Axonal transport and neurological disease

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Abstract | Axonal transport is the process whereby motor proteins actively navigate microtubules to deliver diverse cargoes, such as organelles, from one end of the axon to the other, and is widely regarded as essential for nerve development, function and survival. Mutations in genes encoding key components of the transport machinery, including motor proteins, motor adaptors and microtubules, have been discovered to cause neurological disease. Moreover, disruptions in axonal cargo trafficking have been extensively reported across a wide range of nervous system disorders. However, whether these impairments have a major causative role in, are contributing to or are simply a consequence of neuronal degeneration remains unclear. Therefore, the fundamental relevance of defective trafficking along axons to nerve dysfunction and pathology is often debated. In this article, we review the latest evidence emerging from human and in vivo studies on whether perturbations in axonal transport are indeed integral to the pathogenesis of neurological disease.

Intracellular cargo trafficking is tightly and spatiotemporally regulated to maintain cell organization, homeostasis and survival, and is particularly crucial for nerve cells owing to their extreme anatomical and biochemical polarization. Neurons shuttle diverse substances along axon microtubules through a bidirectional, ATP-dependent process known as axonal transport. Anterograde transport, from the cell body to the axon tip, is driven by the kinesin superfamily of motor proteins[1] and delivers substances such as RNAs, proteins and organelles towards growth cones and synapses[2]. In the opposite direction, retrograde transport is dependent on cytoplasmic dynein[3] and is essential for processes such as neurotrophic factor signalling[4], autophagy–lysosomal degradation[5,6] and the response to nerve injury[7]. Axonal transport thus encompasses various long-distance intracellular trafficking events that require exquisite regulation to preserve neuronal function and viability. The axonal transport machinery, which, in addition to motors and microtubules, includes essential motor adaptor proteins, is controlled through intricate protein kinase signalling pathways[8,9] and post-translational microtubule modifications[10,11] to ensure efficient transport in neurons.

Given the constant energy demands and distances over which cargoes must be mobilized, it is not surprising to find that mutations in the axonal transport machinery, even in genes that are widely expressed, can cause neurological diseases[12–14], as can genetic disruption of closely related cellular processes such as endolysosomal sorting[15], autophagy[5] and mitochondrial dynamics[16]. In addition, impairments in axonal trafficking have been reported in a multitude of neurological diseases, including Alzheimer disease (AD), amyotrophic lateral sclerosis (ALS) and Parkinson disease (PD)[17], as well as inherited and acquired peripheral neuropathies, such as Charcot–Marie–Tooth disease (CMT)[18]. Furthermore, transport can decline with ageing[19,20], which is a major risk factor in many neurodegenerative conditions. However, not all cargoes seem to be equally affected[21], and the relationship between defective axonal transport and neuronal pathology is often complex. For instance, we do not know why mutations in genes involved in axonal transport can cause such a breadth of neuronal disorders, why neurons with the longest axons are not always preferentially affected and why cargo-specific deficiencies can occur. Moreover, for those neurological diseases not linked to transport mutations, the question of whether transport defects impair neuronal homeostasis or are simply a consequence of degeneration remains largely unresolved.

To address these questions and illuminate the nuanced mechanisms that regulate axonal trafficking, the main aim of this Review is to examine the evidence for and against a causative role for axonal transport impairments in human nervous system dysfunction. We highlight putative and definite axonal transport-related genes that have been linked to human neurological conditions. We then discuss the relevance of disturbed trafficking in neuronal disorders that are not directly connected to transport machinery, focusing on

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Key points

- Mutations in various genes encoding components of the axonal transport machinery have been implicated in the pathogenesis of neurological diseases.
- Defective axonal trafficking has been linked to many nervous system disorders, but whether it is a cause or consequence of neuropathology remains largely unresolved.
- Intravital imaging of transport in axons of live mice provides some of the most compelling evidence that trafficking disturbances contribute to neuronal dysfunction.
- Targeting of specific mechanisms of axonal transport might be a valid therapeutic strategy to treat neurological disease.

key findings from patient samples and cells, followed by in vivo models of disease. Finally, we consider the merits of targeting axonal transport as a therapeutic strategy for neurodegeneration.

The axonal transport machinery

The microtubule cytoskeleton and motor proteins. Microtubules are crucial for long-range intracellular transport, and are highly dynamic structures consisting of heterodimers of α-tubulin and β-tubulin, isotypes of which are encoded by seven and eight human genes, respectively[22]. Axonal microtubules have a largely uniform morphology that dictates the directionality of motor protein transport: the growing plus end, which is targeted by the kinesin family of anterograde motors, points towards axon terminals, whereas the stable minus end faces the cell body and directs cytoplasmic dynein transport[23]. Although the microtubules that are found within axons are more stable than those in dividing cells, a considerable fraction is labile[24].

The kinesin superfamily of motor proteins is encoded by 45 mammalian genes, 38 of which are expressed in the nervous system, and is classified into 15 subfamilies (kinesin 1 to kinesin 14b)[1,25]. Kinesin 1, kinesin 2 and kinesin 3 seem to be most important for axonal transport[12,14]. Kinesin 1 motors consist of a dimer of kinesin heavy chains, encoded by *KIF5A*, *KIF5B* and *KIF5C*, as well as a dimer of kinesin light chains, encoded by *KLC1*, *KLC2*, *KLC3* and *KLC4*.

In contrast to the expansive kinesin family, one form of cytoplasmic dynein is essential to retrograde axonal transport. Cytoplasmic dynein is a large (~1.4 MDa) multisubunit motor complex consisting of two dynein heavy chains (encoded by *DYNC1H1*), two intermediate chains (encoded by *DYNC1IC1* and *DYNC1IC2*), two light intermediate chains (encoded by *DYNC1LI1* and *DYNC1LI2*) and three light chain families (Roadblock encoded by *DYNLRB1* and *DYNLRB2*, LC8 encoded by *DYNLL1* and *DYNLL2*, and Tctex encoded by *DYNLT1* and *DYNLT3*)[3]. The core motor is formed from a dynein heavy chain dimer on which the other dynein subunits assemble; the resulting complex binds to microtubules and hydrolyses ATP. Intriguingly, this motor complex by itself lacks major processivity and relies on accessory and adaptor proteins to efficiently transport cargo and carry out its myriad of functions.

Adaptor proteins. Adaptor proteins bind distinct cargoes and are fundamental to both kinesin and dynein function. Dynactin, a 1.1 MDa complex composed of 23 subunits built around a short, actin-like filament made of actin-related protein 1, is considered an essential cofactor for dynein[26]. Dynactin binds dynein and aligns the motors to activate processive movement[27]. Other activating adaptors include BICD2 and Hook proteins[3]. BICD2 is the best-characterized member of the mammalian BICD family of proteins (BICD1, BICD2, BICDR1 and BICDR2) and is a potent enhancer of minus-end-directed transport, substantially increasing processivity of the dynein–dynactin complex[28–30]. The BICD family, like other activating adaptors, features coiled-coil domains, which are vital for the interaction of the proteins with the dynein–dynactin complex[26].

Two other key dynein regulators are LIS1 and NDEL1. LIS1 binds directly to the motor domain of dynein and, depending on its mode of interaction with the dynein complex, can lead to either increased or decreased microtubule binding[31]. Studies also suggest that this protein can both increase and decrease dynein velocity[32,33]. NDEL1 is a coiled-coil-containing protein that interacts with dynein via its intermediate chain and LC8 subunits, and also interacts with LIS1[3]. NDEL1 has been shown to tether LIS1 to the dynein complex; however, it is not clear whether NDEL1 inhibits or enhances LIS1 function[34,35].

Kinesins seem to rely on comparatively few adaptor proteins, perhaps owing to the diversity of kinesin motors compared with the single dynein complex. Most of the kinesin adaptors, including HAP1, JIP1 and TRAK1, are also adaptors for dynein. The shared bidirectional adaptors frequently possess overlapping kinesin and dynein interaction regions, suggesting a binary switching mechanism to dictate the directionality of transport, at least in some scenarios[36].

Fast and slow axonal transport. For many decades, axonal transport has been subdivided into fast and slow categories on the basis of pulse–chase experiments using radiolabelled amino acids[37]. Fast axonal transport occurs at a rate of ~50–200 mm/day and delivers varied cargoes, including vesicles and membrane-bound organelles[38]. Slow axonal transport is crucial for the mobilization of substances such as cytoskeletal proteins (for example, tubulin and actin) and covers distances of ~0.2–10.0 mm/day (REF.[38]). Both fast and slow axonal transport are dependent on the same motor proteins, and the distinction in speed is simply a product of the time that cargoes remain stationary[39]. For a 1-m-long motor neuron, fast axonal transport can convey cargoes between the cell body and the axon tip within a week, whereas slow axonal transport can take longer than a year. However, slow axonal transport is estimated to deliver more than three times as much protein as fast axonal transport, at least in some neuronal subtypes[40]. Although imaging of slow axonal transport is possible, it is technically challenging owing to the timescale over which the process occurs[38,41]; thus, much of what we know about the dynamic properties of cargo trafficking along axons was derived from live-imaging studies of fast axonal transport, beginning in the early 1980s[42,43]. Consequently, in this Review, unless otherwise stated, discussions of axonal transport relate to fast axonal transport.

Processivity
The ability of motor–cargo complexes to undergo axonal transport without dissociation from microtubules.

Coiled-coil domains
A structural motif composed of two or more α-helices wrapped around each other to form a supercoil. The coiled-coil domain of the cytoplasmic dynein–dynactin complex connects the ATPase domain with the microtubule-binding domain.

Mutations in transport machinery genes

The transport of cargoes along axons requires three basic components: the microtubule network; a kinesin motor or the cytoplasmic dynein–dynactin retrograde complex; and various adaptor proteins. A single cytoplasmic dynein heavy chain is required for retrograde axonal transport, whereas more than 40 different kinesins fulfil various roles, including organelle transport, cytoskeletal remodelling and chromosomal dynamics[44]. Missense mutations and small genomic rearrangements in genes encoding these key components of the transport machinery cause various neurological diseases (TABLE 1, FIG. 1). The fact that many of these genes are ubiquitously expressed, yet the disease-causing mutations result only in a neurological phenotype, has been interpreted as evidence that deficits in axonal transport are causative of neurological disease.

Mutations in approximately 20 motor protein-related genes have been reported to cause neuronal disorders, although it is important to state that for many of these genes, no clear role in axonal transport has been demonstrated either in vitro or in vivo. Examining the human phenotype and disease course associated with these mutations, coupled with in vitro and murine disease models, can help us to understand whether and how deficits in axonal transport cause neuropathology. At first glance, disease-causing mutations in putative axonal transport-related genes seem to result in a wide range of complex phenotypes, with mutations even in a single gene, such as *DYNC1H1*, having been linked to various conditions, including cognitive disability, motor dysfunction and epilepsy[45]. However, the reality is more simple, with most disease-causing mutations in the transport machinery giving rise to just a handful of neurodevelopmental disorders (for example, malformations of cortical development (MCDs), congenital fibrosis of extraocular muscles (CFEOM) or spinal muscular atrophy, lower extremity predominant (SMALED)) or neurodegenerative disorders (for example, hereditary spastic paraplegia (HSP), CMT, ALS or parkinsonism). With the exception of *DCTN1*, *KIF1A*, *KIF1C* and *KIF5A*, mutations in all other disease-associated axonal transport genes result in a neurodevelopmental, as opposed to a neurodegenerative, phenotype. In the sections that follow, we discuss the neurodevelopmental phenotypes associated with putative axonal transport-related gene mutations and how these observations support or refute the hypothesis that deficits in axonal transport contribute to neurological disease.

Disorders of neuronal migration. MCDs include the conditions lissencephaly–pachygyria, polymicrogyria and microcephaly, all of which result in severe intellectual disability and are often associated with intractable epilepsy. Although MCDs have various aetiologies, including intrauterine infections and toxin exposure, a genetic cause is being recognized in an increasing number of cases[46]. A clear bias is evident towards genes that encode proteins involved in microtubule-based transport, including kinesins (*KIF5C*, *KIF1A* and *KIF1C*), components of the retrograde transport machinery (*DYNC1H1*, *NDE1* and *LIS1*) and tubulins (*TUBA1A*, *TUBA8*, *TUBB*, *TUBB2A*, *TUBB2B* and *TUBB3*).

Neuronal migration and cerebral cortical development depend on several processes, including interkinetic nuclear migration (INM) and proliferation of radial glial progenitor cells (the precursors of cortical neurons and glia), radial neuronal migration and terminal translocation. Cytoplasmic dynein, LIS1, NDE1 and KIF1A have all been shown to contribute to INM, which is the oscillatory movement of radial progenitor cell nuclei at the ventricular plate between successive cycles of mitosis[47–49]. Loss-of-function mutations and genomic rearrangements in *NDE1* and *LIS1* disrupt this process, resulting in MCDs. Following the progression of neuronal progenitor cells from a multipolar to a bipolar orientation, the dynein–LIS1–NDEL1 complex has an essential role in the microtubule-based transport of the nucleus towards the cortical surface[50]. Perturbations of this process are likely to underlie the cortical lamination defects observed in the 'Legs at odd angles' (*Loa*) mice, which are homozygous for a missense mutation in *Dync1h1* and show normal progenitor cell proliferation but delayed radial somal migration[51].

Importantly, although INM and radial neuronal migration are microtubule-based processes, they occur either before or during the early stages of neuronal polarization, when the axon is specified from neurite precursors. Therefore, although mutations in axonal transport-related genes in MCDs reflect the importance of microtubule-based transport in cell division and nuclear migration during cortical development, they do not provide direct evidence that deficits in axonal transport cause MCDs.

Disorders of axonal pathfinding. CFEOM can occur in the presence of MCD and is characterized by a congenital disorder of eye movement, often accompanied by facial palsy. The fibrosis of the extraocular eye muscles is secondary to a failure of innervation by its corresponding cranial nerve, indicating that CFEOM is a disorder of disrupted axonal guidance. The condition can be caused by missense mutations in the tubulin genes *TUBB2B* and *TUBB3*, which encode the major β-tubulin subunits in the CNS and the PNS, or in the kinesin gene *KIF21A* (REFS[52–54]). The normal function of KIF21A is to reduce the microtubule polymerization rate to prevent microtubule 'catastrophe' (the sudden shrinkage of a microtubule network). CFEOM-causing mutations in *KIF21A* disrupt the autoinhibited state of the protein, leading to a reduction in microtubule polymerization and failure of axonal elongation in the oculomotor nerve. Mutant KIF21A displays enhanced microtubule binding in vitro, but has no effect on the microtubule run length or velocity in vitro, arguing against a role for disrupted axonal transport in CFEOM[55].

Disorders of motor neuron development. Missense mutations in *DYNC1H1* and *BICD2*, which encode two major components of the retrograde transport complex, cause SMALED, a congenital disorder of motor neuron development predominantly affecting the lower limbs[56,57]. In vitro microtubule gliding assays have shown increased and decreased processive movement, respectively, in the presence of disease-causing mutations in *BICD2*

Run length
The total displacement covered by a motor–cargo complex without pausing.

Microtubule gliding assays
An experimental technique that is used to assess the activity of motor proteins, in which microtubules and ATP are applied to motors bound to glass coverslips.

Table 1 | **Axonal transport gene mutations and neurological disease**

Protein complex	Gene	Inheritance	Disease	OMIM entry	Phenotype
Anterograde transport machinery					
Kinesin 1	*KIF5A*	AD	Spastic paraplegia 10 (SPG10)/Charcot–Marie–Tooth disease type 2 (CMT2)	604187	Neurodegenerative
		AD	Neonatal intractable myoclonus (NEIMY)	617235	Neurodevelopmental
		AD	Amyotrophic lateral sclerosis (ALS)	617921	Neurodegenerative
Kinesin 1	*KIF5C*	AD	Complex cortical dysplasia with other brain malformations 2 (CDCBM2)	615282	Neurodevelopmental
Kinesin 3	*KIF1A*	AD	Mental retardation, autosomal dominant 9 (MRD9)	614255	Neurodevelopmental
		AR	Hereditary sensory neuropathy type IIC (HSN2C)	614213	Neurodegenerative
		AR	Spastic paraplegia 30 (SPG30)	610357	Neurodegenerative
Kinesin 3	*KIF1C*	AR	Spastic ataxia 2 (SPAX2)/spastic paraplegia 58 (SPG58)	611302	Neurodegenerative
Kinesin 3	*KIF14*	AR	Meckel syndrome 12 (MKS12)	616258	Neurodevelopmental
		AR	Primary microcephaly 20 (MCPH20)	617914	Neurodevelopmental
Kinesin 3	*KIF16A*	AR	Microcephaly and blindness (single case)	NA	Neurodevelopmental
Kinesin 4	*KIF4A*	XL	Mental retardation (MRX100; single case)	300923	Neurodevelopmental
Kinesin 4	*KIF7*[a]	AR	Acrocallosal syndrome (ACLS)/Joubert syndrome 12 (JBTS12)	200990	Neurodevelopmental
Kinesin 4	*KIF21A*	AD	Congenital fibrosis of extraocular muscles 1/3B (CFEOM1/3B)	135700	Neurodevelopmental
Kinesin 5	*KIF11*	AD	Microcephaly with or without chorioretinopathy, lymphoedema or mental retardation (MCLMR)	152950	Neurodevelopmental
Kinesin 7	*KIF10*[a]	AR	Primary microcephaly 13 (MCPH13)	616051	Neurodevelopmental
Kinesin 9	*KIF6*[a]	AR	Intellectual disability (single case)	NA	Neurodevelopmental
Kinesin 12	*KIF15*	AR	Microcephaly and thrombocytopenia (single case)	NA	Neurodevelopmental
Kinesin 13	*KIF2A*[a]	AD	Cortical dysplasia, complex, with other brain malformations 3 (CDCBM3)	615411	Neurodevelopmental
Kinesin-binding protein	*KIF1BP / KBP*	AR	Goldberg–Shprintzen syndrome (GOSHS)	609460	Neurodevelopmental
Retrograde transport machinery					
Dynein cytoplasmic 1 heavy chain 1	*DYNC1H1*	AD	Mental retardation 13 (MRD13)	614563	Neurodevelopmental
			Spinal muscular atrophy, lower extremity predominant 1 (SMALED1)	158600	Neurodevelopmental
Dynactin 1/P150^{Glued}	*DCTN1*	AD	Distal hereditary motor neuropathy type VIIB (HMN7B)	607641	Neurodegenerative
			Perry syndrome	168605	Neurodegenerative
Bicaudal D2	*BICD2*	AD	Spinal muscular atrophy, lower extremity predominant, 2A (SMALED2A)	615290	Neurodevelopmental
Lissencephaly 1	*LIS1 / PAFAH1B1*	AD	Lissencephaly, subcortical laminar heterotropia	607432	Neurodevelopmental
NudE neurodevelopment protein 1	*NDE1*	AR	Microhydranencephaly (MHAC)	605013	Neurodevelopmental
			Lissencephaly 4 (LIS4) with microcephaly	614019	
Microtubule network					
α1A-Tubulin	*TUBA1A*	AD	Lissencephaly 3 (LIS3)	611603	Neurodevelopmental
α8-Tubulin	*TUBA8*	AR	Complex cortical dysplasia with other brain malformations 8 (CDCBM8)	613180	Neurodevelopmental
B-Tubulin class 1	*TUBB*	AD	Complex cortical dysplasia with other brain malformations 6 (CDCBM6)	615771	Neurodevelopmental
β2A-Tubulin class IIa	*TUBB2A*	AD	Complex cortical dysplasia with other brain malformations 5 (CDCBM5)	615763	Neurodevelopmental
β2A-Tubulin class IIb	*TUBB2B*	AD	Complex cortical dysplasia with other brain malformations 7 (CDCBM7)	610031	Neurodevelopmental

Table 1 (cont.) | **Axonal transport gene mutations and neurological disease**

Protein complex	Gene	Inheritance	Disease	OMIM entry	Phenotype
Microtubule network (cont.)					
β3-Tubulin class III	*TUBB3*	AD	Complex cortical dysplasia with other brain malformations 1 (CDCBM1)	614039	Neurodevelopmental
		AD	Congenital fibrosis of extraocular muscles 3A (CFEOM3A)	600638	Neurodevelopmental
β4B-Tubulin class IVa	*TUBB4A*	AD	Torsion dystonia 4 (DYT4)	128101	Neurodegenerative
		AD	Hypomyelinating leukodystrophy 6 (HLD6)	612438	Neurodegenerative
β4B-Tubulin class IVb	*TUBB4B*	AD	Leber congenital amaurosis with early-onset deafness (LCAEOD)	617879	Neurodegenerative
β6-Tubulin class V	*TUBB6*	AD	Congenital facial palsy with ptosis and velopharyngeal dysfunction (FPVEPD)	617732	Neurodevelopmental
γ1-Tubulin	*TUBG1*	AD	Complex cortical dysplasia with other brain malformations 4 (CDCBM4)	615412	Neurodevelopmental

The table lists genes encoding components of the anterograde and retrograde transport complexes and microtubule network that have been implicated in neurological disease, along with their associated mode of inheritance and the nature of the phenotype (neurodevelopmental or neurodegenerative). AD, autosomal dominant; AR, autosomal recessive; NA, not applicable; OMIM, Online Mendelian Inheritance in Man; XL, X-linked. [a]Mutated transport protein is implicated in non-motile cilia as opposed to intracellular transport.

(REF.[58]) and *DYNC1H1* (REF.[59]). Before the discovery of human disease-causing mutations in *DYNC1H1*, similar missense mutations were reported in three *N*-ethyl-*N*-nitrosourea (ENU) mutant mouse models[60,61]. In the case of the *Loa* mouse, the mutation was associated with a reduction in retrograde axonal transport speeds both in vitro and in vivo[62,63].

At first glance, these observations would seem to provide strong evidence in favour of a causal role for defective retrograde axonal transport in human motor neuron diseases, such as ALS. In humans, the SMALED-linked missense mutations in *DYNC1H1* and *BICD2* cause a developmental disorder of α-motor neurons, whereas in mice, similar but not identical mutations cause a developmental loss of γ-motor and large-diameter 1a sensory neurons. In mice, the sensory neuron number is preserved at embryonic day 15, but dramatic loss of these neurons is observed by postnatal day 1 (REF.[60]), possibly as a result of defective retrograde nerve growth factor signalling[63]. Taken together, these findings suggest that the effects of deficits in retrograde axonal transport are most prominent during early motor and sensory neuron development, and particularly during periods of programmed cell death that are heavily dependent on efficient and timely retrograde neurotrophin signalling. However, in both mice and humans with missense mutations in *DYCN1H1*, the developmental loss of neurons remains static in adulthood with no evidence of progressive axonal degeneration, indicating that postnatal adult axons are impervious to modest perturbations in retrograde axonal transport, perhaps reflecting the differential demands of developing and mature axons.

Mutations in the retrograde transport machinery and neurodegeneration. *DCTN1* encodes the p150^{Glued} CAP-Gly subunit of dynactin, which is required for microtubule binding and processive retrograde transport[3]. Polymorphisms are common in this gene; however, missense mutations in four amino acid residues in the second exon cause distal hereditary motor neuropathy type 7 (DHMN7; G59S mutation) or Perry syndrome (G71A/E/R, T72P or Q74P mutations)[64–66]. Although the mutations are only a few amino acids apart, they give rise to vastly different phenotypes. DHMN7 is an autosomal dominant disorder beginning in the fourth to the sixth decade of life, and is characterized by bilateral vocal cord palsies. In addition, patients develop distal motor weakness often affecting the upper limbs that progresses in a slow and stable manner[64]. Perry syndrome, on the other hand, is characterized by parkinsonism, psychiatric symptoms and hypoventilation. No motor neuron degeneration is observed in this condition, and post-mortem studies reveal neuronal loss in the substantia nigra and TAR DNA-binding protein 43 (TDP43) pathology[67].

The question of whether these disease-specific mutations impair retrograde axonal transport remains unresolved, with independent studies producing conflicting data. In a *Drosophila melanogaster* model of DHMN7, axonal transport of green fluorescent protein-tagged-Rab7 endosomes was not impaired[68]. This observation was replicated in a mouse model of the disease with evidence of motor nerve degeneration[69]. In a separate study, primary mouse sensory neurons overexpressing the G59S mutant protein showed impaired retrograde lysosomal trafficking — an effect that was not replicated with Perry syndrome mutants[70]. A consistent finding in both studies, however, was the accumulation of p150^{Glued} in distal axons. Moreover, in *D. melanogaster* motor axons, the G59S mutant led to the accumulation of dense synaptic vesicles and impaired synaptic transmission at the neuromuscular junction[68]. Thus, missense mutations in *DCTN1* that cause motor nerve degeneration and nigral neuron degeneration do not seem to result in a general disruption of retrograde transport but might affect the trafficking of specific organelles, such as lysosomes, and might be neuron subtype specific.

Human mutations in *DYNC1H1* were initially linked to CMT[71], suggesting that impairments in retrograde axonal transport are involved in peripheral nerve degeneration. However, subsequent clinical descriptions of

N-Ethyl-*N*-nitrosourea (ENU). A potent mutagen that is often used to generate mutant animal models.

Endosomes
Membranous organelles involved in intracellular transport, sorting and delivery of various substances, including growth factors, internalized from the cell exterior.

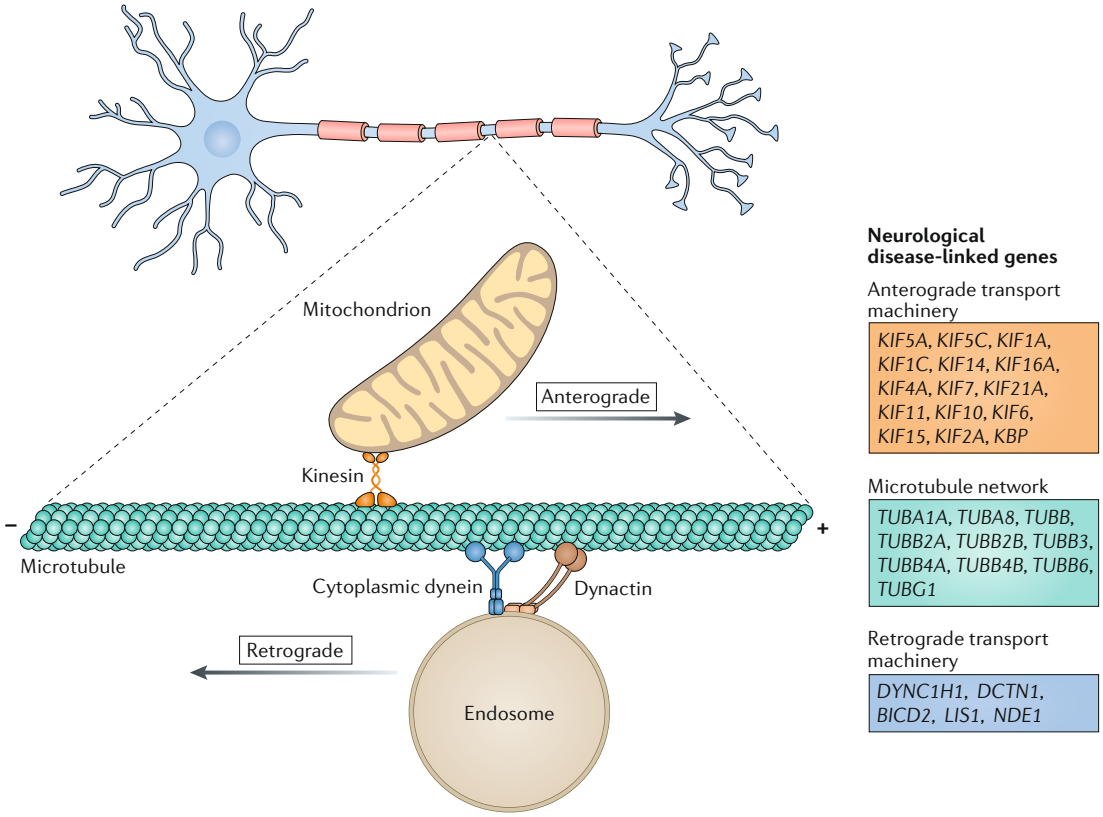

Fig. 1 | **The axonal transport machinery.** Mutations in genes that are integral to the anterograde and retrograde axonal transport machineries, as well as to the microtubule network, have been causally linked to many nervous system diseases (TABLE 1). Note that motor proteins can attach to and traffic many different cargoes and are not restricted to those depicted; for example, kinesin can transport vesicles and dynein can transport mitochondria. The components of the machinery are not shown to scale.

human *DYNC1H1* mutations consistently showed that they cause a developmental and non-progressive loss of motor neurons predominantly affecting the lower limbs, namely, SMALED. In fact, the clinical description of the original published family was of a motor-predominant disorder affecting the lower limbs, in keeping with a diagnosis of SMALED and incorrectly described as CMT.

Mutations in the anterograde transport machinery and neurodegeneration. The strongest genetic evidence implicating impaired axonal transport in neurodegeneration came from mutations in the two kinesin genes, *KIF1A* and *KIF5A*. Mutations in *KIF1A* give rise to various phenotypes: autosomal recessive loss-of-function mutations cause HSP[72], a disease of progressive corticospinal tract degeneration with profound sensory neuropathy, and dominant de novo mutations cause a complex phenotype of MCD, HSP and CMT[73]. KIF1A is required for INM and neuronal progenitor cell proliferation and migration, and dominant-negative effects on this process are likely to explain the MCD[47]. In addition, KIF1A transports the neurotrophin receptor TrkA to sensory axon terminals to promote their survival, and loss of KIF1A in mice and humans results in a sensory neuropathy, providing direct evidence that disrupted axonal transport of a specific cargo can lead to axonal degeneration[74]. However, not all HSP-associated *KIF1A* mutations result in loss of function. Several *KIF1A* mutants were shown to have enhanced motility in vitro, resulting in increased frequency and speed of anterograde movements of synaptic vesicle precursors in *Caenorhabditis elegans* motor neurons in vivo, indicating that motor protein hyperactivity can also contribute to neurodegeneration[75].

Autosomal dominant mutations in *KIF5A*, clustering in the amino-terminal domain, were first described as a cause of HSP and peripheral neuropathy[76]. In vitro studies using microtubule gliding and cargo transport assays revealed that some, but not all, pathogenic mutations caused a reduction in transport velocities[77]. The effects of these HSP-causing mutations on slow axonal transport of cytoskeletal proteins or the cytoplasmic dynein motor or on axonal transport have not yet been investigated in cellular or in vivo models, partly owing to the limited tools available to image slow axonal transport. Therefore, whether mutations in *KIF5A* cause peripheral and corticospinal nerve degeneration through deficits in axonal transport remains unclear. Mutations in the carboxy-terminal, cargo-binding domain of KIF5A have recently been linked to a large number of cases of ALS, suggesting that the anterograde delivery of specific cargoes to axon terminals is impaired in this condition[78,79].

Autosomal dominant mutations in *KIF1B* were originally reported to cause CMT type 2 (CMT2) in a single family[80]; however, the lack of confirmation in

additional families casts doubt on the pathogenicity of these mutations.

Axonal transport and neurodegeneration

The mutations in transport machinery discussed above provide evidence that alterations in axonal trafficking can impair neuronal homeostasis and lead to neurological disease. It is conceivable, therefore, that disturbances in axonal transport, which have been reported in many, if not most, nervous system disorders[17,18], could be a major cause of associated neuropathology. However, compromised transport could also be a consequence of neuronal dysfunction and degeneration, and causation has proved difficult to demonstrate in vitro and in vivo. Several reviews have been published that discuss transport deficiencies in neuronal disorders across model systems[9,12–18]. In this section, focusing principally on human and in vivo studies, we briefly discuss the evidence for and against the idea that transport disruptions contribute to the aetiology of neurological diseases that are not caused by the genetic mutations in transport machinery outlined above.

When axonal transport is perturbed, cargoes are likely to aberrantly accumulate and cause swellings along the axon, as has been shown through genetic disruption of the transport machinery in *D. melanogaster* and mice[81,82]. This phenomenon has also been observed in disease-relevant neurons in post-mortem studies of patients with diverse neurological conditions. For example, the brains of patients with early-stage AD display swellings in basal forebrain axons before amyloid deposition[83], motor axons in patients with ALS accumulate phosphorylated neurofilament proteins and organelles in swellings that can selectively ensnare kinesin[84,85], and axonal accumulations of synaptic vesicles and α-synuclein have been observed in hippocampal neurons of patients with PD[86]. Moreover, a transport deficiency has been indirectly observed in patients with PD, using a method that implements heavy water pulses to assess kinetic biomarkers in cerebrospinal fluid[87].

Consistent with these findings, evidence indicates that quantitative and qualitative alterations in the transport machinery, including the microtubule cytoskeleton, are widespread in neurological disorders. For instance, motor protein expression levels are altered in AD[88], ALS[89], multiple sclerosis[90] and PD[91] patient samples. Moreover, impairments in microtubule stability and function have been reported in the brains of patients with AD[92,93] and in induced pluripotent stem cell (iPSC)-derived dopaminergic neurons from patients with PD[94,95]. These observations have been corroborated by studies in animal and cell models of many neuronal disorders[10,96], suggesting that impairment and/or deregulation of the cytoskeleton is a frequent pathological feature of neurodegeneration. However, although the findings from human cells and tissues are consistent with the idea that axonal transport disruptions are common in neurological disorders, they neither directly demonstrate transport perturbations nor prove causation.

iPSC-derived neurons in the study of axonal transport. Many studies are emerging in which individual fluorescently labelled cargoes were tracked while being transported along the axons of human iPSC-derived neurons. Motor neurons generated from CMT2 patients with dominant *MFN2* or *NEFL* mutations displayed reduced mitochondrial transport velocities but no differences in the percentage of motile mitochondria[97]. By contrast, the percentage mitochondrial mobility, but not the velocity, was affected in motor axons derived from patients with spinal muscular atrophy[98]. This change occurred early in the disease course, was linked to swellings and was specific to the tissue involved in the disease, with no defects being observed in forebrain neurons. These two studies indicate that different aspects of axonal transport are selectively affected by disease, and that analysing various parameters[99] can potentially provide early mechanistic insight into the initial cause of transport disruption, for example, transport initiation versus maintenance, anterograde versus retrograde trafficking or rate versus frequency of transported organelles. The data also suggest that the reported transport disturbances are not likely to be simply due to poor neuronal health, as one would expect multiple dynamic properties of various cargoes to be altered if this was the case. Additional support for this notion is provided when transport defects are progressive and occur before signs of major cellular upheaval, including neuronal death, as has been reported for mitochondrial deficits in motor neurons derived from ALS patients with *FUS* mutations[100].

Mitochondria are the most frequently experimentally tracked axonal cargo; thus, much of what we know about axonal transport comes from this organelle[101]. Mitochondrial transport along axons is typified by frequent pausing, relatively slow speeds and bidirectional movements, in contrast to other cargoes, such as signalling endosomes and autophagosomes, which mainly move in the centripetal direction, and synaptic vesicle precursors and secretory granules, which are powered in the opposite direction. These differences are probably attributable to distinct cargo functions, energy requirements[102], motors and adaptors[12,36], and regulatory mechanisms[8,9]. Therefore, a disturbance in one cargo type does not necessarily imply that other cargoes are affected, as has often been documented[103,104]. However, the disruptions could still have a common cause, such as a microtubule deficit that affects the motility of all motors, or the trafficking impairment could reflect a degenerating neuron that is incapable of maintaining homeostasis. Defective axonal transport of additional cargoes has been reported in human iPSC models of disease, for instance, mRNA in TDP43-associated ALS[103] and amyloid precursor protein-containing vesicles in AD[105]. However, multiple cargoes are yet to be routinely analysed in iPSC-derived neurons, as has been done in other systems[62,103,106,107], and such studies will be paramount if we are to better understand the full contribution of defective axonal transport to neurological disease.

Additional caveats to iPSC experiments include the considerable intrinsic and extrinsic variability that persists in iPSC models, despite continuous improvements[108]. Moreover, we do not yet know how robustly a developmentally reprogrammed and re-differentiated cell can model adult-onset neurodegenerative conditions

in which the disease-targeted neurons can remain viable for many decades in patients. However, induced neurons directly converted from somatic cells provide a promising alternative to circumvent this issue[109].

Studying axonal transport in vivo. In vitro systems and ex vivo tissue preparations do not always accurately replicate the in vivo environment, especially when modelling dynamic, tightly regulated processes such as axonal transport[110–113], which can be influenced by non-cell-autonomous signalling[114,115] and cell–cell interactions[116,117]. This issue is particularly pertinent for discriminating between cell-autonomous and non-cell-autonomous pathomechanisms, which have been shown to contribute to diverse neuropathologies to varying degrees[118]. Moreover, neuronal activity[119,120] and maturation[106] can differentially affect cargo trafficking, as can the location of the cargoes along the axon[113,121]. In vivo transport experiments also have their limitations[99] and similarly struggle with the pervasive difficulty of distinguishing cause from effect. Nonetheless, bona fide in vivo experiments, although often challenging, are likely to yield more consistent results that accurately reflect the in situ situation. In this section, we concentrate on evidence from in vivo models of neurological disease, in which the trafficking of individual cargoes was directly assessed in live organisms.

Selective expression of fluorescent proteins in distinct organelles has facilitated the assessment of axonal transport in a range of genetic model organisms. The combination of fluorescent reporter strains with an ever-expanding repertoire of disease models has provided considerable evidence that impaired axonal transport can at least contribute to neuronal disease. For instance, intravital imaging in filleted *D. melanogaster* larvae has been used to model axonal transport impairments in various neurological diseases, including ALS[103,104], Friedreich ataxia[121] and PD[122,123]. In these studies, imaging was predominantly performed on motor axons of the segmental nerve, which is perhaps more pertinent to modelling of diseases that affect the motor system, such as ALS, than to non-motor neuron disorders, such as AD. Nonetheless, these findings have been corroborated by similar experiments assessing transport in mechanosensory neurons of *C. elegans*[124,125] and motor and dopaminergic axons of zebrafish larvae[126,127]. The latter model has an added advantage over *C. elegans* and flies of being a vertebrate with myelinated axons. However, one must be wary when interpreting results generated from larval-stage, non-mammalian organisms, especially *D. melanogaster*, which lack axonal transport-relevant pathways such as neurotrophin receptor signalling[128] and require major tissue disruption for imaging, and thus might not accurately replicate the complex environment of the human nervous system.

Mouse models of human neurological disease also have limitations. Intravital experiments in which axonal transport can be monitored in live, anaesthetized mice currently provide the most accurate setting in which to assess this dynamic process, and have provided some of the most compelling evidence that disturbed transport can contribute to neurological disease by enabling the identification of transport disturbance before symptom onset and cell death.

In vivo deficits in axonal transport of individual cargoes were first reported in the SOD1^{G93A} mouse model of ALS[62]. Impaired trafficking of neurotrophin-containing signalling endosomes and mitochondria was observed in surgically exposed sciatic nerve axons of pre-symptomatic mutant mice; retrograde endosome transport speeds were significantly reduced before motor neuron loss and became progressively worse, and mitochondria showed an early increase in pausing in anterograde and retrograde directions without alterations in the proportions of moving mitochondria[62]. In addition, injection of a radiolabelled tracer into the ventral horn of the spinal cord in a SOD1^{G93A} mouse strain with a milder disease phenotype revealed pre-symptomatic deficits in slow anterograde transport of cytoskeletal proteins in ventral roots[129]; these results were replicated in two additional mutant SOD1 strains (SOD1^{G37R} and SOD1^{G85R})[130]. These data indicate that SOD1-linked ALS is associated with general disruption to the transport machinery that affects multiple cargoes and both directions of transport, suggesting an alteration in the microtubule network[84,96]. Progressive, pre-symptomatic retrograde mitochondrial transport disturbances were independently verified using a second fluorescent reporter strain crossed with SOD1^{G93A} mice, and were replicated in the TDP43^{A315T} mutant transgenic mouse model of ALS[131].

Impaired retrograde trafficking of signalling endosomes in live motor axons of the sciatic nerve was also reported in a newly developed TDP43^{M337V} mouse model of ALS. Unlike the previously discussed ALS mice, this model expresses the mutant transgene at near-endogenous levels, leading to neuromuscular pathology without motor neuron loss[132]. Deficits in signalling endosome dynamics manifested between 1.5 and 3 months and persisted until at least 9 months of age[133]. These results indicate that transport defects can occur in ALS mice without supraphysiological transgene expression, and that axonal transport disturbances, although intricately linked to neuromuscular phenotypes, do not necessarily cause immediate motor neuron death. Moreover, given that the signalling endosome transport deficit is of a similar severity between SOD1^{G93A} and TDP43^{M337V} mice, which show major differences in neurodegeneration and survival, additional ALS pathomechanisms are likely to operate in these models[134].

The data discussed so far suggest that in vivo transport defects are common to all mouse models of ALS. However, a novel humanized FUS mutant mouse, Fus$^{\Delta 14/+}$, which displays overt and progressive motor neuron loss from 1 year of age[135], shows no clear impairment in axonal transport of signalling endosomes at 3 and 12 months, and only a minor increase in pausing by 18 months[133]. Similarly, motor neuron degeneration was dissociated from transport disruption in ex vivo intercostal and tibialis nerve preparations from SOD1^{G85R} mice, which did not show disturbances in mitochondrial flux or transport dynamics of cholera toxin B subunit-labelled vesicles[136]. This result does not preclude disruption of transport of other cargoes or in motor axons innervating different muscles. However, these studies

suggest that global defects in transport are not common to all ALS mouse models, and that mutations in different genes lead to inherent differences in the pathogenesis of the disease. Moreover, the observation that stark motor neuron loss can occur in the absence of transport disruption in $Fus^{\Delta 14/+}$ mice indicates that degenerating neurons do not always display defects in axonal trafficking before neuronal death. Perhaps counter to expectation, this idea was supported by in vivo observations from a mouse model of spinal and bulbar muscular atrophy, which also shows neuromuscular phenotypes and motor neuron loss yet no disruption in the trafficking of signalling endosomes in sciatic nerve axons[137].

Taken together, these findings suggest that disrupted axonal transport is not simply a non-specific by-product of neurodegeneration, and that the trafficking defects reported in diverse neurological disease models could have a causative and/or contributory role in the pathology. Indeed, the evidence from the mouse models of ALS indicates that transport disturbances are one of the earliest observable phenotypes. Similarly, acute and chronic mouse models of multiple sclerosis displayed defects in both anterograde and retrograde transport of mitochondria and peroxisomes in normal-appearing spinal cord axons in vivo[138]. These defects resulted in diminished organelle supply to the periphery and preceded the development of morphological abnormalities in axons, cargoes and microtubules[138], consistent with the idea that impaired axonal transport contributes to secondary axonal loss in multiple sclerosis.

In contrast to the in vivo results obtained in mice, axonal transport defects have been reported in *D. melanogaster* and iPSC models of mutant FUS-linked ALS[103,104] and in squid axoplasm and ex vivo mouse sciatic nerve models of spinal and bulbar muscular atrophy[139,140]. These discrepancies between models could reflect distinctions in time points or disease-associated mutations, but are probably more likely to be determined by the model system. Therefore, careful consideration must be paid to the experimental model before axonal transport disruption is invoked or disregarded as the cause of neurodegeneration. On balance, the frequency of axonal transport perturbation in disease models suggests that trafficking alterations contribute to neuronal dysfunction in numerous neurological conditions, especially those where broad agreement exists across models, such as mutant TDP43-linked ALS[103,104,131,133,141] and SOD1-linked ALS[62,128,130,136,142–144].

Axonal transport as a drug target

If impairments in axonal transport cause or contribute to neurological disease, targeting of deficient cargo trafficking is an attractive therapeutic strategy. Promisingly, in vivo studies in mouse models of disease indicate that such defects can be acutely reversed[138,144], creating scope to develop and test drugs that modulate transport.

A relatively non-specific approach would be to target neuronal microtubules, the post-translational modification and dynamics of which are altered — thereby possibly exacerbating transport anomalies — in several neurodegenerative diseases[10,11,96]. Microtubule-stabilizing compounds have shown positive effects in models of various diseases, including AD, ALS and PD[96], and also in spinal cord injury[145], the response to which is dependent on axonal transport[7,146]. However, increased microtubule stabilization produced negative effects in the $SOD1^{G93A}$ mouse model of ALS[147]. Similarly, chemical inhibition of histone deacetylase 6 (HDAC6), which removes acetyl groups from microtubules, thereby diminishing their affinity for motor proteins[148], has been shown to reverse axonal transport deficits in models of ALS[100], Huntington disease[149] and CMT[150,151]. Consistent with these findings, genetic deletion of *Hdac6* can extend $SOD1^{G93A}$ mouse survival and improve motor axon integrity[152]. HDAC6 also affects transport through deacetylation of a protein called MIRO1, which is crucial for the calcium-dependent recruitment of motor complexes to mitochondria to facilitate their trafficking[153]; thus, the transport-related effects of HDAC6 inhibition are likely to be multifactorial.

Disease-related impairments in axonal transport are frequently motor protein specific and/or cargo specific, or affect particular dynamic properties such as the percentage mobility, speed or overall flux of cargoes. Therefore, broad modulation of axonal transport might have undesired consequences, such as those associated with the delivery of superfluous cargoes or increases in transport speeds above normal levels, as reported in models of CMT2B (REFS[154,155]) and HSP[75]. This issue is particularly relevant for mitochondria, which, unlike most transported organelles, pause frequently and become anchored at specific points along the axon that require a constant energy supply or calcium buffering[101]. A twofold increase in the motility of mitochondria, through genetic knockout of the mitochondria-specific docking protein syntaphilin, had no impact on disease progression in $SOD1^{G93A}$ mice[156], although the caveat remains that syntaphilin ablation has not been confirmed to modify mitochondrial transport in motor axons or in vivo. Negative stress-related signals can be retrogradely transported along axons[115] and, if enhanced, might also prove detrimental[157]. Thus, global modification of axonal transport, especially affecting cargoes that are not altered in a disease (either due to cargo specificity or axonal transport being completely unaffected), might not be an ideal treatment strategy for all neurological conditions, and a tailored approach aimed at particular transport mechanisms and organelles could prove more beneficial.

Protein kinases are vital for efficient axonal transport, as they directly phosphorylate many key components of the transport machinery[8,9]. For example, extracellular signal-regulated kinase ERK1/2 can phosphorylate the dynein intermediate chain to specifically enhance the retrograde transport of signalling endosomes, but not of mitochondria[114]; JNK1-mediated phosphorylation of adaptor protein JIP1 stabilizes the interaction of JIP1 with kinesin, thereby promoting anterograde transport of amyloid precursor protein-containing vesicles over retrograde transport[158]; and CDK5 can phosphorylate neurofilaments, thereby inhibiting their slow axonal transport[159]. Disruptions in these and other kinase signalling pathways that are crucial for the maintenance of axonal trafficking have been reported in several

neurological conditions[8,9]. For instance, p38 mitogen-activated protein kinase (MAPK) can negatively regulate axonal transport through phosphorylation of motor and cytoskeletal proteins[142,160], and its overactivation in the spinal cord in patients and mice with ALS might contribute to the impairments in fast and slow axonal transport that have been reported in this disease[142,161–163]. Inhibition of p38 MAPK can preserve motor neuron integrity and marginally increases SOD1^{G93A} mouse survival[163]. This strategy was shown to restore signalling endosome transport dynamics in both in vitro primary motor neurons and in vivo sciatic nerve axons of SOD1^{G93A} ALS mice[144]. Although the long-term impact of this treatment could not be assessed owing to the systemic toxicity of the experimental drug, this study provides proof of principle that pharmacological modification of disease-implicated kinases is a promising therapeutic avenue for neurological conditions. However, whether such a strategy can be used to treat transport deficiencies when the targeted kinase is not directly implicated in pathology remains to be seen.

Modification of protein kinases in the nervous system has its challenges[164], including drug traversal of the blood–brain barrier. In addition, many protein kinases involved in axonal trafficking have multiple transport-related and non-transport-related targets, are involved in diverse processes across cell types and show considerable crosstalk in downstream signalling cascades[165–167]. Other important considerations are that drugs can differentially influence transport depending on the axonal location, at least in cultured neurons[107], and that retrograde and anterograde transport are not mutually independent; for example, kinesin 1 delivers cytoplasmic dynein to distal axons[41] and, accordingly, malfunctioning of one motor can affect transport in the opposite direction[168,169]. Therefore, although targeting of axonal transport is in theory a promising therapeutic strategy for neurological disease, the long-term viability and impact of such an approach requires considerable investigation. Encouragingly, with a detailed understanding of disease pathways, multiple key nodes of kinase signalling pathways might be modulated to achieve amelioration of neurological disease phenotypes[170]. In the interim, studies in which the ramifications of pharmacological transport correction can be assessed will be invaluable in further determining the importance of axonal transport to neurological diseases. Similarly, kinetic biomarkers, such as those assessed in cerebrospinal fluid of patients with PD[87], might provide a method to monitor the disruption of axonal transport in humans while providing a sensitive measure of neuronal dysfunction and the impact of treatment.

Conclusions

The delivery of substances to precise subcellular locations is vital to maintaining cell function and viability, and nowhere is this more important than in the body's longest and arguably most polarized and energy-demanding cell type, the neuron. Axons can reach well over 1 m in length in humans and require specialized mechanisms to orchestrate the intricate, bidirectional distribution of multiple cargoes between cell bodies and axon terminals. Therefore, it is not surprising that deficiencies in axonal transport have been invoked as a major causative factor in a range of neurological diseases. However, the evidence is perhaps not as decisive as one might initially expect, not least because of difficulties in deciphering whether early trafficking disturbances cause neuronal dysfunction or whether nerve degeneration leads to transport defects. Although the latter scenario can ultimately contribute to neuropathology and exacerbate the demise of a degenerating neuron, it is not consistent with defective transport playing a central role in disease aetiology and substantially reduces the potential impact of targeting this basic neuronal process for therapeutic intervention. Nevertheless, we have highlighted several diseases in which considerable evidence across model systems supports a role for disturbed axonal transport in neuropathology, including some examples where defective axonal trafficking is one of the first identifiable phenotypes in the in vivo models. Given the importance of non-cell-autonomous mechanisms to axonal transport regulation, developments in longitudinal intravital imaging should be incorporated into future studies of this process.

As we have discussed, mutations in many genes that encode constituents of axonal transport machinery have been linked to human diseases. Most of these conditions are neurological or display a strong neuronal component, which is indicative of the importance of transport machinery to neuron integrity. Additional mutations in other key transport genes are likely to be identified, but owing to the essential nature of axonal transport, complete loss-of-function mutations in such genes are likely to be embryonic lethal.

Numerous therapeutic strategies that augment axonal transport have been tested in disease models and have shown signs of efficacy. The feasibility of gene therapy to combat transport deficiencies has been demonstrated in ALS mice[171] and is an appealing area for future research, both to help further elucidate transport mechanisms and to highlight potentially viable therapeutic strategies for currently incurable nervous system disorders.

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