p150Glued and DIC subunits. Dynein binds MTs via the tip of the stalk of DHC, while dynactin also associates with MTs via the N-terminal globular domain of p150Glued. Dynein–dynactin motor complex transports cargoes retrogradely (toward – end of the MTs; arrow).

Abbreviations: KHC, kinesin heavy chain; MTs, microtubules; KLC, kinesin light chain; DHC, dynein heavy chain; DIC, dynein intermediate chain; DLC, dynein light chain.

dimerize, and the C-terminal tail domain interacts with the kinesin light chain (KLC) subunit.^{22,25-29} Classical studies using truncated fragments of *Drosophila* KHC expressed in *Escherichia coli* revealed that KHC is able to exclusively induce movement along MTs.³⁰ Fragments that specifically contained the motor domain were capable of inducing MT motility,³⁰ indicating that the KHC motor domain is required for movement.

Loss of function of KHC in *Drosophila* showed larval paralysis and axonal accumulations of synaptic vesicles in larval segmental nerves with lethality at early third instar larval stages.³¹⁻³⁴ In mice, *KIF5B* is expressed ubiquitously while *KIF5A* and *KIF5C* are neuronal specific. Targeted disruption of the *KIF5B* or *KIF5A* gene in mice caused embryonic lethality,^{35,36} while knockout of the *KIF5C* gene caused a reduction in brain size with no gross changes in the nervous system. These mice were viable.³⁷ Primary cultures from *KIF5B* null embryonic cells exhibited striking phenotypes of mitochondrial and lysosomal distribution, indicating that *KIF5B* may be essential for mitochondrial and lysosomal dispersion.³⁵ A postnatal knockout of *KIF5A* exhibited seizures and death at approximately 3 weeks of age

with neurofilament proteins accumulating in the cell bodies of peripheral sensory neurons, accompanied by a reduction in sensory axon caliber.³⁶ Older animals also developed age-dependent sensory neuron degeneration, accumulation of neurofilaments in cell bodies, reductions in axons, and hind limb paralysis. Recent studies indicated that *KIF5A* is also essential for gamma-aminobutyric acid receptor transport and that deletion of *KIF5A* caused epileptic phenotypes.³⁸ Interestingly, a missense mutation in the mammalian *KIF5A* gene was identified in a family with hereditary spastic paraplegia, an inherited neurodegenerative disease characterized by lower limb spasticity and weakness.³⁹ Thus far, 19 of the 21 hereditary spastic paraplegia (SPG) disease mutations identified in humans are localized to the motor domain of *KIF5A*,^{39,40} indicating that these mutations cause disease by perturbing the proper transport of components to the synapse. Indeed, the hereditary spastic paraplegia mutation N256S, which occurs within the *KIF5A* gene, severely disrupted MT-based transport in *Drosophila*.¹³ Furthermore, a *KIF5A* mutation was also detected in a Charcot–Marie–Tooth type 2 patient belonging to a SPG mutant family.⁴⁰ Thus, mutations in *KHC* can lead to neurodegeneration due to deficits in axonal transport.

The *KLC* subunit contains three domains: an N-terminal coiled-coil domain or heptad repeats which bind *KHC*; a tetratricopeptide repeat (TPR) domain; and a C-terminal domain. The TPR and C-terminal domains bind cargo and function as a linker between *KHC* and the transported cargoes.⁴¹ The TPR domain, which is a protein–protein interaction domain, has been shown to interact with a variety of adaptor proteins, such as the JNK-interacting proteins (JIP), huntingtin (HTT)-associated protein (HAP) 1,^{42,43} and amyloid precursor protein (APP).⁴⁴ Structurally, TPR is composed of tandem repeats of 34 amino acids that each form a helix–turn–helix arrangement and a superhelical conformation of multiple TPR repeats.⁴⁵ While *Drosophila* has only one *KLC* gene, mammals have three *KLC* genes – *KLC1*, *KLC2*, and *KLC3*. *KLC1* is predominantly expressed in neuronal tissues while *KLC2* shows a more ubiquitous pattern of expression.⁴⁶ Removal of functional *KLC1* resulted in significantly smaller mutant mice that exhibited pronounced motor disabilities.⁴⁵ *KLC2* was shown to play a role in Na/K-ATPase-containing vesicles in alveolar epithelial cells.⁴⁷ Interestingly, *KLC3* appears to play a unique and specialized role in spermatids.⁴⁸ Loss of function of the single *KLC* gene in *Drosophila* showed paralysis phenotypes, axonal accumulations, and a third instar larval lethality.³⁴ Interestingly, a polymorphism in

KLC was found to associate with Alzheimer’s disease (AD),⁴⁹ indicating that mutations in *KLC* may also lead to neuronal dysfunction.

Cytoplasmic dynein motor

Dynein, the retrograde motor, (Figure 1), is a multisubunit complex that consists of two catalytic heavy chains (DHC), two intermediate chains (DIC), and several light chains (DLC: Tctex1; roadblock; and LC8 subfamilies).^{14,50,51} In mammals, DHC is encoded by a single gene (*Dync1h1*), DIC is encoded by two genes (*Dync1i1*, *Dync1i2*), and DLC is encoded by a family of three genes (*Dynl1*, *Dynl3*, *Dynlrb1*, *Dynlrb2*, *Dynll1*, *Dynll2*).^{51,52} The motor domain is localized in the C-terminal region of DHC.^{53,54} Structurally, the C-terminus of DHC is comprised of six tandemly linked AAA (ATPases Associated with diverse cellular Activities) domains,^{53–56} with the first four AAA domains essential for dynein movement.^{57,58} Force production and translocation are achieved through the N-terminal region of DHC, also known as the “stem domain.”⁵⁹ In vitro studies of the stem domain in *Dictyostelium* revealed that the dimerization of DHC and the binding of DIC and DLC can be achieved through this region.⁶⁰ Collectively, the association of the smaller dynein subunits with the N-terminal region (tail domain) of DHC forms the cargo-binding complex for retrograde motility.

Cytoplasmic dynein associates with a protein complex called dynactin (Figure 1) for retrograde motility. Dynactin is composed of: p150Glued; dynamitin (p50); p62; actin-interacting protein (Arp)1 and Arp11; actin; p24; p25; p27; and capZ α and β ;⁶¹ and plays an important role in dynein’s ability to bind to vesicles.^{61,62} Dynactin also binds to MTs via p150Glued,^{63,64} the largest subunit that interacts with both MTs and dynein. The N-terminal of p150Glued binds MTs while the two coiled-coil regions are required for its dimerization and interaction with DIC.^{63,65,66} Mutations in the MT-binding domain of p150Glued showed decreased affinity for MTs resulting in neuronal abnormalities.⁶⁷ Binding of the dynein–dynactin complex to vesicles is mediated by the Arp1 filament, which has been shown to bind to the spectrin matrix on organelles.^{68–71}

Both dynein and dynactin are required for viability. Using targeted disruption mice homozygous for loss of *DHC* died early, while heterozygous mice showed no abnormalities.⁷² However, in vitro cultures of *DHC*–/– blastocysts showed defects in Golgi vesiculation and lysosome dispersion, suggesting that DHC is required for cell proliferation and proper distribution of endosomes and lysosomes.⁷³

In flies, dynein was also essential for survival. Loss of function of *DHC* or *DLC* caused lethality while partial loss of function mutations caused pleiotropic morphogenetic defects in bristle and wing development and resulted in female sterility.^{74–76} Most of these defects were associated with loss of cellular shape and structure due to a disorganization of the cytoskeleton.⁷⁵ Functions for *DLC* in the regulation of axonogenesis have also been shown, indicating a role of cellular motors in growth cone guidance.⁷⁷ Interestingly, missense point mutations in *DHC* resulted in decreased retrograde transport and progressive motor neuron degeneration accompanied by the formation of Lewy-like inclusion bodies.⁷ Two of these missense point mutations, *legs at odd angles* (*Loa*) and *cramping 1* (*Cra1*) occurred in the DIC binding site and in the homo dimerization site of *DHC*.

Mutations in *p150Glued* are also linked to neurodegeneration. Mutations in the *p150Glued* dynactin subunit were identified in familiar forms of motor neuron disease, including amyotrophic lateral sclerosis (ALS) and distal spinal and bulbar muscular atrophy.^{78–83} A mutation in *p150Glued* was also identified in Perry syndrome, a rare atypical form of Parkinson's disease resistant to L-3,4-dihydroxyphenylalanine (L-DOPA).⁸⁴ Interestingly, postnatal overexpression of the *p50* dynactin subunit induced late onset motor neuron disease in mice.⁷⁸ Motor nerves from specimens obtained from ALS patients showed decreased transport of organelles, such as mitochondria.⁸⁵ Interestingly, in vivo studies in intact sciatic nerves from the G93A superoxide dismutase 1 (SOD1) transgenic ALS mouse model showed decreased retrograde transport, which preceded visible disease symptoms.^{86,87} Collectively, these observations indicate that defects in dynein/dynactin function can lead to progressive neurodegeneration.

Regulatory models of bidirectional movement

Since both kinesin-1 and dynein motors are found together on mobile vesicles,^{88,89} experimental emphasis has been placed on elucidating the mechanisms of how these motors coordinate to achieve bidirectional motility.⁹⁰ Thus far, three models have been proposed to explain bidirectional transport – the exclusionary, the tug-of-war, and the coordination models.

The exclusionary model of transport suggests that there is only one type of motor or motor complex attached to a vesicle at a given time, and so these vesicles move unidirectionally. In this model, different motors would have to bind/unbind to achieve the bidirectional movement seen with many cargoes. Alternatively, the entire complex could hop onto opposite polarity MTs in areas where MTs are not unipolar, such as

dendrites.⁹¹ However, recent in vivo evidence has shown that there are, in fact, stable populations of both kinesin-1 and dynein motors on APP vesicles,⁹² suggesting that this model is incorrect, at least for APP vesicles under physiological conditions. Furthermore, in retrogradely moving early endosomes in *Ustilago maydis*, dynein-containing vesicles also contained the kinesin-3 complex, while kinesin-3 was located on vesicles moving in both directions.⁹³ Therefore, although more than one type of kinesin together with dynein could be associated with cargo during motility under physiological conditions, only one motor might be active at a given time.

The tug-of-war model suggests that both kinesin-1 and dynein motors are simultaneously active on vesicles and that the motors producing the highest net force ultimately move vesicles in their respective direction,⁹⁴ similar to a tug-of-war, with back-and-forth motility, ultimately resulting in the net movement in one direction. Much of the evidence for this model comes from studies done in vitro using purified motors. In contrast, the coordination model of bidirectional motility proposes that opposite-polarity motors work in regulated concert to achieve the observed complex vesicle motility rather than a simple tug-of-war mechanism. In this context, at one particular time, one motor is active for movement while the other motor is inactive. While the active motor dictates net movement in one direction, there is little back-and-forth motility.

Part of the reasoning behind the tug-of-war model lies in the observation that many transported vesicles will often change directions quickly in a process called “reversing”.⁹⁵ In 2009, Soppina et al⁹⁶ using *Dictyostelium* cells showed that endosomes experience elongation and slowed velocities during reversals, which is consistent with a tug-of-war mechanism. However, these results do not preclude the possibility of a transient tug-of-war mechanism that could result from delayed inactivation of externally regulated motors. Several studies have used various mathematical models, such as Monte Carlo simulations and models based on a lipid droplet system^{97,98} to show that the tug-of-war model is not only cooperative, but it can also be used to explain the dynamics of bidirectional movement, such as reversing.^{97,98}

Research using origami DNA⁹⁹ showed that opposite-polarity motors work to stall synthetic DNA cargoes and seem to follow a cooperative tug-of-war model. Furthermore, the motility of purified vesicles reconstituted in vitro closely resembled the movement of LysoTracker-positive vesicles in primary neurons, where bidirectional motility was interrupted with frequent directional switches, diffusional movement, and pauses. Quantitative analysis indicated that these vesicles copurified with a low number of stably bound motors, suggesting that a small complement of tightly bound motors,

and small changes in the number of engaged motors can mediate large changes in the motility of cargo. These observations are consistent with predictions for a motor coordination via a stochastic tug-of-war model, where transport could be driven by the force-dependent kinetics of opposing motors in the absence of external regulation.¹⁰⁰

Recently, many researchers have leaned toward a motor coordination model with motor regulation to explain bidirectional motility in vivo. This is, in part, due to the relatively high velocities of vesicle motility observed during in vivo analysis, which contradicts with the in vitro velocities. Since bidirectional motility must occur in a crowded environment, many cargoes must maneuver in both directions on a single microtubule track. In vivo studies of peroxisomes in *Drosophila* S2 cells indicated that peroxisomes can move at very fast velocities, reaching speeds much faster than those observed during in vitro experiments that contained only one type of motor polarity (ie, [+] ended motors, such as kinesin-1).¹⁰¹ In vivo analysis of APP vesicles in *Drosophila* larvae showed that APP velocities were dependent on the amount of kinesin available, as 50% reduction of KHC resulted in decreased velocities.⁹²

Reduction of dynein affected both anterograde and retrograde velocities,^{92,128} indicating that the functions of kinesin-1 and dynein are interdependent and that loss of either motor can lead to the impairment of bidirectional transport. Indeed, several studies support this proposal. Barkus et al showed that mutations in *Drosophila* Unc-104, a kinesin-3 protein, also inhibited retrograde movement.¹⁰² Likewise, in axonal neurofilaments, knockouts of kinesin-1 in mice and reductions of dynein motors inhibited both anterograde and retrograde transport.¹⁰³ Pilling et al showed that mitochondria are not only transported by kinesin-1, but that kinesin-1 mutants drastically reduced retrograde transport.⁸⁹ Furthermore, replacement of either kinesin-1 or

dynein motors with motors of similar polarity (such as Unc-104 for kinesin-1) was able to activate cargo movement in the opposite direction.¹⁰⁴ However, mutated motors with active heads but lacking motility failed to initiate retrograde transport. As a result, it was suggested that the opposing force generated by the anterograde motor could activate retrograde movement. Furthermore, a complete and stable kinesin-1 holoenzyme not only mediated anterograde movement of prion protein-containing vesicles, but it also activated its retrograde movement.¹⁰⁵

Together, these studies indicate an interdependence of opposite-polarity motors on their activation and motor function and suggest that precise regulation is needed to maintain complex motility in axons. Perhaps activation of both kinesin-1 and dynein/dynactin via phosphorylation may be an important mechanism by which coordination of these motors occur under physiological conditions.^{108,208} Indeed, both KHC and KLC subunits are known to be phosphorylated and contain sites for several kinases.^{245,256} While little is known about dynein phosphorylation, putative phosphorylation sequences are seen in dynein subunits.²⁰⁸ While binding of dynactin to dynein activates retrograde motility,^{106,107} evidence also suggests that kinesin-1 can mechanically interact with dynein to coordinate motor activity and movement.^{88,92,105,128} Therefore, several mechanisms of motor regulation and coordination must exist in vivo for proper bidirectional motility of several cargoes on a single microtubule track.

Motor-cargo complexes in neurodegenerative disease

Recent work suggests that several motor-cargo complexes are transported on a single microtubule track. Several of these motor-cargo complexes contain proteins involved in neurodegenerative disease (Table 1). Here we will discuss a few of these motor-cargo complexes with an emphasis on the

Table 1 Vesicle-motor complexes associated with neurodegenerative diseases

Cargo/vesicle complex	Motor protein involved	Complex components	Disease	References
APP	KHC, KLC, dynein	PS1, GAP-43, BACE, TrKA, synapsin, JIP1, JIP3	Alzheimer's disease	36, 98, 100, 101, 102, 119
HTT	KIF5, KLC, dynein	HAPI, HAP40, GAPDH, Rab11, BDNF	Huntington's disease	35, 124, 127, 128, 129, 190, 218, 219
Mitochondria	KIF5, KIF1B-alpha, dynein	Milton-Miro complex, JIP1, RanB2, pink-parkin, Mfn2, syntabulin	Parkinson's disease, CMT2A	138, 142, 145-148, 157, 208, 209
mRNA-protein complex	KIF5, dynein	FMRP, La mRNA	Fragile X syndrome	165, 172, 175

Abbreviations: APP, amyloid precursor protein; HTT, huntingtin; KHC, kinesin heavy chain; KLC, kinesin light chain; PS1, presenilin 1; GAP43, growth-associated protein 43; BACE, beta-site amyloid precursor protein cleaving enzyme; TrKA, tyrosine kinase receptor type A; JIP1, c-Jun-amino-terminal kinase-interacting protein 1; JIP3, c-Jun-amino-terminal kinase-interacting protein 3; HAPI, huntingtin-associated protein 1; HAP40, huntingtin-associated protein 40; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; BDNF, brain-derived neurotrophic factor; Mfn, mitofusin; CMT, Charcot-Marie-Tooth; FMRP, Fragile X mental retardation protein.

normal function of these disease proteins to determine how defects in vesicle motility result in neuronal dysfunction.

APP-cargo complex

APP, the AD protein, is found in many tissues, but it is highly enriched in the brain.^{109,110} Although the function of APP remains unclear, there is evidence that it can function as a receptor for kinesin-1 during axonal transport. While APP can bind to components of the extracellular matrix, the structural and sequence analysis of APP gives insight into several possible functional domains, including domains for protease inhibition.¹¹¹ Further, APP can function as an extracellular receptor^{112,113} much like Notch because of its similarity in structure and the ability of proteases, such as secretases, to cleave full-length APP into fragments. Similarly, APP has also been implicated in cell adhesion through the binding of extracellular matrix proteins.^{114,115} There is also evidence for APP to function as a regulator of neuronal outgrowth and aptogenesis during differentiation^{116–118} and neuronal repair following trauma.¹¹⁹

Concurrent with roles in neurite growth and synaptogenesis, APP is transported by fast axonal transport via KLC, either directly since the C-terminus of APP has been found to bind to KLC^{120–122} or indirectly via an interaction between KLC and JNK interacting protein 1 (JIP1).^{121,247} Biophysical evidence indicated that 15aa of the C-terminus of APP was sufficient to promote the transport of APP in squid axons¹²² and that APP beads had a high-binding affinity for kinesin-1.¹²³ In addition, genetic evidence showed that excess human APP or *Drosophila* APP-like (APPL) and the loss of function of *Drosophila* APPL resulted in phenotypes similar to motor protein mutants, indicating a function for APP in axonal transport.¹²⁴

Interestingly, the C-terminal deletions of human APP or APPL (including the kinesin-binding region) disrupted APP transport and failed to interact functionally with kinesin-1.¹²⁴ JIP1 has also been shown to interact preferentially with APP that is phosphorylated at threonine 668 (Thr668) and affects the transport of phosphorylated (p)APP vesicles¹²⁵ (Figure 2A). Recently, subpixel colocalization analysis suggested that APP vesicles contain both kinesin-1 and dynein,¹²⁶ indicating that APP utilizes both kinesin-1 and dynein motors for bidirectional motility within axons. Furthermore, studies have also shown that the aberrant processing of APP by either β or γ secretase/presenilin can lead to the impaired transport of APP along axons which, in turn, may alter its normal function, leading to synaptic degeneration.^{127,128}

During axonal transport, APP is thought to be contained within a subclass of vesicles that contain the proteins:

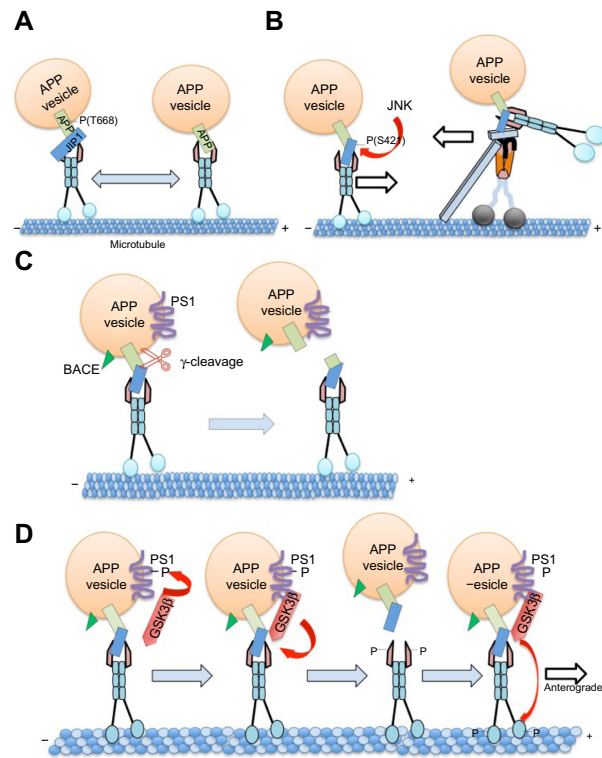


Figure 2 Models of APP-cargo complexes and their regulation during transport.

Notes: (A) APP can associate with kinesin-1 in two ways. APP associates with kinesin-1 via interactions with JIP1. This interaction may require APP to be phosphorylated at T668. Alternatively, the C-terminus of APP can associate with kinesin-1 via KLC through its TPR domains. (B) JNK phosphorylation of JIP1 at S421 regulates APP association with kinesin-1 and dynein. Phosphorylation of JIP1 is thought to promote anterograde transport, while dephosphorylation promotes retrograde transport. Arrows indicate direction of motility. (C) APP is in a vesicular compartment that contains BACE, PS1, GAP43, synapsin, and TrkA. PS1 is the catalytic component of gamma (γ) secretase that is known to cleave (scissor) APP and may serve to release APP vesicles from motors. (D) PS1 contains two putative glycogen synthase kinase 3-beta (GSK-3 β) phosphorylation sites in its loop region. Phosphorylated PS1 may serve as a scaffold for GSK-3 β to associate with PS1 similar to the β -catenin-PS interaction, thus enabling GSK-3 β to phosphorylate kinesin, which may release the vesicle from kinesin-1 or regulate motility of APP vesicles. It remains unclear whether these events are specific to APP vesicles or are general to all vesicles.

Abbreviations: APP, amyloid precursor protein; T668, threonine 668; S421, serine 421; JIP1, Jun N kinase interacting protein 1; KLC, kinesin light chain; TPR, tetratricopeptide repeat; JNK, Jun N kinase; BACE, beta-site amyloid precursor protein cleaving enzyme; PS1, presenilin; GAP43, growth-associated protein 43; TrkA, tyrosine kinase receptor type A.

presenilin 1 (PS1), the catalytic component of γ -secretase); beta-site APP cleaving enzyme 1 (BACE1); synapsin 1; tyrosine kinase receptor type A (TrkA); and growth-associated protein 43 (GAP43).¹²⁰ In addition, APP functionally interacts with PS1, BACE1, KLC, and c-Jun-amino-terminal kinase-interacting protein 1 (JIP1),^{125,128–130} and some of these proteins have also been shown to have enzymatic activity on APP itself, illustrating the importance of APP as an axonal protein. Evidence that APP is not transported in vesicles containing synaptotagmin^{120,128} further solidifies APP as a marker for a distinct subset of fast axonal transport vesicles. APP has also been shown to be cotransported with calsynenin-1, which

is a ligand for KLC.¹³¹ Further, reduction of calyntenin-1 impaired APP transport and caused accumulations of A β ,¹³¹ perhaps due to the fact that BACE1 and PS1 form a complex with APP.¹²⁹ Although a study using fluorescent imaging failed to find colocalization of APP and BACE1 in retinal ganglion cells,¹³² BACE1 activity has also been observed in axons.¹³² While enhanced β -secretase processing altered APP axonal transport,¹²⁷ a recent study showed that BACE1 and APP vesicles converge via an endocytosis dependent pathway in neurons.¹³³ In cultured hippocampal neurons, dendritic APP/BACE1-containing vesicles were largely segregated in physiologic states, where BACE1 was sorted into acidic recycling endosomes, and APP was conveyed in Golgi-derived vesicles. However, upon activity induction, a known trigger of the amyloidogenic pathway, APP was routed into BACE1-positive recycling endosomes via a clathrin-dependent mechanism,¹³³ suggesting that BACE1 and APP can be together in a particular functional pathway.

Recent work has demonstrated that APP vesicle motility is regulated through several different mechanisms. Anterograde velocities of APP vesicles are dependent on kinesin-1 concentration, and DIC may play a role in the coordinated regulation of APP motility.⁹² APP is phosphorylated and, although the role of phosphorylation is still uncertain, it may play a role in the regulation of APP vesicles during axonal transport. APP is phosphorylated at residue Thr668, although it is unclear which kinase is responsible for this phosphorylation. Previous studies have shown that cyclin dependent kinase (CDK5), and c-Jun amino-terminal kinase (JNK), are potential kinases responsible for APP phosphorylation at Thr668.^{134,135}

Further work has shown that JIP1 prefers to interact with pAPP, such that reductions in JIP1 impaired transport of pAPP, but not APP.¹²⁵ More recently,¹³⁶ JIP1 has been shown to potentially regulate the anterograde/retrograde movements of APP. In vitro experiments showed that JNK can phosphorylate JIP1 at S421 to promote anterograde movement and that lack of JIP1 phosphorylation caused retrograde movement via interactions with dynein (Figure 2B). However, short interfering RNA experiments in cultured rat cortical neurons showed no changes in APP transport with reductions of JIP1.¹³⁷

Recent in vivo experiments have shown that GSK-3 β can negatively regulate APP transport via kinesin-1 by regulating the numbers of active motors on these vesicles.¹³⁸ GSK-3 β is thought to phosphorylate KLC, causing vesicles to release from motors.¹⁰⁸ (Figure 2C). Alternatively, GSK-3 β can phosphorylate KHC to regulate vesicle motility.²⁰⁸ Evidence

has also shown that GSK-3 β -mediated activities on APP may be via an action on presenilin,^{128,208} which is found on APP-containing vesicles¹²⁹ and is known to cleave APP.^{139,140} In vivo imaging showed that presenilin also negatively regulates APP motility, similar to GSK-3 β .¹²⁸ Loss-of-function presenilin mutants resulted in increased APP vesicle velocities in both the anterograde and retrograde directions along MTs.¹²⁸ In contrast to cell culture data, in vivo analysis showed that presenilin can increase the activity of GSK-3 β , which may lead to increased kinesin-1 and dynein binding to APP vesicles, causing uncoordinated motility.²⁰⁸ Perhaps PS1 and GSK-3 β may form a complex with motors to regulate motility in vivo. Indeed, PS1 associates with GSK-3 β ¹⁴¹ and can act as a potential scaffold to bring GSK-3 β within close proximity of β -catenin (Figure 2D). Similarly, perhaps PS1 can also act as a scaffold that brings GSK-3 β into close proximity of kinesin-1 and dynein, thereby regulating the motility of APP vesicles. Alternatively, since PS1 is the catalytic component of the gamma secretase complex, perhaps PS1 may cleave APP vesicles from motors^{139,140} (Figure 2C) and may serve as another form of regulation of APP motility. Indeed, high levels of APP peptides were shown to impair transport in squid axons,¹²² which may be a mechanism of disrupting transport in some forms of familial Alzheimer's disease.

Since the transport of APP and its associated proteins may be regulated through several mechanisms, it is not surprising that misregulation of these complexes can lead to the impairment of axonal transport, which may be an early trigger in disease pathology. Indeed, axonopathy and transport deficits were observed in mouse models of AD that preceded known disease-related pathology by more than a year and in the early stages of AD in humans.¹⁴² Furthermore, in Down syndrome, where an increased expression of APP is seen, in the context of trisomy, abnormal transport of nerve growth factor and cholinergic neurodegeneration was observed,¹⁴³ and these transport defects were thought to be independent of A β production.¹⁴⁴

Taken together, these studies suggest that defects in axonal transport may be an early event in the progression of AD and may result, in part, due to the misregulation of axonal transport.

HTT cargo complex

HTT, the protein involved in Huntington's disease (HD), is a ubiquitously expressed protein found in many cell types, but it is concentrated in neurons. HTT is comprised of two expanded regions of polyglutamine (polyQ) and polyproline, at the N-terminus and approximately 28–36 HEAT repeat

domain motifs,¹⁴⁵ which likely form a superhelical structure¹⁴⁶ that is involved in protein–protein interactions. The polyQ tract can expand to longer lengths of CAG repeats (normally, approximately 35 repeats) that tend to correspond to the age of HD onset. X-ray crystallography of the N-terminus of HTT¹⁴⁷ has given insight into the structure of the polyQ region, showing various conformations depending on the surrounding proteins that could potentially affect its binding capabilities. In addition, the predicted structure of pathogenic and nonpathogenic N-terminal fragments of HTT aligned with this X-ray structure suggests an aggregation model for the pathogenesis of HD.¹⁴⁸

Like APP, the primary function of HTT is still unclear. However, HTT is essential for development, since *HTT* null mice are embryonic lethal and heterozygous mice contain a myriad of defects, including cognitive deficits and neurodegeneration.¹⁴⁹ Despite the lack of clarity on function, HTT interacts with a variety of different proteins, including huntingtin-interacting proteins and HAPs. These proteins appear to have various functions, including regulation of transcription, cell signaling, and cargo trafficking.¹⁵⁰ The structure of HTT and the fact that HTT interacts with many different proteins implies that HTT may act as a scaffolding protein that is essential for cellular trafficking.

HTT has also been shown to have important functions in axonal transport and is transported along MTs.¹⁵¹ Both biochemical and genetic evidence suggest that HTT interacts directly with both kinesin^{9,152} and the dynein/dynactin complex.^{9,153} HAP1, which binds to HTT,^{154,155} has also been shown to interact with p150Glued¹⁵⁶ and KLC.⁴³ These findings indicate that HAP1 may act to tether HTT (and its cargo) to the antero- and retrograde motors. Furthermore, HTT has been shown to transport vesicles containing brain-derived neurotrophic factor (BDNF) (Figure 3A) through its interaction with both HAP1 and P150Glued.¹⁵⁷ Interestingly, HAP1 has also recently been shown to associate with APP, since deletion of HAP1 resulted in an increased in motionless APP vesicles¹⁵⁸ and impaired the anterograde transport of APP.⁴³ Furthermore, HTT may regulate organelle dynamics by influencing different Rab proteins (Figure 3A). Indeed, Pal et al showed that HTT can mediate the transport of Rab5–HAP40–HTT containing early endosomes on actin filaments.¹⁵⁹ HTT was also linked to Rab8 through FIP2 (family interacting protein 2), and this interaction regulated cell polarization and morphogenesis.¹⁶⁰ Further, impairment of Rab11 caused the defective formation of vesicles in HD models,^{161,162} membrane binding of Rab11 was decreased with knockout of HD,¹⁶³ and the axonal transport of Rab11 was

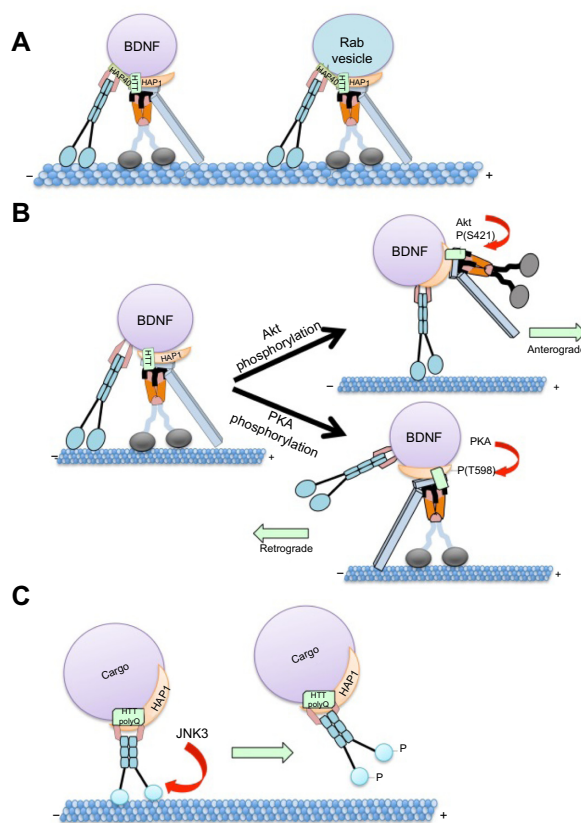


Figure 3 Models of HTT-cargo complexes and their regulation during transport. **Notes:** (A) HTT may attach to BDNF or Rab protein containing vesicles and associate with kinesin-I and dynein via interactions with HAP1 and HAP40. (B) Akt can phosphorylate HTT at S421 causing it to stabilize kinesin-I and increase anterograde transport of BDNF vesicles. Dephosphorylation of HTT at S421 and possibly phosphorylation of HAP1 by PKA may destabilize kinesin, enabling HAP1 to associate with p150Glued and increase retrograde transport. (C) Pathogenic HTT (HTT polyQ) activates JNK3, a neuronal specific kinase, to phosphorylate KHC and release kinesin-I from MTs.

Abbreviations: HTT, huntingtin; polyQ, polyglutamine; BDNF, brain-derived neurotrophic factor; HAP1, huntingtin-associated protein 1; HAP40, huntingtin-associated protein 40; S421, serine 421; PKA, protein kinase A; JNK3, Jun N kinase 3; KHC, kinesin heavy chain; MTs, microtubules.

perturbed with reduction of HTT,¹⁶⁴ indicating that HTT may mediate the transport of Rab11 vesicles on MTs and defects in Rab11 transport may contribute to the early stages of the synaptic dysfunction in HD. Consistent with this proposal, early synaptic dysfunction,¹⁶⁵ dendritic spine loss, and neurodegeneration¹⁶⁶ observed in HD models were reversed by overexpression of Rab11.

Much like APP, phosphorylation is also associated with the regulation of HTT. Akt has been shown to act as a molecular switch by phosphorylating HTT at serine 421.¹⁶⁷ This modification caused HTT to recruit kinesin to the BDNF vesicles, and possibly to dynactin, resulting in increased anterograde transport of BDNF vesicles in cultured cortical neurons. Conversely, lack of HTT phosphorylation caused kinesin to release from MTs and to promote retrograde transport.¹⁶⁸ As

a result, perhaps pHTT may stabilize the interaction between kinesin and dynactin to promote anterograde transport. Similarly, HAP1 is thought to be regulated via phosphorylation since protein kinase A (PKA) can phosphorylate HAP1 at T598, causing a decrease in the association of HAP1 with dynactin and KLC.¹⁶⁹ Interestingly, this study also showed that nerve growth factor stimulation led to an increase in activated Akt and enrichment of HAP1 at neurites, indicating an increase in HAP1 anterograde transport which is consistent with Zala et al.¹⁶⁸ Together, these findings suggest a mechanism by which HTT and HAP1 work together to coordinate vesicle directionality through two competing kinase pathways (PKA and Akt) (Figure 3B).

More recently, HTT has been implicated as a scaffold for vesicular glycolysis machinery that regulates vesicle transport by providing onboard energy for the transport of a subset of vesicles.¹⁷⁰ They showed that HTT can tether glycolytic proteins, such as glyceraldehyde 3-phosphate dehydrogenase (GAPDH), onto BDNF vesicles,¹⁷⁰ suggesting that the HTT-mediated glycolytic machinery provides the ATP that is used by BDNF vesicles for axonal transport. These observations suggest that HTT is not only important for carrying cargoes to target regions, but that HTT is also required for the proper transport of potentially any vesicle that relies on ATP generated by glycolysis.

Since HTT influences both kinesin-1 and dynein motors via a series of different complexes that include Rab proteins¹⁶⁴ and BDNF,¹⁵⁷ it is not surprising that misregulation of these HTT vesicular complexes could also lead to an impairment of axonal transport, which may be an early trigger in disease pathology. Expression of mutant HTT also caused neuritic aggregates, which blocked protein transport in neurites and caused neuritic degeneration before nuclear DNA fragmentation occurred. Simultaneously, expression of cytoplasmic polyQ proteins resulted in axonal blockages, retinal degeneration, and larval lethality in a *Drosophila* model of HD.⁹ Together, these studies suggest that the early neuropathology of HD can originate from axonal dysfunction mediated by defects in axonal transport. Indeed, mutant HTT can activate JNK3 (a neuronal specific c-Jun kinase) that phosphorylates the kinesin-1 motor, resulting in the disassociation of kinesin from MTs¹⁷² (Figure 3C) perturbing transport.

Mitochondria complex

Distal compartments, such as synapses, depend on mitochondrial transport to supply ATP for cellular processes like synaptic transmission.¹⁷³ Mitochondria is known to undergo both anterograde and retrograde transport^{89,174,175}

by utilizing kinesin-1 and cytoplasmic dynein motors.^{89,176–179} Evidence suggests that a specific protein complex is involved in anchoring mitochondria to KHC. Genetic screens in *Drosophila* identified a Rho guanosine triphosphate (GTP) ase, Miro, and trafficking protein Milton, which form a complex for mitochondrial transport.^{173,177,180} (Figure 4A). While *Drosophila* contains only one *Milton* gene,^{173,180} mammals have two orthologs named *TRAK1/OIP106/Milton1* and *GRIF1/Milton2*.¹⁷⁷ Biochemical evidence showed interactions between Miro and Milton.^{173,181}

While the overexpression of Miro recruited Milton to mitochondria and regulated its trafficking in hippocampal neurons,¹⁸¹ the loss of Miro impaired the kinesin-mediated transport of mitochondria.¹⁸² Evidence using yeast two-hybrid interaction assays, coimmunoprecipitation, and colocalization indicated that GRIF1/Milton2 directly interacts with KHC through its C-terminal cargo-binding domain.¹⁸³ Furthermore, the Milton interaction with KHC is independent of KLC.¹⁷³ Therefore, Milton functions as an adaptor that links KHC to Miro-mediating mitochondrial transport.

Since mitochondria also undergo retrograde transport in axons, a Miro complex could exist with dynein. Loss or overexpression of Miro in *Drosophila* reduced retrograde transport of mitochondria by impairing both kinesin and dynein-mediated movement.¹⁸¹ Fractionation studies in *Drosophila* suggested a physical association between cytoplasmic dynein and mitochondria, and mutations in DHC-altered mitochondrial retrograde transport.⁸⁹ Furthermore, dynein colocalized with mitochondria in both anterograde and retrograde directions.¹⁸⁴ Although a physical association between dynein and Miro is unknown, together, these studies suggest that dynein is a key player in the retrograde transport of mitochondria. Furthermore, the *Drosophila* homolog of JIP1, APP-like protein interacting protein 1 (APLIP1), has also been implicated in mitochondrial transport.¹⁸⁵ While a mutation in APLIP1 caused larval paralysis, swelling in axons, and reduced both anterograde and retrograde vesicle transport,¹⁸⁵ only the retrograde transport of mitochondria was decreased, suggesting an inhibition of the dynein motor. However, it remains unclear whether APLIP1 acts as an adaptor for the dynein-mediated retrograde transport of mitochondria. Perhaps several adaptor proteins may link mitochondria to dynein.¹⁸⁶

Several proteins have been implicated in the regulation of mitochondrial movement^{187–190} (Figure 4B). Mitofusins (Mfn1 and Mfn2), which are located on the outer membrane of mitochondria¹⁸⁷ and are essential for mitochondria fusion,^{191,192} are thought to be involved in mitochondrial transport.¹⁸⁷ Biochemical evidence suggested that Mfn1/Mfn2 interacts

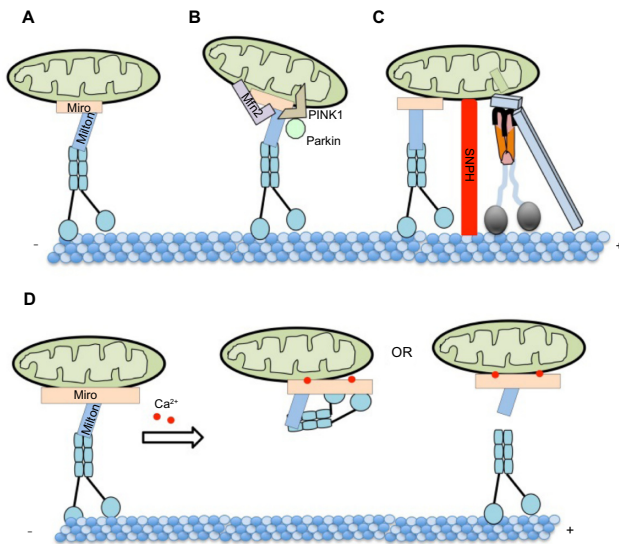


Figure 4 Proposed models of mitochondrial cargo complexes and their regulation during transport.

Notes: (A) Rho-GTPase Miro and Milton form a complex in which Milton serves as an adaptor to link the Miro/mitochondria complex to KHC. (B) Mfn2 associates with the Miro/Milton complex. PINK1 phosphorylates Miro at S156 to release damaged mitochondria from kinesin-1. (C) SNPH acts as an anchor to halt transport of mitochondria in the presence of Ca^{2+} . (D) Ca^{2+} binding to the EF-hand helix-loop-helix structural domain of Miro induces KHC to bind Miro instead of MT or Ca^{2+} binding to Miro detaches the Milton/Miro complex from KHC, resulting in mitochondria to becoming stationary.

Abbreviations: GTP, guanosine triphosphate; Mfn, mitofusin; KHC, kinesin heavy chain; PINK, PTEN induced kinase 1; SNPH, syntaphilin; MT, microtubules.

with the Milton/Miro complex.¹⁸⁷ Furthermore, defects in anterograde and retrograde mitochondrial transport were seen in neurons from mice with a knockout of Mfn2 or mice with a Charcot-Marie-Tooth (CMT2a) disease mutant in Mfn2 (R94Q),¹⁸⁷ suggesting that Mfn has a role in mitochondrial motility. Another mitochondrial-associated protein, PINK1, has been proposed to form a multiprotein complex with Milton/Miro.¹⁹³ Biochemical and genetic evidence indicated that Miro/Milton and PINK1 could interact with each other.¹⁸⁵ Furthermore, PINK1 can regulate mitochondrial motility in motor neurons.¹⁹⁴ Overexpression of PINK1 decreased mitochondrial flux and velocities in both directions, while the loss of PINK1 had the opposite effect. Altering PINK1 activity with RNA interference affected the distribution of mitochondria in motor neuron axons.¹⁹⁴ Interestingly, Wang et al suggested that PINK1 can phosphorylate Miro on Ser156, and this can activate the proteasomal degradation of Miro in a parkin-dependent manner.¹⁸⁹ Perhaps removal of Miro via proteasomal degradation is a potential mechanism for the detachment of kinesin motors from mitochondria, targeting dysfunctional mitochondria for mitophagy.¹⁸⁹ Additionally, syntaphilin (SNPH), an axonally targeted protein, was identified as a mitochondria-docking receptor to MTs (Figure 4C). Deletion of SNPH increased the percent-

age of motile mitochondria, while overexpression abolished motility.¹⁹⁵ Furthermore, neuronal activity and Ca^{2+} promoted the recruitment of SNPH to mitochondria and was necessary to maintain the balance between stationary and mobile mitochondria.¹⁹⁶ Together, these observations propose an engine-switch- and-brake model, in which SNPH may function as an engine-off switch by sensing Miro- Ca^{2+} and as a brake by anchoring mitochondria to MTs.¹⁹⁶

While little is known about the regulatory mechanisms of mitochondrial motility, work has shown that Ca^{2+} may be important. Miro contains two EF-hand Ca^{2+} binding domains and is a Ca^{2+} sensor that regulates mitochondrial motility.^{176,186,197-199} While the assembly of the KHC-Milton-Miro protein complex increased mitochondrial motility along axons, elevated cytosolic Ca^{2+} , as a result of neuronal activity inhibited mitochondrial motility. The inhibition of mitochondrial motility in neurons with Miro mutations in the two EF hands that cannot bind Ca^{2+} ^{197,198} suggests that Miro-mediated Ca^{2+} levels play a key role in the regulation of mitochondrial motility. Perhaps a Miro- Ca^{2+} sensing mechanism exists whereby the binding of Ca^{2+} to Miro releases KHC from mitochondria^{181,186,197} (Figure 4D).

Interestingly, impairment in mitochondrial transport has been observed in many neurodegenerative diseases, such as AD.²⁰⁰⁻²⁰² Impaired mitochondrial motility is observed in $\text{A}\beta$ -rich environments,^{203-206,208} implicating axonal mitochondrial changes in synaptic dysfunction in AD neurons. Interestingly, *in vitro* studies in hippocampal neurons indicate a potential role for PKA and GSK-3 β on $\text{A}\beta$ and its action on mitochondrial transport.²⁰⁷ While the inhibition of PKA had no effect on $\text{A}\beta$ -mediated mitochondrial transport, activation via forskolin completely eliminated mitochondrial transport defects induced by $\text{A}\beta$. On the other hand, GSK-3 β inhibition via lithium also completely abolished $\text{A}\beta$ -induced mitochondrial defects,²⁰⁸ while excess of GSK-3 β inhibited the bidirectional movement of mitochondria *in vivo*.²⁰⁸ Similarly, $\text{A}\beta$ oligomers impaired mitochondrial motility in hippocampal neurons, which appeared to be mediated by N-methyl-D-aspartate receptors.²⁰³

Considering that PKA inhibits GSK-3 β via phosphorylation,²⁰⁷ and GSK-3 β affects the phosphorylation state of KHC,²⁰⁹ a potential mechanism may exist whereby PKA suppresses $\text{A}\beta$ -induced mitochondrial transport defects by modulating GSK-3 β activity.

Work has also shown mitochondrial transport defects in HD.²¹⁰⁻²¹² Mitochondria were progressively immobilized with frequent stops in neurons from HD transgenic animals, and these defects occurred early during development prior

to the onset of measurable neurological or mitochondrial abnormalities.²¹⁰ Further, changes in the levels of electron transport chain genes and mitochondrial structural genes were thought to be responsible for the abnormal mitochondrial dynamics observed in the cortices of HD patients.²¹¹ Moreover, mutant HTT associated with the mitochondrial protein Drp1 influenced axonal transport.²¹² Perhaps mutant HTT may induce aberrant associations that impair mitochondrial dynamics, decreasing mitochondrial function in HD.

RNA cargo complex

Several lines of evidence suggest that molecular motors are responsible for the spatial localization of RNA, which regulates protein production at specific sites within a cell.²¹³ In *Drosophila* oocytes, kinesin-1 is responsible for the transport of Oskar messenger RNA (mRNA) to the posterior pole,^{214,215} while dynein mediates the anterior-dorsal localization of gurken mRNA and anterior localization of bicoid mRNA.^{216,217} Similarly, intracellular mRNA localization is also a conserved mechanism for spatially regulating protein production in polarized neurons since local translation of mRNAs is thought to be essential for many neuronal functions, such as the regulation of axon growth, axon maintenance, and regeneration after axonal injury.

Recent imaging studies have shown that distinct mRNA-containing complexes, including granules and mitochondrial mRNA, are transported long distances within neuronal projections.²¹⁸ Neuronal RNA granules are essentially ribonucleoprotein particles whose roles involve transporting mRNA along MTs and modulating local protein synthesis in response to synaptic activity.^{219–221} Further, the mRNA encoding the translational repressor Nanos (nos) forms ribonucleoprotein (RNP) particles that are dendritically localized in dendritic arborization neurons in *Drosophila*.²²²

Interestingly, the RNA-binding protein Rumpelstiltskin and the germ plasm protein Oskar, also functions in dopaminergic neurons to assemble and to transport nosRNP particles mediated by dynein.²²² Although the exact mechanisms of how these RNP complexes associate with kinesin-1 or dynein for localization is still unclear, these studies show that localization factors are adaptable and can regulate and target transcripts in different cellular contexts.

Biochemical, imaging, and immunofluorescence studies on the Fragile X mental retardation (FMR) mRNA complex have provided evidence for its involvement in axonal transport. FMR mRNA, which encodes the Fragile X mental retardation protein (FMRP), forms granules containing its own RNA and moves along neurites.²²³ Interestingly,

FMRP was found in the same complex as KHC in mouse homogenates.²²⁴ The *Drosophila* homolog of FMRP, dFMRP, was shown to associate with both KHC and DHC and the RNAi knockdown of either motor blocked transport of FMRP-containing granules.²²⁵ Moreover, while one study found that the FMRP interaction with kinesin-1 was specific to KHC and not KLC,²²⁵ another study showed that the C-terminal domain of FMRP can directly interact with KLC.²²⁶ Together, these studies indicate that both motors are required for the transport of FMRP within axons. Furthermore, FMRP is thought to negatively regulate the number of mitochondria in axons and in neuromuscular junction synapses, affecting mitochondrial flux and processivity.²²⁷

Work on the La RNA chaperone protein exposed an interesting mechanism for RNA transport.²²⁸ In dorsal root ganglion neurons, sumoylation of La at lysine 41 was essential for its interaction with dynein but not kinesin.²²⁸ Furthermore, sumoylated La moved retrogradely in ligated sciatic nerves from adult rats causing distal accumulations of sumoylated La.²²⁸ This study suggests that modifying protein–protein interactions may control the directionality of RNA transport via sumoylation and may constitute an important regulatory mechanism. However, whether this is the central mechanism that governs all RNA transport is unclear, given that only a few RNA-binding proteins have been shown to undergo sumoylation.²²⁹ Thus, it is becoming evident that long-distance mRNA transport requires microtubule-dependent motors, but the molecular mechanisms underlying the sorting and trafficking of mRNAs into axons or dendrites remains elusive. While the roles of RNPs are still unclear, their sequestration into transported RNP particles and their specific localization is likely the result of specific targeting rather than passive diffusion. Therefore, local translation of RNA must be a rapid response mechanism to signals at the synapses to mediate synaptic plasticity^{230–232} during development and regeneration.

Other cargo complexes

Many more potential disease protein complexes are emerging that may rely on axonal transport to maintain their normal functions in neurons. Two of these are briefly discussed.

Potential spinal muscular atrophy (SMA)-cargo complex

The survival motor neuron (SMN) protein is vital for the maturation of small nuclear ribonucleoproteins²³³ and, possibly, the functional localization of messenger RNPs.²³⁴ Lack of SMN due to SMN deletion is one cause of SMA. Although it is unclear what complexes SMN is a part of, SMN has been

found in axonal termini and growth cones.²³⁵ Furthermore, SMN granules were observed to be transported in cultured primary neurons with speeds that were consistent with fast axonal transport ($1.8 \mu\text{ms}^{-1}$).²³⁶ Since associations between SMN and mRNA binding proteins HuD²⁴⁰ and FMRP²⁴¹ have been shown, perhaps SMN functions in RNA motility. Interestingly, several mutations in the tail domain of DHC (DYNC1H1) that disrupt dynein function have been found in patients with SMA.²³⁷ More recently, two other mutations in the neck and motor domain of dynein were associated with SMA.²³⁸ Interestingly, pharmacological compounds known to rescue kinesin-mediated larval locomotion in *Drosophila* stimulated neurite growth in rat spinal neurons and rescued motor neuron development in a Zebra fish model of SMA.²³⁹ Taken together, these observations suggest that SMN has a role in axonal transport and may form the unique cargo complex that interacts with MT motors.

Potential ALS-cargo complex

ALS is a motor neuron disease caused by mutations in several genes, including *SOD1* and *TDP-43*. Recent work has shown that mutant superoxide dismutase (SOD1) can induce defects in axonal transport.²⁴⁹ KAP3 (kinesin-associated protein 3), a subunit of the kinesin 2 family, was shown to bind misfolded-mutant SOD1.²⁴⁴ KAP3 is known to bind to choline acetyltransferase (ChAT), which requires kinesin-2 for transport in axons.^{242,243} Tateno et al suggested that misfolded SOD1 was able to sequester kinesin-2 motors and impair ChAT transport.²⁴⁴ Indeed, a similar mechanism, the impairment of axonal transport via sequestration of motors, was previously proposed in a *Drosophila* model of HD⁹ and was recently theorized to occur in ALS through computational analysis.²⁴⁵ Computational analysis indicated that defects in axonal transport in SOD1 models of ALS result in protein aggregation and motor depletion. Furthermore, it was also shown that misfolded SOD1 impaired acetylcholine release, which was rescued via overexpression of KAP3.²⁴⁴ Together, these results suggest that disrupting the transport of acetylcholine- and ChAT-containing vesicles may lead to aberrant protein accumulations, leading to the progression of ALS, postulating a ChAT/acetylcholine cargo complex in the context of ALS.

Alternatively, similar to SMA, disruption in RNA transport could also lead to the progression of ALS. A pathological hallmark of ALS is the cytoplasmic inclusions containing hyperphosphorylated and ubiquitinated TDP-43.²⁵¹ TDP-43 is a ubiquitous DNA/RNA-binding protein with a nuclear role in pre-mRNA splicing, and mutations have recently been linked to the development of ALS.²⁵⁰ Recent work has

shown that TDP-43 is localized and actively transported in motor neuron axons and colocalizes with well-studied axonal mRNA-binding proteins, such as FMRP, HuD, and SMN.²⁵⁰

Perhaps similar to SMA, a TDP-43-RNA complex also exists. These observations suggest that TDP-43 has an important role in the regulation of axonal growth, and that impairment in the posttranscriptional regulation of mRNAs in the cytoplasm of motor neurons may be a major factor in the development of ALS.

Summary

It is intriguing that a vast majority of disease-related proteins have been shown to interact with molecular motors or to be present on transported cargo complexes. Many of these disease-related proteins also have roles – either during axonal transport or they are dependent on axonal transport for their proper localization and function. Further, the motility of these cargo complexes appears to be highly regulated at many levels; regulation of the motors themselves and the regulation of the association of these complexes with motors by a myriad of adaptor proteins and/or post translational modifications. Moreover, in some diseases where more than one gene or protein is involved in causing the disease, all of the disease proteins appear to converge in the axonal transport pathway. Either they are contained in the same vesicle or they are in different types of vesicle or RNA granules. Thus highlighting the importance of the axonal transport pathway and implicating perturbations in axonal transport in the initiation and progression of many neurodegenerative diseases. Therefore, it is not surprising that mutations in motor proteins cause neurodegeneration defects in transport have been shown to precede behavioral defects and neuropathology in many human neurodegenerative diseases. Thus, unraveling the identity of these motor-cargo complexes, the mechanisms of transport and how normal disease protein complexes function are all critical in understanding how problems in transport contribute to disease states. Such analysis has important implications for potential early diagnosis of neuronal disease and for the development of therapeutic interventions before the onset of neuronal death.

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Disclosure

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