The lifecycle of the neuronal microtubule transport machinery.

Alison E. Twelvetrees[1]

1. Sheffield Institute for Translational Neuroscience, University of Sheffield, 385 Glossop Road, Sheffield S10 2HQ, UK

Abstract

Neurons are incredibly reliant on their cytoskeletal transport machinery. During development the cytoskeleton is the primary driver of growth and remodelling. In mature neurons the cytoskeleton keeps all components in a constant state of movement, allowing both supply of newly synthesized proteins to distal locations as well as the removal of aging proteins and organelles for recycling or degradation. This process is most challenging within axons as large distances need to be covered between synthesis and degradation, but it is essential as the lifetime of any single protein is much shorter than the lifetime of the neuron and its synapses. However, the transport machinery itself also has to be actively transported, recycled and degraded in order to localise properly and perform within neurons. This review provides an overview of the lifecycle of cytoskeletal components in neurons, focusing on its spatial organisation over time in the axon.

Keywords:

cytoskeleton; microtubule motors; neuronal cell biology; axonal transport; slow transport; local translation

1 Introduction

Neurons are polarised, incredibly long and last a lifetime - facts that are often repeated, but no less remarkable for it. Although neurons have to be maintained for many years, neurons are made from proteins, lipids and RNAs that typically have a lifespan much shorter than our own. Our neurons are like the Ship of Theseus (or Trigger's broom), where each individual component must be replaced as it ages to maintain the whole. Neurons achieve this by keeping everything in a constant state of movement. This allows quality control and recycling as well as removal by degradation. This fundamental rule applies to the cellular machinery of transport itself, the processive motors and tracks of the microtubule cytoskeleton (see Figure 1), not just the cargo it carries.

The challenge of transport in neurons is most pressing in axons, which can be a metre long in the human body. The continuous synthesis and anterograde delivery of new material to the axon is essential for neuronal survival [1,2] and disruptions in axonal transport are extensively linked to neurodegenerative disease [3–6]. Axon degeneration is a common feature of age related neurodegeneration [6] and several studies show that axonal transport declines with age [7,8]. What is less clear is how normal aging impacts axonal transport. How mature neurons manage the turnover of the cytoskeleton is critically important to understanding why this system is a weak point in neurodegenerative disease.

Using recent insights into local translation, the cell biology of the cytoskeleton and protein degradation in neurons, I review current evidence for how the protein life cycle of the transport machinery is organised in adult axons.

2 Life in the axon, from synthesis to degradation

2.1 Sites of protein synthesis

The majority of protein synthesis in neurons takes place in the cell body, but substantial evidence now supports local synthesis in the axon [9]. However, it's worth stating that even if most axonal protein was synthesised locally, the machinery of local translation, including mRNA, would still need to be transported out from the cell body. Transcriptomes and translatomes of axons show that the pool of locally translated proteins changes dramatically during development [10,11], including changes in key components of the microtubule cytoskeleton (see Table 1). There is also

a developmental program of ribosome removal from axons [12], but ribosomes are still localised in adult presynaptic terminals [13].

2.2 Microtubule mediated axonal transport

The microtubule transport machinery is the foundation of polarised long distance transport and neuronal function. This is because the end on end assembly of hetero-dimeric tubulins creates polarised microtubule filaments. The organization of microtubule polarity in neurons is reviewed extensively elsewhere [14]; briefly, in axons microtubules assemble with uniform polarity with all plus ends pointing away from the cell body whilst in dendrites microtubules have mixed polarity, particularly in the proximal dendrite. This means that processive kinesins and cytoplasmic dynein motor proteins walk towards the distal axon and the cell body respectively (Figure 1).

There are two broad categories of anterograde (away from the cell body) axonal transport in neurons, fast and slow [3,15]. Fast transport is typically membrane bound vesicles and organelles. Slow transport is largely made of cytosolic proteins and the cytoskeleton, and carries at least three times the material of fast transport, but ten to a hundred times more slowly [15]. The slow anterograde axonal transport of protein is often further subdivided into two speed categories: slow component a (SCa) moving 0.2–1 mm/day and slow component b (SCb) moving 1–10 mm/day. Transit time directly influences the half-life of proteins. A protein moving 2 mm per day along the 50 mm human optic nerve will be a month old in the distal axon, at 0.2 mm per day it could be a year old (Figure 1). This is exemplified by the axon specific microtubule associated protein tau. Tau does not appear in transcriptomes or translatomes of adult animals [10,11] and is well characterised to travel in the slowest phase of axonal transport [16]. Accordingly, the measured half life of tau radioactively labelled in three week old primary cultures is estimated to be over 100 days [17]. Critically, transport rates slow further in older neurons [7,8].

2.3 Degradation

The major routes to cytosolic protein degradation in cells are the ubiquitin-proteasome and autophagy-lysosome systems. The autophagy pathway shows clear spatial regulation in axons with axon terminals the preferred site for autophagosome biogenesis [18]. It is less clear whether the activity of the proteasome is spatially regulated in the same way, but neurons keep tight control of the proteasome in response to signalling and development [19].

3 Microtubules and tubulin

3.1 Tubulin synthesis

3.1.1 Autoregulation of tubulin synthesis

Free tubulin concentration is tightly controlled in the cell and the concentration of free tubulin subunits directly modulates tubulin mRNA stability [20,21]. At least part of this autoregulation is down to the conserved first four amino acid residues emerging during nascent tubulin translation, Met-Arg-Glu-Cys/Ile, which serve as a regulatory tag [22]. This nascent tag is now known to be recognised by tetratricopeptide repeat protein 5 (TTC5) for both α and β-tubulin [23] (Figure 2). Several steps in this autoregulatory pathway remain unidentified [24], but it's clear that autoregulated synthesis is a critical cellular mechanism. Gasic et al recently found robust differential gene expression signatures of tubulin genes that had opposite responses to microtubule stabilising and destabilising drugs. By developing a tubulin mRNA stability signature they went on to query the Gene Expression Omnibus (GEO) database, discovering many other perturbations that trigger tubulin differential expression [21].

3.1.2 Tubulin chaperones

Tubulin monomers are heavily dependent on chaperones and cofactors to ensure correct folding after translation (Figure 2). Unfolded tubulin is first transferred to the cytosolic chaperonin TRiC by the prefoldin complex [25,26]. Prefoldin is a hexamer of two α and four β subunits with a jellyfish-like appearance [27]. From here, tubulin relies on the chaperonin TRiC (for TCP-1 Ring Complex, also called CCT for Chaperonin Containing TCP1) for folding in vivo [28]. TRiC is a large structure of two stacked eight-membered hetero-oligomeric rings, each composed of eight separate subunits, CCT1-8 [29,30].

Once folded, tubulin monomers are assembled into functional αβ-heterodimers by essential postchaperonin tubulin folding cofactors (Figure 2). This requires five conserved tubulin cofactors, TBCA-E, and ADP ribosylation factor–like 2 (Arl2) [31]. Data supports a model whereby TBCD and TBCE form a heterotrimeric cage-like chaperone in complex with Arl2 termed TBC-DEG [32]. TBC-DEG binds either to soluble αβ-heterodimers in the cytosol or nascent dimers formed through the delivery of tubulin monomers by TBCA and TBCB.

Subsequent binding of TBCC to the TBC-DEG-αβ-tubulin complex activates the Arl2 GTPase, releasing polymerisation competent αβ-heterodimers [32].

3.1.3 Local tubulin synthesis in neurons

There is evidence to support local synthesis of tubulin in the axon, however this corresponds to a limited selection of tubulin isotypes, particularly in adult axons. The transcriptomes of DRG neurons show *tubb3* mRNA disappearing from adult axons [10], whilst the translatome of adult optic nerves drops from seven to two tubulin isotypes during development [11]. This means that adult axons, compared to developing axons, must rely much more heavily on the delivery of tubulin from the cell body by slow axonal transport [33].

Having outlined the biosynthesis pathway of tubulin above, it's clear that to support local translation neurons need not only mRNA and the translation machinery, but also tubulin chaperones and cofactors. For example, local synthesis of tubulin monomers without cofactor mediated assembly into αβ-heterodimers, could be toxic to microtubule polymerisation [31]. Here again there is a clear decrease during development of the axon to locally translate this machinery. Prefoldin and the tubulin co-factors in particular seem to be entirely absent in adult axon transcriptomes and translatomes (summarised in Table 1). mRNAs for the TRiC subunits are present in adult DRG neurons, but they are absent from the adult translatome of the optic nerve (Table 1). TRiC is one of the few characterised cargos to be transported along axons by slow transport [34], so even without local translation adult axons are likely well supplied with chaperones.

3.2 Microtubule stability in neurons

Even once synthesised, the life of a tubulin heterodimer incorporated into the microtubule lattice is far from straight forward. Microtubules undergo dynamic instability, switching between phases of growth and disassembly, regulated by post-translational modifications of tubulin [35,36] and microtubule binding proteins [14]. Any individual tubulin heterodimer could undergo many cycles of microtubule incorporation.

Experimental evidence shows subpopulations of microtubules within neurons, with different propensities for microtubule dynamics [37]. As microtubules accumulate post-translational modifications with time, the most dynamic microtubules have few modifications, becoming

richer in modifications with increasing stability. The most stable microtubules in neurons, often termed cold-stable microtubules (a reference to tubulin purification protocols from brain), are rich in polyaminated tubulin and do not undergo dynamics [38]. Unlike other posttranslational modifications, polyaminated tubulin is directly involved in stabilising microtubules [38]. Another key factor shown to directly stabilize microtubules is decoration with microtubule-associated protein 6 (MAP6 or stable tubule only polypeptide, STOP) [39–41]. Some stable microtubules can still undergo plus end dynamics and microtubules can have patches of different stabilities [42,43]. The most stable of neuronal microtubules are also resistant to common depolymerizing drugs [42].

3.3 Microtubule and tubulin transport

Tubulin was one of the first polypeptides identified to be moved by axonal transport, in part because of its abundance [44–46]. These large quantities of tubulin move from cell body to distal axon at a rate of 0.5-2 mm/day, coincidently the speed of axon regeneration (Figure 1). Two opposing models have been proposed for the transport of tubulin, either as tubulin monomers or microtubule polymers [47,48]. Analysis and interpretation of original experiments in this area are complicated by polymerization and depolymerization rates outlined above. However, the movement of short microtubule fragments within axons has been observed [49,50] and this movement is motor dependent [51]. Conversely, there is also evidence for the transport of soluble tubulin dimers in a kinesin-dependent manner [52,53]. As outlined above, both soluble pools of tubulin and very stable microtubules exist and are necessary in axons, so it seems more likely that both forms of transport exist to maintain microtubule homeostasis along the axon length.

3.4 Damage, repair and degradation of microtubules

It is now established that both shear and mechanical stress cause defects in the microtubule lattice, and that microtubules have the capacity for repair [54–56]. Microtubules under shear stress in microfluidic systems decrease in stiffness with cycles of bending, but can recover stiffness when free tubulin is present [55]. This recovery happens by incorporating replacement tubulin mid-lattice rather than at the polymerising microtubule ends. In fact, the turnover of tubulins within the lattice happens even without external forces [57]. Similarly, motor protein walking along the microtubules removes individual tubulins from the lattice through molecular wear and tear [54] (Figure 3). When microtubules are not chemically stabilised, degradation of the lattice can happen very quickly unless free tubulin is also present in the system [56]. Although these

experiments were carried out with *in vitro* reconstituted systems, mid lattice repair has been observed in cells [58]. Rather than relying solely on free tubulin for repair, cells are likely to make use of proteins currently best known for suppressing catastrophe, such as CLASP [59]. However, this observation highlights the importance of maintaining free tubulin pools in distal axons in order to support repair and transport.

Critically, mid-lattice repair sites can act as rescue points during microtubule depolymerisation [58]. Defects in the lattice use GTP-bound tubulin for repair, creating islands of GTP-tubulin distant to the GTP-cap [58] (Figure 3). This not only permits repolymerisation of microtubules, but when damage accrues successively along the lattice, actually increases microtubule lifetime and length *in vitro* [58]. In cells, repeated laser damage at the periphery promoted microtubule growth in this region.

Tubulin degradation pathways have not been studied in detail, but current evidence supports it's removal through the ubiquitin proteasome pathway. Both α and β tubulins are ubiquitinated by the E3 ligase parkin, and overexpression of parkin accelerates the removal of tubulins from HEK 293 cells [60]. In support of a role for parkin in microtubule homeostasis, neurons in parkin knockout mice accumulate markers of stable microtubules at a younger age compared to wild-type mice [61]. The removal of tubulin from cells likely also includes it's disassembly from heterodimers back into monomers. Tubulin heterodimers are extremely stable and the rate of αβ-tubulin heterodimer dissociation is very slow [62]. It has been suggested that the TBC-DEG chaperone system could stimulate dissociation through TBCC-mediated Arl2 GTP hydrolysis [31,32], but the role of chaperones in the turnover of tubulin remains to be characterised.

4 Microtubule motor proteins

Despite being the power behind almost all long distance travel in the axon, we know surprisingly little about the life of microtubule motors within neurons in their own right. This is largely because they are most often studied through the prism of their cargos [3], leaving significant gaps in our knowledge about the synthesis, degradation and life in transit.

4.1 Kinesin

4.1.1 Kinesin synthesis

Similarly to tubulins we can deduce that most processive kinesins in axons must be synthesised in the cell body in adults, as both axonal transcriptomes and translatomes show a marked decrease in their synthesis during development (Table 1). In a recent study profiling the transcriptome of excitatory presynaptic terminals from adult mice, mRNAs for kinesin-1 subunits, KIF5C and KLC1, as well as the kinesin-3 genes KIF1A and B were isolated [13], so it may be the case that small and functionally relevant quantities are transcribed at synapses.

4.1.2 Kinesin-1 isoforms and auto-regulation

The kinesin-1 sub-family is the most studied of the processive kinesins that function in axonal transport (Figure 4). Kinesin-1 is formed of two heavy chains (KIF5A–KIF5C) and two light chains (KLC1–KLC4). The heavy chains have an N-terminal motor head domain, which hydrolyses ATP and binds microtubules, an extended coiled-coil stalk, and a C-terminal tail domain which binds the light chains and cargo. KIF5A and KIF5C have brain specific expression, although all three heavy chains are highly expressed in the brain. Similarly KLCs 1, 2 and 4 show enrichment in the brain, but it is still unclear whether the heavy chains require light chains for all their functions in mammalian neurons or not [63].

Kinesin-1, when not bound to cargo, exists in a folded autoinhibited conformation [64–66], mediated by direct interactions between the heavy chain head and tail [64,67] (Figure 4). There is a central hinge in the heavy chain that allows the head and tail to come into contact [65]. KLCs themselves are also autoinhibited and contribute to the inhibition of motor activity [68–70]. Cargo binding has a complex role to play in releasing autoinhibition; cargo binding to the light chains is critical [68–71], as is binding to the C-terminal cargo binding domain of the heavy chain [68,72–74]. There may also be an additional requirement of cargo binding further down the stalk of kinesin-1 to stabilise activation [73,74]. Although autoinhibition means kinesin cannot bind to microtubules without first binding to cargo, some studies suggest that membrane bound cargo can carry motors in an inactive state [75]. Structural studies of kinesin-cargo complexes are yet to confirm if this is possible, and similarly if the autoinhibitory QIAK motif in the C-terminus of the heavy chain is still accessible to the motor domains when cargo is bound.

4.1.3 Kinesin-1 transport in axons

Kinesin-1 motors are the driving force behind both fast and slow axonal transport. Kinesin-1 is well characterised to be important for the fast transport of vesicular cargos such as amyloid precursor protein (APP) [76], TrkB [77] and lysosomes [78] amongst many others. Similarly, kinesin-1 is known to be the main motor for key slow transport cargo such as neurofilaments [79], chaperones [80] and dynein [81]. Conversely, *in vitro* studies show that constitutively active kinesin motors have a very consistent velocity as they walk along microtubules [65]. *In vivo* radiolabelling studies agree that the vast majority of kinesin-1 is transported into the axon as a coherent wave, equivalent to its maximum velocity and not at slow transport speeds [82]. This means firstly, that kinesin dependent slow transport is driven by only short bursts of kinesin motility, and secondly, that kinesin is almost always in motion from one end of the axon to the other. In the longest axons of the human body, this implies near constant motor stepping for almost two weeks.

4.1.4 Kinesin-1 lifetime and degradation

Although it seems likely that tubulin proteins are recycled and repaired many times in their long lifetime, the same is not true for kinesin-1. In differentiated neuron-like cells, there is evidence that kinesin can diffuse back to the cell body on relevant time scales [83], but this would not be the case in very long axons where diffusion would take too long to be meaningful. *In vivo* radiolabelling from the optic nerve agrees with this assumption as the vast majority of radiolabelled kinesin does not persist in the optic nerve over the longer time periods that would result from diffusion based recycling [82]. The most likely model is that kinesin is made in the cell body, delivered at the maximum velocity of its own motility and degraded distally, however how kinesin is targeted for degradation is unknown.

4.2 Dynein

4.2.1 Dynein synthesis

Cytoplasmic dynein is a complex multisubunit machine (Figure 5). The core cytoplasmic dynein motor is formed of two heavy chains (DHC) that bind to microtubules and hydrolyse ATP. A dimerization domain forms between the two heavy chains and DHC binds directly to a pair each of dynein intermediate chains (DICs) and dynein light intermediate chains (DLICs) [84,85]. The

DICs themselves bind to DHC with a C-terminal WD40 domain, while the N-terminus forms an extended and mobile structure that does not interact with DHC, supporting three pairs of light chains: LC7, LC8 and TcTex [85]. The light chains restrain the DICs, which in turn ensure a near parallel arrangement of DHCs [86]. The molecular mass of the assembled dynein motor is ~1.5 MDa.

There is one cytoplasmic heavy chain gene, expressed in all cells, but the other dynein subunits show more complex patterns of expression. For example, the intermediate chain genes are extensively spliced, with DYNC1I1 and most splice forms of DYNC1I2 being brain specific [87]. Work on recombinant dynein purification highlights the complexity of it's assembly into a functional motor; although a processive dynein motor can be purified from the brain [88–90], reconstituting processive motors *in vitro* is much more complex [91–93]. Current evidence does not support the local synthesis of the dynein heavy chain in adult axons, indicating that all functional heavy chains are synthesised in the cell body of neurons (Table 1).

4.2.2 Dynein transport in the axon

Dynein is synthesised in the cell body, but given the uniform microtubule polarity of the axon, dynein cannot move itself distally. It is essential that transport competent dynein reaches the distal axon, as it is required for essential functions such as growth cone extension [94,95], axon elongation [96], retrograde neurotrophic signaling [97,98] and autophagy [99]. Radiolabelling studies demonstrate that 80% of newly synthesized dynein moves outward as a component of slow axonal transport, with a velocity in the region of 1–10 mm/day [100,101] (Figure 1). We recently demonstrated that the localisation of dynein in the distal axon is dependent on direct interactions with kinesin-1 [81]. Our study made use of the dynein-GFP mouse, a knockin model with a GFP tag fused in frame to the neuron-specific isoform of the dynein intermediate chain, DIC1 [102]. Imaging dynein-GFP in axons showed that the total pool of dynein, not just newly synthesized motors, was moving in the anterograde direction [81]. One immediate consequence of this is that far more dynein is made and transported than is needed for retrograde transport alone. On the surface this is a wasteful 'over supply', which may be necessary to ensure sufficient functional dynein after it has aged in transit. On the other hand, it may reflect a function of dynein related to microtubule transport and maintaining microtubule polarity [51,96,103].

4.2.3 Dynein lifetime and degradation

The slow axonal transport of dynein implies that dynein is synthesised in the cell body to be degraded in the distal axon [81]. The E3 ubiquitin ligase TRIM58 ubiquitinates the dynein intermediate chain in erythrocytes, triggering the proteasomal degradation of the whole dynein complex [104]. However, TRIM58 is not expressed in the brain, so it is unclear how dynein is targeted for degradation in the axon.

5 Discussion

By considering the lifecycle of the key cytoskeletal elements of axonal transport in turn, three key themes emerge. Firstly, there is a decline in the local synthesis of the microtubule cytoskeleton in adult axons. Secondly, that the whole machinery moves from cell body to distal axon, but at different speeds. Thirdly, from the available evidence it seems likely that this same machinery is specifically degraded in the distal axon, although the mechanisms are unclear. Consequently there is a large separation in space between the site of synthesis and degradation; the majority of the building blocks of the microtubule cytoskeleton are built in the cell body, moved along the axon and then degraded distally. Given that the transit in the axon is slowest for tubulins and dynein, potentially on the order of years, it seems likely that the adult distal axon has a significant pool of aged transport machinery.

It is unknown how the process of aging in transit, from one end of the axon to the other, affects the function of the material it carries. It's clear that microtubules have a self repair strategy, but this relies on a consistent supply of functional tubulin heterodimers - not an insignificant hurdle. The strategy that neurons employ to monitor dynein function will be particularly important to decipher. Just as microtubules experience mechanical stress from motor proteins that requires repair (see section 3.5), so the motors themselves experience strain in constant movement. Consequently, a key question is whether dynein motor domains are active in the axon, held in inactive conformations or repaired in transit to maintain motile properties (see Figure 5). A related issue is understanding how carefully degradation is monitored along the axon and whether refolding in transit can be prioritised over degradation.

There is a limited capacity for local translation to renew the cytoskeleton in the distal axon of adult neurons. Resolving and improving how the axonal transport machinery renews itself will be

critical for developing effective treatments for neurodegeneration, as there can be no recovery of the axon without it.

Acknowledgements

This work was supported by a Vice-Chancellor's Fellowship from the University of Sheffield.

6 References

[1] J.A. Kleim, R. Bruneau, K. Calder, D. Pocock, P.M. VandenBerg, E. MacDonald, M.H. Monfils, R.J. Sutherland, K. Nader, Functional organization of adult motor cortex is dependent upon continued protein synthesis, Neuron. 40 (2003) 167–176.

[2] L.E. Cromberg, T.M.M. Saez, M.G. Otero, E. Tomasella, M. Alloatti, A. Damianich, V. Pozo Devoto, J. Ferrario, D. Gelman, M. Rubinstein, T.L. Falzone, Neuronal KIF5b deletion induces striatum-dependent locomotor impairments and defects in membrane presentation of dopamine D2 receptors, J. Neurochem. 149 (2019) 362–380.

[3] S. Maday, A.E. Twelvetrees, A.J. Moughamian, E.L.F. Holzbaur, Axonal Transport: Cargo-Specific Mechanisms of Motility and Regulation, Neuron. 84 (2014) 292–309.

[4] K.J. De Vos, M. Hafezparast, Neurobiology of axonal transport defects in motor neuron diseases: Opportunities for translational research?, Neurobiol. Dis. 105 (2017) 283–299.

[5] J.N. Sleigh, A.M. Rossor, A.D. Fellows, A.P. Tosolini, G. Schiavo, Axonal transport and neurological disease, Nat. Rev. Neurol. 15 (2019) 691–703.

[6] N. Salvadores, M. Sanhueza, P. Manque, F.A. Court, Axonal Degeneration during Aging and Its Functional Role in Neurodegenerative Disorders, Front. Neurosci. 11 (2017) 451.

[7] I.G. McQuarrie, S.T. Brady, R.J. Lasek, Retardation in the slow axonal transport of cytoskeletal elements during maturation and aging, Neurobiol. Aging. 10 (1989) 359–365.

[8] S. Milde, R. Adalbert, M.H. Elaman, M.P. Coleman, Axonal transport declines with age in two distinct phases separated by a period of relative stability, Neurobiol. Aging. 36 (2015) 971–981.

[9] C.E. Holt, K.C. Martin, E.M. Schuman, Local translation in neurons: visualization and function, Nat. Struct. Mol. Biol. 26 (2019) 557–566.

[10] L.F. Gumy, G.S.H. Yeo, Y.-C.L. Tung, K.H. Zivraj, D. Willis, G. Coppola, B.Y.H. Lam, J.L. Twiss, C.E. Holt, J.W. Fawcett, Transcriptome analysis of embryonic and adult sensory axons reveals changes in mRNA repertoire localization, RNA. 17 (2011) 85–98.

[11] T. Shigeoka, H. Jung, J. Jung, B. Turner-Bridger, J. Ohk, J.Q. Lin, P.S. Amieux, C.E. Holt, Dynamic Axonal Translation in Developing and Mature Visual Circuits, Cell. 166 (2016) 181–192.

[12] R.O. Costa, H. Martins, L.F. Martins, A.W. Cwetsch, M. Mele, J.R. Pedro, D. Tomé, N.L. Jeon, L. Cancedda, S.R. Jaffrey, R.D. Almeida, Synaptogenesis Stimulates a Proteasome-Mediated Ribosome Reduction in Axons, Cell Rep. 28 (2019) 864–876.e6.

[13] A.-S. Hafner, P.G. Donlin-Asp, B. Leitch, E. Herzog, E.M. Schuman, Local protein synthesis is a ubiquitous feature of neuronal pre- and postsynaptic compartments, Science. 364 (2019). https://doi.org/10.1126/science.aau3644.

[14] L.C. Kapitein, C.C. Hoogenraad, Building the Neuronal Microtubule Cytoskeleton, Neuron. 87 (2015) 492–506.

[15] S. Roy, Seeing the unseen: the hidden world of slow axonal transport, Neuroscientist. 20 (2014) 71–81.

[16] M. Mercken, I. Fischer, K.S. Kosik, R.A. Nixon, Three distinct axonal transport rates for tau, tubulin, and other microtubule-associated proteins: evidence for dynamic interactions of tau with microtubules in vivo, J. Neurosci. 15 (1995) 8259–8267.

[17] A.R. Dörrbaum, L. Kochen, J.D. Langer, E.M. Schuman, Local and global influences on protein turnover in neurons and glia, Elife. 7 (2018) e34202.

[18] S. Maday, Mechanisms of neuronal homeostasis: Autophagy in the axon, Brain Res. 1649 (2016) 143–150.

[19] H.-C. Tai, H. Besche, A.L. Goldberg, E.M. Schuman, Characterization of the Brain 26S Proteasome and its Interacting Proteins, Front. Mol. Neurosci. 3 (2010). https://doi.org/10.3389/fnmol.2010.00012.
[20] D.W. Cleveland, M.A. Lopata, P. Sherline, M.W. Kirschner, Unpolymerized tubulin modulates the level of tubulin mRNAs, Cell. 25 (1981) 537–546.
[21] I. Gasic, S.A. Boswell, T.J. Mitchison, Tubulin mRNA stability is sensitive to change in microtubule dynamics caused by multiple physiological and toxic cues, PLoS Biol. 17 (2019) e3000225.
[22] T.J. Yen, P.S. Machlin, D.W. Cleveland, Autoregulated instability of beta-tubulin mRNAs by recognition of the nascent amino terminus of beta-tubulin, Nature. 334 (1988) 580–585.
[23] Z. Lin, I. Gasic, V. Chandrasekaran, N. Peters, S. Shao, T.J. Mitchison, R.S. Hegde, TTC5 mediates autoregulation of tubulin via mRNA degradation, Science. 367 (2020) 100–104.
[24] O. Shoshani, D.W. Cleveland, Gene expression regulated by RNA stability, Science. 367 (2020) 29–29.
[25] I.E. Vainberg, S.A. Lewis, H. Rommelaere, C. Ampe, J. Vandekerckhove, H.L. Klein, N.J. Cowan, Prefoldin, a chaperone that delivers unfolded proteins to cytosolic chaperonin, Cell. 93 (1998) 863–873.
[26] D. Gestaut, S.H. Roh, B. Ma, G. Pintilie, L.A. Joachimiak, A. Leitner, T. Walzthoeni, R. Aebersold, W. Chiu, J. Frydman, The Chaperonin TRiC/CCT Associates with Prefoldin through a Conserved Electrostatic Interface Essential for Cellular Proteostasis, Cell. 177 (2019) 751–765.e15.
[27] R. Siegert, M.R. Leroux, C. Scheufler, F.U. Hartl, I. Moarefi, Structure of the molecular chaperone prefoldin: unique interaction of multiple coiled coil tentacles with unfolded proteins, Cell. 103 (2000) 621–632.
[28] H. Sternlicht, G.W. Farr, M.L. Sternlicht, J.K. Driscoll, K. Willison, M.B. Yaffe, The t-complex polypeptide 1 complex is a chaperonin for tubulin and actin in vivo, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 9422–9426.
[29] L.A. Joachimiak, T. Walzthoeni, C.W. Liu, R. Aebersold, J. Frydman, The structural basis of substrate recognition by the eukaryotic chaperonin TRiC/CCT, Cell. 159 (2014) 1042–1055.
[30] M. Jin, W. Han, C. Liu, Y. Zang, J. Li, F. Wang, Y. Wang, Y. Cong, An ensemble of cryo-EM structures of TRiC reveal its conformational landscape and subunit specificity, Proc. Natl. Acad. Sci. U. S. A. 116 (2019) 19513–19522.
[31] J. Al-Bassam, Revisiting the tubulin cofactors and Arl2 in the regulation of soluble αβ-tubulin pools and their effect on microtubule dynamics, Mol. Biol. Cell. 28 (2017) 359–363.
[32] S. Nithianantham, S. Le, E. Seto, W. Jia, J. Leary, K.D. Corbett, J.K. Moore, J. Al-Bassam, Tubulin cofactors and Arl2 are cage-like chaperones that regulate the soluble αβ-tubulin pool for microtubule dynamics, Elife. 4 (2015). https://doi.org/10.7554/eLife.08811.
[33] A.N. Kar, S.J. Lee, J.L. Twiss, Expanding Axonal Transcriptome Brings New Functions for Axonally Synthesized Proteins in Health and Disease, Neuroscientist. 24 (2018) 111–129.
[34] G.J. Bourke, W. El Alami, S.J. Wilson, A. Yuan, A. Roobol, M.J. Carden, Slow axonal transport of the cytosolic chaperonin CCT with Hsc73 and actin in motor neurons, J. Neurosci. Res. 68 (2002) 29–35.
[35] M.M. Magiera, P. Singh, C. Janke, SnapShot: Functions of Tubulin Posttranslational Modifications, Cell. 173 (2018) 1552–1552.e1.
[36] M.M. Magiera, P. Singh, S. Gadadhar, C. Janke, Tubulin Posttranslational Modifications and Emerging Links to Human Disease, Cell. 173 (2018) 1323–1327.
[37] P.W. Baas, A.N. Rao, A.J. Matamoros, L. Leo, Stability properties of neuronal microtubules, Cytoskeleton . 73 (2016) 442–460.

[38] Y. Song, L.L. Kirkpatrick, A.B. Schilling, D.L. Helseth, N. Chabot, J.W. Keillor, G.V.W. Johnson, S.T. Brady, Transglutaminase and polyamination of tubulin: posttranslational modification for stabilizing axonal microtubules, Neuron. 78 (2013) 109–123.
[39] L. Guillaud, C. Bosc, A. Fourest-Lieuvin, E. Denarier, F. Pirollet, L. Lafanechère, D. Job, STOP proteins are responsible for the high degree of microtubule stabilization observed in neuronal cells, J. Cell Biol. 142 (1998) 167–179.
[40] T. Slaughter, M.M. Black, STOP (stable-tubule-only-polypeptide) is preferentially associated with the stable domain of axonal microtubules, J. Neurocytol. 32 (2003) 399–413.
[41] E. Tortosa, Y. Adolfs, M. Fukata, R.J. Pasterkamp, L.C. Kapitein, C.C. Hoogenraad, Dynamic Palmitoylation Targets MAP6 to the Axon to Promote Microtubule Stabilization during Neuronal Polarization, Neuron. 94 (2017) 809–825.e7.
[42] P.W. Baas, M.M. Black, Individual microtubules in the axon consist of domains that differ in both composition and stability, J. Cell Biol. 111 (1990) 495–509.
[43] C. Janke, M. Kneussel, Tubulin post-translational modifications: encoding functions on the neuronal microtubule cytoskeleton, Trends Neurosci. 33 (2010) 362–372.
[44] B. Grafstein, B.S. McEwen, M.L. Shelanski, Axonal transport of neurotubule protein, Nature. 227 (1970) 289–290.
[45] P.C. Hoffmann, R.J. Lasek, The slow component of axonal transport. Identification of major structural polypeptides of the axon and their generality among mammalian neurons, J. Cell Biol. 66 (1975) 351–366.
[46] H. Mori, Y. Komiya, M. Kurokawa, Slowly migrating axonal polypeptides. Inequalities in their rate and amount of transport between two branches of bifurcating axons, J. Cell Biol. 82 (1979) 174–184.
[47] P.W. Baas, A. Brown, Slow axonal transport: the polymer transport model, Trends Cell Biol. 7 (1997) 380–384.
[48] N. Hirokawa, S.T. Funakoshi, S. Takeda, Slow axonal transport: the subunit transport model, Trends Cell Biol. 7 (1997) 384–388.
[49] L. Wang, A. Brown, Rapid movement of microtubules in axons, Curr. Biol. 12 (2002) 1496–1501.
[50] K.E. Miller, H.C. Joshi, Tubulin transport in neurons, J. Cell Biol. (1996). http://jcb.rupress.org/content/133/6/1355.abstract.
[51] Y. He, F. Francis, K.A. Myers, W. Yu, M.M. Black, P.W. Baas, Role of cytoplasmic dynein in the axonal transport of microtubules and neurofilaments, J. Cell Biol. 168 (2005) 697–703.
[52] S. Terada, M. Kinjo, N. Hirokawa, Oligomeric tubulin in large transporting complex is transported via kinesin in squid giant axons, Cell. 103 (2000) 141–155.
[53] J.A. Galbraith, T.S. Reese, M.L. Schlief, P.E. Gallant, Slow transport of unpolymerized tubulin and polymerized neurofilament in the squid giant axon, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 11589–11594.
[54] E.L.P. Dumont, C. Do, H. Hess, Molecular wear of microtubules propelled by surface-adhered kinesins, Nat. Nanotechnol. 10 (2015) 166–169.
[55] L. Schaedel, K. John, J. Gaillard, M.V. Nachury, L. Blanchoin, M. Théry, Microtubules self-repair in response to mechanical stress, Nat. Mater. 14 (2015) 1156–1163.
[56] S. Triclin, D. Inoue, J. Gaillard, Z.M. Htet, M. De Santis, D. Portran, E. Derivery, C. Aumeier, L. Schaedel, K. John, C. Leterrier, S. Reck-Peterson, L. Blanchoin, M. Thery, Self-repair protects microtubules from their destruction by molecular motors, bioRxiv. (2018) 499020. https://doi.org/10.1101/499020.
[57] L. Schaedel, S. Triclin, D. Chrétien, A. Abrieu, C. Aumeier, J. Gaillard, L. Blanchoin, M. Théry, K. John, Lattice defects induce microtubule self-renewal, Nat. Phys. 15 (2019) 830–

838.
[58] C. Aumeier, L. Schaedel, J. Gaillard, K. John, L. Blanchoin, M. Théry, Self-repair promotes microtubule rescue, Nat. Cell Biol. 18 (2016) 1054–1064.
[59] A. Aher, D. Rai, L. Schaedel, J. Gaillard, K. John, L. Blanchoin, M. Thery, A. Akhmanova, CLASP mediates microtubule repair by promoting tubulin incorporation into damaged lattices, bioRxiv. (2019) 809251. https://doi.org/10.1101/809251.
[60] Y. Ren, J. Zhao, J. Feng, Parkin binds to alpha/beta tubulin and increases their ubiquitination and degradation, J. Neurosci. 23 (2003) 3316–3324.
[61] D. Cartelli, A. Amadeo, A.M. Calogero, F.V.M. Casagrande, C. De Gregorio, M. Gioria, N. Kuzumaki, I. Costa, J. Sassone, A. Ciammola, N. Hattori, H. Okano, S. Goldwurm, L. Roybon, G. Pezzoli, G. Cappelletti, Parkin absence accelerates microtubule aging in dopaminergic neurons, Neurobiol. Aging. 61 (2018) 66–74.
[62] M. Caplow, L. Fee, Dissociation of the tubulin dimer is extremely slow, thermodynamically very unfavorable, and reversible in the absence of an energy source, Mol. Biol. Cell. 13 (2002) 2120–2131.
[63] K.J. Verhey, N. Kaul, V. Soppina, Kinesin assembly and movement in cells, Annu. Rev. Biophys. 40 (2011) 267–288.
[64] D.L. Coy, W.O. Hancock, M. Wagenbach, J. Howard, Kinesin's tail domain is an inhibitory regulator of the motor domain, Nat. Cell Biol. 1 (1999) 288–292.
[65] D.S. Friedman, R.D. Vale, Single-molecule analysis of kinesin motility reveals regulation by the cargo-binding tail domain, Nat. Cell Biol. 1 (1999) 293–297.
[66] M.F. Stock, J. Guerrero, B. Cobb, C.T. Eggers, T.G. Huang, X. Li, D.D. Hackney, Formation of the compact confomer of kinesin requires a COOH-terminal heavy chain domain and inhibits microtubule-stimulated ATPase activity, J. Biol. Chem. 274 (1999) 14617–14623.
[67] H.Y.K. Kaan, D.D. Hackney, F. Kozielski, The structure of the kinesin-1 motor-tail complex reveals the mechanism of autoinhibition, Science. 333 (2011) 883–885.
[68] T.L. Blasius, D. Cai, G.T. Jih, C.P. Toret, K.J. Verhey, Two binding partners cooperate to activate the molecular motor Kinesin-1, J. Cell Biol. 176 (2007) 11–17.
[69] K.J. Verhey, D.L. Lizotte, T. Abramson, L. Barenboim, B. Schnapp, T.A. Rapoport, Light chain-dependent regulation of Kinesin's interaction with microtubules, J. Cell Biol. 143 (1998) 1053–1066.
[70] Y.Y. Yip, S. Pernigo, A. Sanger, M. Xu, M. Parsons, R.A. Steiner, M.P. Dodding, The light chains of kinesin-1 are autoinhibited, Proceedings of the National Academy of Sciences. 113 (2016) 201520817–201522423.
[71] M.P. Dodding, R. Mitter, A.C. Humphries, M. Way, A kinesin-1 binding motif in vaccinia virus that is widespread throughout the human genome, EMBO J. 30 (2011) 4523–4538.
[72] K.-I. Cho, H. Yi, R. Desai, A.R. Hand, A.L. Haas, P.A. Ferreira, RANBP2 is an allosteric activator of the conventional kinesin-1 motor protein, KIF5B, in a minimal cell-free system, EMBO Rep. 10 (2009) 480–486.
[73] M.-M. Fu, E.L.F. Holzbaur, JIP1 regulates the directionality of APP axonal transport by coordinating kinesin and dynein motors, J. Cell Biol. 202 (2013) 495–508.
[74] A.E. Twelvetrees, F. Lesept, E.L.F. Holzbaur, J.T. Kittler, The adaptor proteins HAP1a and GRIP1 collaborate to activate the kinesin-1 isoform KIF5C, J. Cell Sci. 132 (2019). https://doi.org/10.1242/jcs.215822.
[75] M.-M. Fu, E.L.F. Holzbaur, Integrated regulation of motor-driven organelle transport by scaffolding proteins, Trends Cell Biol. 24 (2014) 564–574.
[76] A. Kamal, G.B. Stokin, Z. Yang, C.-H. Xia, L.S.B. Goldstein, Axonal transport of amyloid precursor protein is mediated by direct binding to the kinesin light chain subunit of kinesin-

I, Neuron. 28 (2000) 449–459.
[77] S.-H. Huang, S. Duan, T. Sun, J. Wang, L. Zhao, Z. Geng, J. Yan, H.-J. Sun, Z.-Y. Chen, JIP3 mediates TrkB axonal anterograde transport and enhances BDNF signaling by directly bridging TrkB with kinesin-1, Journal of Neuroscience. 31 (2011) 10602–10614.
[78] G.G. Farías, C.M. Guardia, R. De Pace, D.J. Britt, J.S. Bonifacino, BORC/kinesin-1 ensemble drives polarized transport of lysosomes into the axon, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) E2955–E2964.
[79] A. Uchida, N.H. Alami, A. Brown, Tight functional coupling of kinesin-1A and dynein motors in the bidirectional transport of neurofilaments, Mol. Biol. Cell. 20 (2009) 4997–5006.
[80] S. Terada, M. Kinjo, M. Aihara, Y. Takei, N. Hirokawa, Kinesin-1/Hsc70-dependent mechanism of slow axonal transport and its relation to fast axonal transport, EMBO J. 29 (2010) 843–854.
[81] A.E. Twelvetrees, S. Pernigo, A. Sanger, P. Guedes-Dias, G. Schiavo, R.A. Steiner, M.P. Dodding, E.L.F. Holzbaur, The Dynamic Localization of Cytoplasmic Dynein in Neurons Is Driven by Kinesin-1, Neuron. 90 (2016) 1000–1015.
[82] R.G. Elluru, G.S. Bloom, S.T. Brady, Fast axonal transport of kinesin in the rat visual system: functionality of kinesin heavy chain isoforms, Mol. Biol. Cell. 6 (1995) 21–40.
[83] T.L. Blasius, N. Reed, B.M. Slepchenko, K.J. Verhey, Recycling of Kinesin-1 Motors by Diffusion after Transport, PLoS One. 8 (2013) e76081.
[84] L. Urnavicius, K. Zhang, A.G. Diamant, C. Motz, M.A. Schlager, M. Yu, N.A. Patel, C.V. Robinson, A.P. Carter, The structure of the dynactin complex and its interaction with dynein, Science. 347 (2015) 1441–1446.
[85] S. Chowdhury, S.A. Ketcham, T.A. Schroer, G.C. Lander, Structural organization of the dynein-dynactin complex bound to microtubules, Nat. Struct. Mol. Biol. 22 (2015) 345–347.
[86] A.P. Carter, A.G. Diamant, L. Urnavicius, How dynein and dynactin transport cargos: a structural perspective, Curr. Opin. Struct. Biol. 37 (2016) 62–70.
[87] A. Kuta, W. Deng, A. Morsi El-Kadi, G.T. Banks, M. Hafezparast, K.K. Pfister, E.M.C. Fisher, Mouse cytoplasmic dynein intermediate chains: identification of new isoforms, alternative splicing and tissue distribution of transcripts, PLoS One. 5 (2010) e11682.
[88] R. Mallik, D. Petrov, S.A. Lex, S.J. King, S.P. Gross, Building complexity: an in vitro study of cytoplasmic dynein with in vivo implications, Curr. Biol. 15 (2005) 2075–2085.
[89] S. Ayloo, J.E. Lazarus, A. Dodda, M.K. Tokito, E.M. Ostap, E.L.F. Holzbaur, Dynactin functions as both a dynamic tether and brake during dynein-driven motility, Nat. Commun. 5 (2014) 4807.
[90] J.L. Ross, K. Wallace, H. Shuman, Y.E. Goldman, E.L.F. Holzbaur, Processive bidirectional motion of dynein–dynactin complexes in vitro, Nat. Cell Biol. 8 (2006) 562–570.
[91] M. Trokter, N. Mücke, T. Surrey, Reconstitution of the human cytoplasmic dynein complex, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 20895–20900.
[92] R.J. McKenney, W. Huynh, M.E. Tanenbaum, G. Bhabha, R.D. Vale, Activation of cytoplasmic dynein motility by dynactin-cargo adapter complexes, Science. 345 (2014) 337–341.
[93] M.A. Schlager, H.T. Hoang, L. Urnavicius, S.L. Bullock, A.P. Carter, In vitro reconstitution of a highly processive recombinant human dynein complex, EMBO J. 33 (2014) 1855–1868.
[94] P.W. Grabham, G.E. Seale, M. Bennecib, D.J. Goldberg, R.B. Vallee, Cytoplasmic dynein and LIS1 are required for microtubule advance during growth cone remodeling and fast axonal outgrowth, Journal of Neuroscience. 27 (2007) 5823–5834.
[95] K.A. Myers, I. Tint, C.V. Nadar, Y. He, M.M. Black, P.W. Baas, Antagonistic forces generated by cytoplasmic dynein and myosin-II during growth cone turning and axonal retraction,

Traffic. 7 (2006) 1333–1351.
[96] D.H. Roossien, P. Lamoureux, K.E. Miller, Cytoplasmic dynein pushes the cytoskeletal meshwork forward during axonal elongation, J. Cell Sci. 127 (2014) 3593–3602.
[97] H.M. Heerssen, M.F. Pazyra, R.A. Segal, Dynein motors transport activated Trks to promote survival of target-dependent neurons, Nat. Neurosci. 7 (2004) 596–604.
[98] H. Yano, F.S. Lee, H. Kong, J.-Z. Chuang, J. Arevalo, P. Perez, C. Sung, M.V. Chao, Association of Trk neurotrophin receptors with components of the cytoplasmic dynein motor, Journal of Neuroscience. 21 (2001) RC125.
[99] S. Maday, K.E. Wallace, E.L.F. Holzbaur, Autophagosomes initiate distally and mature during transport toward the cell soma in primary neurons, J. Cell Biol. 196 (2012) 407–417.
[100] J.F. Dillman, L.P. Dabney, K. Pfister, Cytoplasmic dynein is associated with slow axonal transport, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 141–144.
[101] J.F. Dillman, L.P. Dabney, S.B. Karki, B.M. Paschal, E.L.F. Holzbaur, K. Pfister, Functional analysis of dynactin and cytoplasmic dynein in slow axonal transport, Journal of Neuroscience. 16 (1996) 6742–6752.
[102] J. Zhang, A.E. Twelvetrees, J.E. Lazarus, K.R. Blasier, X. Yao, N.A. Inamdar, E.L.F. Holzbaur, K. Pfister, X. Xiang, Establishing a novel knock-in mouse line for studying neuronal cytoplasmic dynein under normal and pathologic conditions, Cytoskeleton. 70 (2013) 215–227.
[103] A.N. Rao, A. Patil, M.M. Black, E.M. Craig, K.A. Myers, H.T. Yeung, P.W. Baas, Cytoplasmic Dynein Transports Axonal Microtubules in a Polarity-Sorting Manner, Cell Rep. 19 (2017) 2210–2219.
[104] C.S. Thom, E.A. Traxler, E. Khandros, J.M. Nickas, O.Y. Zhou, J.E. Lazarus, A.P.G. Silva, D. Prabhu, Y. Yao, C. Aribeana, S.Y. Fuchs, J.P. Mackay, E.L.F. Holzbaur, M.J. Weiss, Trim58 degrades Dynein and regulates terminal erythropoiesis, Dev. Cell. 30 (2014) 688–700.
[105] D. Sehnal, A.S. Rose, J. Koča, S.K. Burley, Mol* towards a common library and tools for web molecular graphics, On Molecular Graphics …. (2018). https://doi.org/10.2312/molva.20181103),.
[106] K. Zhang, H.E. Foster, A. Rondelet, S.E. Lacey, N. Bahi-Buisson, A.W. Bird, A.P. Carter, Cryo-EM Reveals How Human Cytoplasmic Dynein Is Auto-inhibited and Activated, Cell. 169 (2017) 1303–1314.e18.
[107] T. Torisawa, M. Ichikawa, A. Furuta, K. Saito, K. Oiwa, H. Kojima, Y.Y. Toyoshima, K. Furuta, Autoinhibition and cooperative activation mechanisms of cytoplasmic dynein, Nat. Cell Biol. 16 (2014) 1118–1124.

Table 1: Evidence of local translation, during development and in adults, for key long distance microtubule transport machinery.

Translatome data summarised from [11] and transcriptome data from [10]. To avoid having to account for the sensitivity of compartment specific preparations, only studies that directly compare developmental and adult tissue are included. Where no entry is recorded, subunits were not seen in either development or adulthood.

	Axonal translatome		Axonal transcriptome	
Gene	**development**	**adult**	**development**	**adult**
Tubulin chaperones and cofactors				
Prefoldin (PFDN1-6)			some	no
TRiC (CCT1-8)	yes	no	yes	yes
Tubulin Cofactors (TBCA-E, ARL2)			yes	no
Tubulins				
α-tubulin (TUBA1A)	yes	no	yes	yes
α-tubulin (TUBA1B)	yes	no	yes	yes
β-tubulin (TUBB2A)	yes	yes	yes	yes
β-tubulin (TUBB2B)	yes	no	yes	yes
β-tubulin (TUBB3)	yes	yes	yes	no
β-tubulin (TUBB4A)	yes	no		
β-tubulin (TUBB4B)	yes	no		
Processive kinesins				
Kinesin-3 (KIF1A)	yes	no	yes	yes
Kinesin-3 (KIF1B)			yes	no
Kinesin-3 (KIF16B)			no	yes
Kinesin-1 (KIF5A)	yes	no	yes	no
Kinesin-1 (KIF5B)	yes	no	yes	no
Kinesin-1 (KIF5C)	yes	no	yes	no
Kinesin-1 (KLC1)			yes	yes
Cytoplasmic dynein subunits				
DHC (DYNC1H1)	yes	no	yes	no
DIC1 (DYNC1I1)	yes	no	yes	no
DIC2 (DYNC1I2)			yes	yes
DLIC1 (DYNC1LI1)			yes	yes
DLIC2 (DYNC1LI2)			yes	yes
Tctex (DYNLT1)			yes	yes
Tctex (DYNLT3)			yes	yes
LC8 (DYNLL1)	yes	no	yes	yes
LC8 (DYNLL2)	no	yes	yes	yes
Roadblock (DYNLRB1)	yes	no	yes	yes

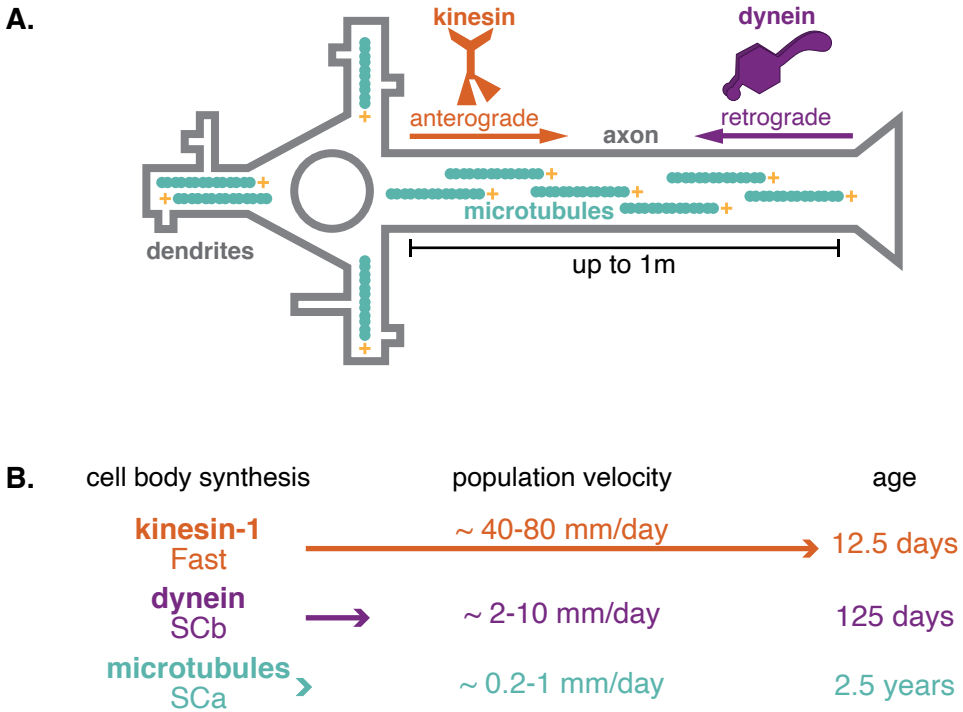

Figure 1: Axonal transport of the key elements of the microtubule transport machinery

A. The organisation of the cytoskeleton in adult axons. Microtubules in the axon have uniform polarity with 'plus' ends (yellow) facing away from the cell body. This means kinesin always walks away from the cell body to power anterograde transport. Conversely, when dynein hydrolyses ATP to walk along microtubules, it moves towards the cell body to power retrograde transport.

B. Summary of the velocity and age for key components of the microtubule transport machinery after synthesis in the cell body. Arrows represent the proportional distance travelled in the same unit time. Kinesin-1 moves at Fast transport speeds and the youngest kinesin in a distal one metre axon would be 12.5 days old. Dynein moves in slow component b (SCb) and the youngest proteins would be over 100 days old. Tubulin and microtubules move in slow component a (SCa) and a conservative estimate of protein age would be at least 2.5 years old in the distal end of a one metre axon.

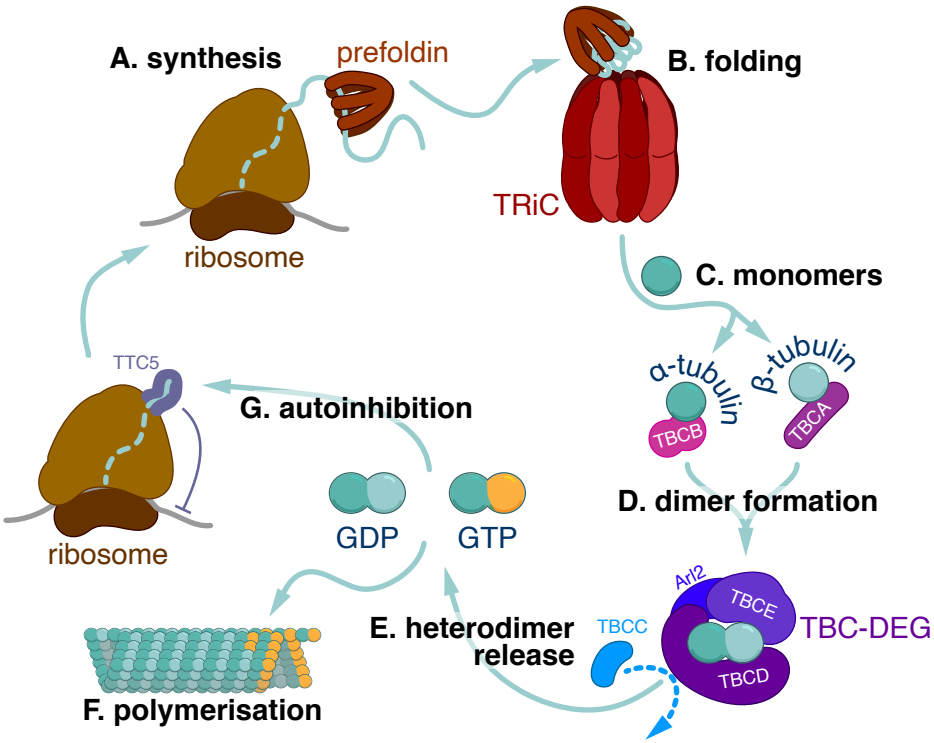

Figure 2: Tubulin biogenesis and microtubule formation

Unfolded tubulin monomers are synthesised (**A**) and transferred to the TRiC folding complex by prefoldin (**B**). Folded α- and β-tubulin monomers are complexed with the tubulin folding cofactors TBCB and TBCA respectively (**C**), which deliver monomers to the TBC-DEG complex (TBCD, TBCE and Arl2) to allow heterodimer formation as tubulin monomers form a heterodimer that cannot associate (or dissociate) in the absence of cofactors (**D**). Heterodimers are released by the action of TBCC (**E**). The GTP bound to β tubulin (yellow) is exchangeable and hydrolysable. Heterodimers are either in the cytosolic pool or polymerised into microtubules (**F**). Tubulin synthesis is autoinhibited by unpolymerised tubulin, in part through the recognition of the nascent tubulin peptide by TTC5 (**G**).

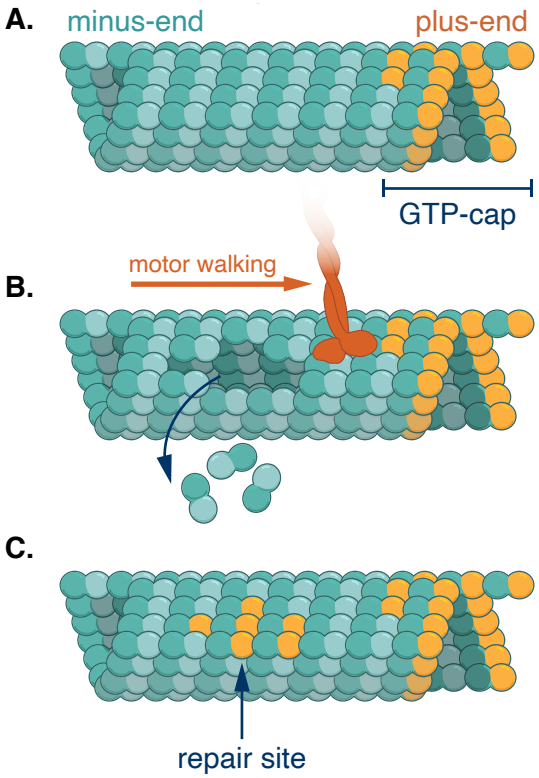

Figure 3: Microtubule polarity, damage and repair

A. As GTP bound heterodimers are incorporated into the growing microtubule, the GTP is hydrolysed to GDP. This creates a 'GTP-cap' of the most recently added hetero-dimers at the plus end of the microtubule.

B. Microtubules experience mechanical force during their life in the axon, particularly from the passage of motor proteins. This can cause damage to the microtubule lattice through the loss of tubulin heterodimers.

C. Damaged microtubules can be repaired, incorporating GTP-tubulin as they do so. These GTP islands can act as rescue sites during normal microtubule dynamics, promoting microtubule growth.

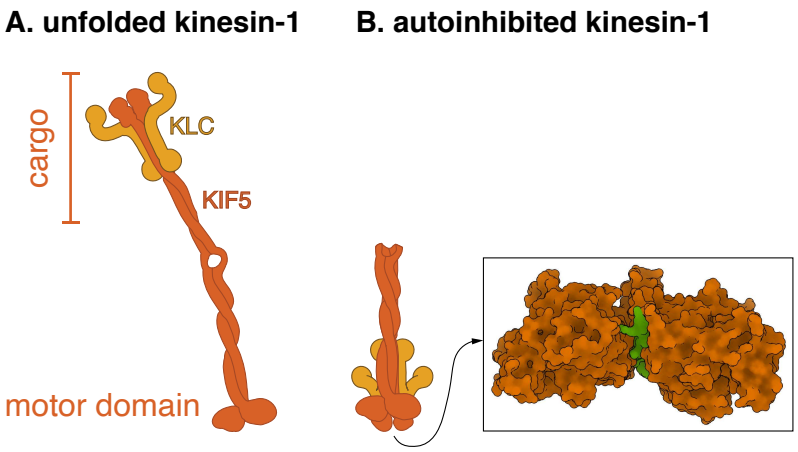

Figure 4: Kinesin-1 structure and autoinhibition

A. Kinesin-1 is a heterotetramer formed of two heavy chains (KIF5A–KIF5C) and two light chains (KLC1–KLC4). The heavy chains have an N-terminal motor head domain, extended coiled-coil regions and globular C-terminal tail. A pair of light chains bind at the C-terminus. Cargo can bind to the C-terminus of the heavy chains and to the light chains and is necessary to relieve autoinhibition.

B. Both the heavy chains and the light chains of kinesin are autoinhibited when not bound to cargo and autoinhibited kinesin exists in a folded conformation. Given the fast travel time of kinesin in the axon, autoinhibition is likely a relatively rare event. Inset shows the autoinhibition of the motor domains (orange) by a single QIAK motif from the C-terminal tail of the heavy chain (green), which locks both motor domains in place (Motor domain structure image of 2Y65 [67], created with Mol* [105]).

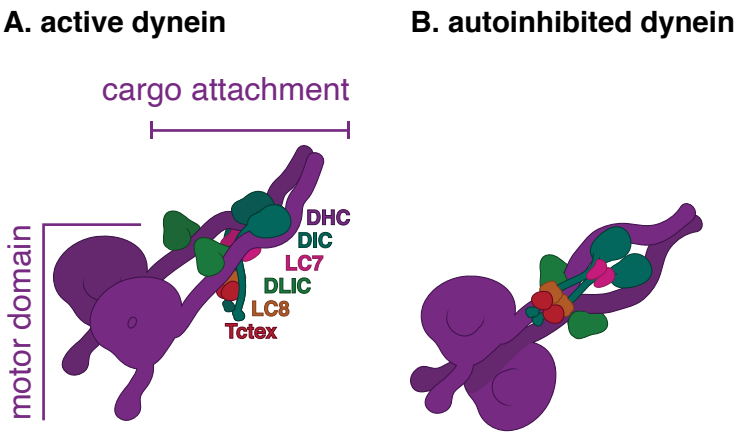

Figure 5: Cytoplasmic dynein structure and autoinhibition

A. Cytoplasmic dynein is a complex multisubunit machine. Two heavy chains (DHC) bind to microtubules and hydrolyse ATP through the C-terminal motor domains. The first N-terminal third of DHC forms the 'tail', and dimerises the motor with the help of other subunits; a pair each of dynein intermediate chains (DICs), dynein light intermediate chains (DLICs) and the light chains LC7, LC8 and TcTex [84,85].

B. Dynein has an autoinhibited conformation termed the phi-particle, which is released by cargo binding [106,107]. In addition, the crossed stalks of the motor domain lock the motors into a conformation with a weak affinity for microtubules.