

Regulation of Axonal Transport by Protein Kinases

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Abstract

The intracellular transport of organelles, proteins, lipids and RNA along the axon is essential for neuronal function and survival. This process, called axonal transport, is mediated by two classes of ATP-dependent motors, kinesins and cytoplasmic dynein, which carry their cargoes along microtubule tracks. Protein kinases regulate axonal transport through direct phosphorylation of motors, adapter proteins and cargoes, and indirectly, through modification of the microtubule network. The misregulation of axonal transport by protein kinases has been implicated in the pathogenesis of several nervous system disorders. Here, we review the role of protein kinases acting directly on axonal transport and discuss how their deregulation affects neuronal function, paving the way for the exploitation of these enzymes as novel drug targets.

Axonal transport in neuronal function and survival

Neurons are highly polarized cells, with axons that can reach over a meter away from the cell body in large mammals. This unique morphology makes neurons particularly dependent on active intracellular transport along the axon. Axonal transport is essential for the delivery of newly synthesized proteins, lipids and RNA, as well as organelles, to growth cones and synapses. It is also required for the removal of damaged cellular components and organelles for degradation and recycling. Furthermore, axonal transport has been shown to play a key role in long-distance communication between the distal region of the axon and the cell body, including neurotrophic and injury response signaling [1-3] and the regulation of axonal length [4]. Congruent with the crucial role of axonal transport in neuronal homeostasis, disruptions in axonal transport have been linked to neuronal dysfunction and degeneration [5, 6].

The complex spatial and temporal regulation of axonal transport is essential for controlled delivery of selected **cargoes** (see **Glossary**) to specific neuronal domains. Whilst a complete understanding of axonal transport

1 regulation is still lacking, it has been known for some time that
2 phosphorylation plays an important role in this process [7, 8]. However,
3 deciphering which **protein kinases** are responsible for axonal transport
4 modulation has been difficult, as kinase cascades display considerable
5 crosstalk and play multiple roles in neuronal homeostasis [9-12]. Recently,
6 significant progress has been made in dissecting out the role of individual
7 kinases in this process and identifying their molecular targets.
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14 Protein kinases are known to regulate axonal transport through direct
15 phosphorylation of motors, adapter proteins and cargoes, and indirectly,
16 through modification of the **microtubule** network. The diversity of protein
17 kinases implicated in axonal transport regulation most likely underlies the
18 complexity of this process and explains why transport characteristics are
19 distinct between different types of neurons (for example, axonal transport is
20 slower in sensory versus motor neurons) [13] and cargoes (for example,
21 mitochondria move bidirectionally with frequent pauses, whilst signaling
22 endosomes undergo sustained retrograde transport) [13, 14].
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33 Here, we review the role of protein kinases that have been implicated in the
34 direct regulation of axonal transport and discuss how kinase deregulation can
35 affect neuronal function.
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40 **The axonal transport machinery**

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43 Axonal transport can be classified based on the speed at which cargoes
44 move. Vesicles, organelles and RNA are transported by fast axonal transport
45 at a speed of 50-200 mm per day (0.5-3 $\mu\text{m/s}$), whilst cytoskeletal
46 components (such as **neurofilaments** and tubulin) and some soluble proteins
47 are transported by slow axonal transport and move at rates of 0.1–3 mm per
48 day [6].
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56 Both types of transport are mediated by two classes of ATP-dependent
57 motors - kinesin and cytoplasmic dynein - which carry their cargoes along
58 microtubule tracks running the length of the axon [15]. Kinesin and dynein are
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1 assisted by adapter proteins, which link the motors to their cargoes and
2 regulate the activity of the motors. Microtubules impart an intrinsic polarity to
3 neurons, with their “plus” ends located at the axon tips and their “minus” ends
4 within the cell body (**Key Figure**). This polarity confers directionality to axonal
5 transport.
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8 9 10 *Anterograde axonal transport*

11 Anterograde axonal transport refers to the movement of cargoes from the cell
12 body towards the axon tips and is mediated by kinesin motor proteins.
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18 Kinesin-1 motors are responsible for the fast anterograde axonal transport of
19 most vesicles, organelles, proteins and RNA particles and the slow axonal
20 transport of cytoskeletal proteins. Kinesin-1 family members are
21 heterotetramers composed of two heavy chains (KIF5A-C) and two light
22 chains (KLC1/2). KIFs comprise the motor domains and contain both ATP and
23 microtubule binding motifs. KLCs interact with the tails of the KIFs and are
24 thought to regulate cargo binding and motor activity [16] (see **Box 1**).
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33 Kinesin-2 and kinesin-3 motors are also important for axonal transport.
34 Members of the kinesin-2 family (KIF3A/KIF3B/KAP3) mediate the transport of
35 late endosome-lysosomes [17, 18], whilst kinesin-3 motors (KIF1A and
36 KIF1B β) carry synaptic vesicle precursors and dense core vesicles [19, 20].
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40 41 42 *Retrograde axonal transport*

43 Retrograde axonal transport refers to the movement of cargoes from the axon
44 tips to the cell body and is mediated by cytoplasmic dynein. Dynein is
45 composed of two heavy chains and additional intermediate, light intermediate
46 and light chains [15]. The heavy chain contains ATP and microtubule-binding
47 motifs at its carboxy terminus, whilst the amino terminus interacts with the
48 additional chains to form the cargo-binding domain. Dynein is found in a
49 complex with dynactin [16], a multi-subunit cofactor that is essential for its
50 function (see **Box 1**), and other adapter proteins, such as lissencephaly-1
51 (Lis1) and huntingtin (htt) [6].
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Kinases implicated in the regulation of axonal transport

Protein kinases regulate axonal transport through direct phosphorylation of motors, adapters and cargoes (summarized in **Table 1**). Importantly, protein kinases also phosphorylate several factors involved in the regulation of microtubule stability, as discussed in detail elsewhere [21].

Glycogen synthase kinase-3 beta (GSK3 β)

GSK3 β was originally identified as a regulator of glycogen metabolism [22], but has since been shown to be a multifunctional kinase with numerous roles in the nervous system [9]. In both invertebrate and rodent models, GSK3 β acts as a negative regulator of anterograde and retrograde axonal transport (**Figure 1** and **2**) [23-28]. In *Drosophila*, overexpression of GSK3 inhibits the axonal transport of mitochondria [24, 25], amyloid precursor protein (APP)-containing vesicles [27] and lipid droplets in larval segmental nerves [27]. There are conflicting views over whether GSK3 also inhibits the axonal transport of synaptic vesicles in these nerves [25, 27]. Stimulation of GSK3 β by pathological proteins, such as presenilin 1 and amyloid beta (A β) (see **Relevance to CNS function and disease** section) also inhibits bidirectional axonal transport in mammalian neurons [29, 30] (**Table 1**).

Pharmacological inhibition of GSK3 β in hippocampal and sensory (DRG) neurons has been shown to stimulate the axonal transport of BDNF-containing secretory vesicles, mitochondria and acidic organelles [24, 28]. Similar results are also seen in *Drosophila* larval segmental nerves carrying loss-of-function mutations in GSK3 [27]. However, in **squid axoplasm**, inhibition of GSK3 β did not alter the axonal transport of membrane-bound organelles [26], indicating that this regulatory mechanism is not conserved between all species.

GSK3 β regulates axonal transport through direct phosphorylation of motors and their adapters. In squid axoplasm, GSK3 β was shown to phosphorylate KLC2, stimulating its release from cargoes, without influencing the microtubule binding or ATPase activity of the motor [26]. However, optical trap

1 experiments in *Drosophila* indicate that GSK3 β may instead act to reduce the
2 number of active kinesins working at any one time, rather than the total
3 number of motors bound to the cargo [27]. These contrasting results may
4 represent differences in the regulation of axonal transport between species.
5 GSK3 β directly phosphorylates dynein intermediate chain 1B (DIC1B) and 2C
6 (DIC2C), leading to inhibition of the retrograde axonal transport of acidic
7 organelles [28]. GSK3 β -mediated phosphorylation of DIC1B was shown to
8 reduce its interaction with Ndel1 and Lis1, which enhance dynein force
9 production [31].
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18 GSK3 β has also been shown to regulate axonal transport through
19 phosphorylation of motor adapter proteins. Collapsin response mediator
20 protein-2 (CRMP-2) acts as a linker between kinesin-1 and specific cargoes
21 such as tubulin heterodimers and TrkB-containing endosomes [32]. GSK3 β -
22 mediated phosphorylation of CRMP-2 disrupts its association with KLC1 and
23 is proposed to perturb the anterograde axonal transport of its cargoes [33]
24 (**Figure 1**). However, this effect has yet to be demonstrated in live axonal
25 transport assays.
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35 Finally, GSK3 β has been found to have differential effects on slow axonal
36 transport, depending on the type of cargo being transported. For example,
37 GSK3 β directly phosphorylates tau [34], increasing its affinity for kinesin-1
38 and thereby accelerating its anterograde axonal transport [35]. By contrast,
39 GSK3 β -mediated phosphorylation of neurofilament heavy chain (NF-H)
40 increases neurofilament bundling and reduces their anterograde axonal
41 transport [36].
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49 *Mitogen-activated protein kinases (MAPKs)*

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51 MAPKs are serine/threonine protein kinases that coordinate cellular
52 responses to diverse extracellular stimuli. In its simplest form, MAPK signaling
53 can be considered as a cascade of three tiers of protein kinases: MAPK
54 kinase kinases (ASK1, TAK1, RAF1, MLK, MEKK4), MAPK kinases (MEK1,
55 MKK4/7 and MKK3/6) and MAPKs (ERKs, JNKs and p38 MAPKs). However,
56 representing the MAPK cascade as three isolated pathways is an
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oversimplification; the MAPK signaling network functionally interacts with many other pathways via shared scaffolding proteins, which target them to specific cellular locations [37]. In addition, there are several isoforms of each MAPK, each with distinct functions [37].

ERK1/2. Extracellular-signal-regulated kinases 1 and 2 (ERK1/2, also known as p44/42 MAPK) are activated in response to mitogens and growth factors, including neurotrophins [38], and have been shown to play a key role in neuronal differentiation, plasticity and survival [39, 40]. ERK1/2 directly phosphorylate both kinesin-1 (**Figure 1**) and dynein (**Figure 2**) and thereby regulate their association with cargoes [41, 42]. In rodent cortical neurons, ERK1/2-mediated phosphorylation of DIC1B and DIC2C increases the association of dynein with TrkB/Rab7-positive signaling endosomes, leading to an increase in the number of signaling endosomes moving in the retrograde direction (**Figure 2**) [42]. Interestingly, phosphorylation of DIC1B and DIC2C by ERK1/2 does not alter the ability of dynein to bind to mitochondria in these neurons, suggesting that mitochondria utilize a different mechanism to recruit dynein [42]. By contrast, ERK1/2-induced phosphorylation of KLC1 at Ser460 prevents binding of the motor to APP-labeled vesicles [41, 43], leading to a decrease in their anterograde transport frequency (**Figure 1**) [41]. ERK1/2-mediated phosphorylation of KLC1, however, has no effect on the axonal transport of other cargoes, such as CRMP2 or the neurotrophin receptor scaffolding protein Kidins220/ARMS [41]. This suggests that ERK1/2 only mediate the fast retrograde axonal transport of specific cargoes.

Interestingly, activated ERK1/2 are also cargoes of signaling endosomes and are transported to the cell body where they induce changes in neurotrophin-dependent gene expression [44]. This raises the question of how the transport machinery is protected from spurious modification by transported kinases. Although the exact mechanism is currently unknown, it could be speculated that the interaction of ERK1/2 with vimentin, which prevents dephosphorylation and deactivation of the kinases [45], could also preclude ERK1/2 from phosphorylating the transport machinery *en route*.

1 ERK1/2 have also been shown to facilitate the slow anterograde axonal
2 transport of neurofilaments [36]. ERK1/2 attenuate GSK3 β -mediated bundling
3 of neurofilaments, leading to an increase in their transported pool [36].
4 However, ERK1/2 activity has no effect on the slow axonal transport of tau,
5 indicating this regulatory mechanism is also cargo-specific [46].
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10 *p38 MAPKs.* p38 MAPKs are activated in response to pro-inflammatory
11 cytokines, such as tumor necrosis factor α (TNF α) and interleukin (IL)-1 β , and
12 cellular stresses, such as hypoxic, genotoxic or oxidative stress [47]. There
13 are four p38 MAPK isoforms: α , β , γ and δ . p38 MAPK α and β are considered
14 to be the major isoforms expressed in the central nervous system [48].
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21 p38 MAPK is a negative regulator of both fast and slow axonal transport. In
22 squid axoplasm, p38 MAPK α directly phosphorylates KIF5C at
23 Ser175/Ser176 and thereby inhibits the fast anterograde axonal transport of
24 membrane-bound organelles (**Table 1**) [48]. In contrast, p38 MAPK β inhibits
25 both anterograde and retrograde axonal transport in this system [48]. p38
26 MAPK-mediated inhibition of fast axonal transport has also been described for
27 mitochondria in rodent hippocampal neurons [48, 49] and synaptic vesicles in
28 *Drosophila* segmental nerves [50] (**Figure 1 and 2**), suggesting that this
29 regulatory mechanism is conserved between species. However, the targets of
30 this kinase in the mammalian transport machinery are presently unknown.
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41 Activation of p38 MAPK has also been shown to inhibit the slow anterograde
42 axonal transport of neurofilaments [51]. p38 MAPK α phosphorylates the tail
43 domains of neurofilament medium (NF-M) and NF-H chains and regulates
44 their attachment to motor proteins [52]. In rat cortical neurons, increased
45 phosphorylation of NF-M and NF-H slows their axonal transport and induces
46 bundling, leading to large accumulations of neurofilaments in the cell body
47 and proximal axons [53].
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56 *JNKs.* The c-Jun N-terminal kinases (JNKs) are encoded by three genes:
57 JNK1 and 2 are expressed ubiquitously, while JNK3 is only expressed in
58 neurons [11]. Similarly to p38 MAPK, JNKs are activated by cellular stress
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1 and pro-inflammatory cytokines [54]. In addition to their physiological roles in
2 neuronal development and synaptic plasticity, over-activation of JNKs has
3 been implicated in several neurological disorders [11].
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7 JNKs regulate axonal transport via direct phosphorylation of motors, adapter
8 proteins and cargoes. In squid axoplasm, JNK3 inhibits fast anterograde
9 axonal transport via direct phosphorylation of KIF5C at Ser176, reducing
10 kinesin-1 function and its ability to bind to microtubules [55, 56]. Activation of
11 JNK also inhibits the axonal transport of synaptic vesicles and mitochondria in
12 rodent hippocampal neurons (**Figure 1 and 2**) [57]. In these studies, JNK was
13 found to directly phosphorylate KIF5B and induce its dissociation from
14 microtubules [57]. The mechanism responsible for JNK-mediated inhibition of
15 retrograde axonal transport remains unclear (**Figure 2**).
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25 JNK also regulates fast axonal transport via phosphorylation of JNK
26 interacting protein 1 (JIP1). JIPs not only act as signaling platforms for JNKs
27 [58], but also serve as adapter proteins for both dynein and kinesin [59, 60]
28 (**Figure 1 and 2**). In rodent neurons, phosphorylation of JIP1 at Ser421
29 stabilizes its interaction with kinesin-1 and promotes the anterograde axonal
30 transport of APP, whilst expression of a non-phosphorylatable JIP1 mutant
31 favors retrograde axonal transport [60]. Thus, JNK-mediated phosphorylation
32 of JIP1 could act as a molecular switch between retrograde and anterograde
33 axonal transport [60].
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43 Similarly to ERK1/2, JNK is also a signaling endosome cargo and its
44 retrograde axonal transport is essential for the response to axonal injury [61].
45 Given the inhibitory effect of JNK on retrograde axonal transport, there must
46 be additional regulatory mechanisms in place to allow its processive transport
47 along the axon. It has been suggested that JIP-1 modulates JNK signaling
48 during transport via association with protein phosphatases [62]. For example,
49 JIP1 has been shown to bind the dual-specificity phosphatase MAPK
50 phosphatase 7 (MKP7), which dephosphorylates and inactivates JNK [63].
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Finally, JNK1 has been shown to negatively regulate slow anterograde axonal transport. In chick sensory neurons, JNK1 was found to directly phosphorylate neurofilaments, leading to inhibition of their slow axonal transport [64].

Cyclin Dependent Kinase 5 (Cdk5)

Cdk5 was first discovered based on its sequence homology to other cyclin-dependent kinases such as Cdc2 and Cdk2 [65, 66]. However, unlike other cyclin-dependent kinases, Cdk5 does not play a direct role in cell cycle control. Instead, Cdk5 regulates several processes in the nervous system, including neurite outgrowth, synaptic activity and neuronal migration [12], and its deregulation has been linked to neurodegeneration [67, 68].

The role of Cdk5 in the regulation of axonal transport is controversial, with dispute over whether the kinase regulates axonal transport under normal conditions or only in response to cellular stress. In squid axoplasm and *C. elegans* motor neurons, inhibition of Cdk5 was reported to disrupt the anterograde axonal transport of membrane-bound organelles [69, 70] and the retrograde axonal transport of synaptic vesicles [71]. Similarly, expression of kinase-dead Cdk5 led to inhibition of both anterograde and retrograde axonal transport of acidic organelles in rodent DRGs [72]. Experiments in squid axoplasm suggest that Cdk5 regulates axonal transport indirectly via inhibition of GSK3 β (see GSK3 β section) [70]. Cdk5 has been shown to activate lemur tyrosine kinase-2 (LMTK2), which in turn phosphorylates and activates protein phosphatase 1 (PP1), thus preventing GSK3 β activation [70, 73, 74]. Cdk5 has also been described to regulate fast retrograde axonal transport through phosphorylation of the dynein adapter protein Ndel1. In rodent DRGs, phosphorylation of Ndel1 by Cdk5 increases its association with Lis1, leading to enhanced retrograde axonal transport of acidic organelles (**Figure 2**) [72].

However, a more recent study performed in rodent DRGs has failed to reproduce these results. In this study, expression of kinase-dead Cdk5 did not affect the axonal transport of lysosomes, mitochondria or TrkB-containing endosomes [75], suggesting that Cdk5 does not play a role in the regulation of axonal transport under normal conditions. By contrast, increasing Cdk5

1 activity disrupted the axonal transport of these cargoes [75] suggesting that
2 Cdk5 modulates axonal transport only under cellular stress. Although the
3 reason for these conflicting results is unclear, it may be related to differences
4 in the protocol used for DRG isolation and maintenance [72, 75].
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9 Cdk5 has also been shown to negatively regulate the slow anterograde
10 axonal transport of neurofilaments [36, 46, 76, 77]. Phosphorylation of
11 neurofilaments by Cdk5 is proposed to disrupt their anterograde axonal
12 transport by promoting bundling [36, 78], reducing binding to kinesin [79] and
13 inhibiting the activity of ERK1/2 through phosphorylation-mediated
14 deactivation of its upstream activator MEK1 [46]. Cdk5 also disrupts the slow
15 anterograde axonal transport of tau, although the mechanism responsible
16 remains unclear [46].
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25 Crucially, the level of crosstalk between different kinases known to
26 phosphorylate neurofilament subunits remains to be addressed, together with
27 the physiological implications of the resulting combinatorial phosphorylation
28 patterns.
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34 *Akt*

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36 The serine/threonine kinase Akt (also known as protein kinase B) has been
37 shown to regulate the directionality of fast axonal transport. Activation of Akt
38 in rodent hippocampal neurons leads to the preferential anterograde axonal
39 transport of BDNF-containing secretory vesicles, APP-containing vesicles and
40 synaptic vesicles through phosphorylation of the adapter protein htt (**Figure 1**)
41 [80]. Akt-mediated phosphorylation of htt at Ser421 increases the recruitment
42 of kinesin-1 to vesicles and also increases the amount of kinesin heavy chain
43 bound to microtubules [80]. Inhibition of Akt causes an increase in the rate of
44 retrograde axonal transport of BDNF-containing vesicles (**Figure 2**) [80]. Akt
45 can also inactivate GSK3 β and thereby release the inhibitory effect of GSK3 β
46 on the fast axonal transport of mitochondria in hippocampal neurons [24, 81].
47 It will be interesting to determine whether these regulatory mechanisms are
48 conserved across species and neuronal types.
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Additional kinases implicated in the regulation of axonal transport

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There is a small body of evidence implicating other protein kinases in the regulation of axonal transport (**Key Figure**). In invertebrate models, both protein kinase A (PKA) and casein kinase 2 (CK2) inhibit fast anterograde axonal transport via direct phosphorylation of kinesin and reduction of its affinity for vesicular cargoes [19, 82]. In addition, CK2 has been shown to inhibit retrograde axonal transport via an unknown mechanism [82].

However, these findings have not been confirmed in mammalian neurons. In contrast to its effects in an invertebrate model [19], PKA has been shown to facilitate the fast axonal transport of mitochondria in rodent DRGs [83]. It is unclear whether this reflects a difference in regulatory mechanisms between species or between different cargoes (mitochondria versus vesicular cargoes).

Protein kinase C has been demonstrated to be a negative regulator of both retrograde [84] and anterograde [85] fast axonal transport, but further work is required to confirm the PKC isoform(s) responsible, their target(s) and the mechanism of action at the molecular level.

Finally, overexpression of PTEN-induced putative kinase 1 (PINK1) has been shown to inhibit the bidirectional axonal transport of mitochondria through phosphorylation of the adapter protein Miro (**Figure 1 and 2**), which targets it for degradation [86].

Relevance to CNS function and disease

The regulation of axonal transport by protein kinases is essential for neuronal function and development. This mechanism has been shown to play an essential role in regulating axon integrity during pathfinding [87], establishing neuronal polarity [88] and regulating synaptic formation and maintenance [89]. Protein kinases also play a key role in the regulation of growth factor and stress signaling [90, 91] and the supply and clearance of proteins, lipids and organelles to the distal axon and synapse [27, 49, 80]. It is therefore not

1 surprising that the deregulation of axonal transport by protein kinases has
2 been implicated in several nervous system disorders (**Table 2**).

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5 *Amyotrophic lateral sclerosis (ALS)*
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7 The early appearance of axonal transport deficits in ALS mouse models [13,
8 92] suggests that they play a key role in disease pathogenesis. p38 MAPK is
9 over-activated in spinal motor neurons of an ALS mouse model (SOD1^{G93A})
10 [48, 93] and is responsible for defects in fast anterograde axonal transport
11 induced by ALS-associated SOD1 mutants [48]. In addition, deregulation of
12 p38 MAPK and Cdk5 is linked to the hyperphosphorylation of neurofilaments
13 and subsequent disruption of their slow axonal transport observed in ALS [51,
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23 *Huntington's disease (HD)*
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25 Mutant huntingtin (polyQ-htt) stimulates JNK3-mediated phosphorylation of
26 KIF5C and induces deficits in both anterograde and retrograde axonal
27 transport in squid axoplasm [56]. Interestingly, Akt-mediated phosphorylation
28 of polyQ-htt at Ser421 can restore fast axonal transport to wild type levels
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36 *Alzheimer's disease (AD)*
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38 A β is proposed to inhibit fast axonal transport through Akt inhibition [96] and
39 activation of CK2 [82], GSK3 β [29, 96, 97] and p38 MAPK [49]. AD-
40 associated presenilin 1 mutations and hyperphosphorylated tau have also
41 been shown to induce axonal transport defects through activation of GSK3 β
42 [25, 96, 98]. Correcting fast axonal transport defects by reducing GSK3 β
43 activity was found to be beneficial in AD models [99, 100]. Deregulation of
44 Cdk5 is also linked to defects in the slow axonal transport of neurofilaments in
45 AD [77].
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54 *Spinal bulbar muscular atrophy (SBMA)*
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56 SBMA is caused by a CAG repeat expansion in the first exon of the androgen
57 receptor (polyQ-AR). Perfusion of the polyQ-AR in squid axoplasm inhibits the
58 fast axonal transport of membrane-bound organelles through stimulation of
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JNK3-mediated phosphorylation of the kinesin heavy chain [55]. However, the role of axonal transport deficits in SBMA is controversial, since alterations in axonal transport were not found *in vivo* in a mouse model of SBMA expressing endogenous levels of polyQ-AR [101].

Charcot-Marie-Tooth type 2F (CMT2F)

CMT2F-mutant HSPB1 has been shown to induce alterations in the slow anterograde axonal transport of neurofilaments [79]. Mutant HSPB1 activates Cdk5, leading to neurofilament bundling and reduced association with kinesin-1.

Over-activation of protein kinases and the subsequent disruption of axonal transport are a common occurrence in neurodegenerative diseases [102]. However, the direct demonstration that kinase-induced disruptions in axonal transport trigger disease pathogenesis is still lacking. The identification of pharmacological inhibitors of key protein kinases, such as GSK3 β , JNK3 and p38 MAPK, able to restore their activity to basal levels and normalize axonal transport, will help answer this question and determine whether protein kinases represent effective therapeutic targets for neurodegenerative diseases.

Concluding remarks and future perspectives

The complex scenario emerging from these studies highlights the crucial role played by protein kinases in the modulation of axonal transport. Motors, adapters and cargoes are clearly key targets for differential regulation by phosphorylation; something we are only just beginning to understand. Many intriguing questions still remain to be answered (**see Outstanding Questions Box**).

Given the key role that axonal transport plays in neuronal function, it is not surprising that alterations in protein kinase activity disrupt the axonal transport of selected cargoes and may lead to the severe pathological features of many nervous system disorders. Protein kinases may therefore represent key

1 therapeutic targets for several neurological diseases that currently lack
2 effective therapies. However, targeting protein kinases in neurological
3 diseases presents a number of challenges [103]. In particular, there are likely
4 to be problems with off-target effects, as protein kinases are often involved in
5 the regulation of many cellular processes and participate in multiple signaling
6 pathways. Understanding how to control the phosphorylation of specific
7 substrates will aid the design of novel kinase inhibitors with fewer off-target
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Box 1. The structure of kinesin-1 and dynein/dynactin motor complexes

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3 **Kinesin-1.** Structural studies have revealed that kinesin-1 is a rod-shaped
4 protein, with globular heads connected by a stalk to a tail domain. The
5 carboxy terminal domains of the two KIF5 subunits associate with two KLCs
6 and mediate cargo binding. The amino terminal globular heads of KIF5 form
7 the motor domains and contain ATP and microtubule binding sites. Kinesin-1
8 is able to take steps along the microtubule by alternately detaching and
9 advancing each of its two globular heads [104]. High-resolution X-ray
10 crystallography and cryo-EM microscopy of kinesin-1 in complex with tubulin
11 in both ATP-bound and nucleotide-free states have provided insights into how
12 the motor moves [104]. ATP binding to the “front” motor domain not only
13 allows high affinity binding to microtubules, but also triggers conformational
14 changes in the “rear” globular head that allows it to detach from microtubules
15 and take a step. Steps are driven by conversion of the neck linker domain
16 from a disordered state to a well-defined structure.
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30 **Cytoplasmic dynein/dynactin.** Dynein is composed of two heavy chains
31 (DHCs) and additional intermediate (DICs), light intermediate (DLICs) and
32 light chains (DLCs). The carboxy terminal region of DHC consists of a ring of
33 six putative AAA+ ATPase domains (AAA1-6). ATP binding to AAA1 lowers
34 the affinity of the microtubule-binding domain for microtubules so that the
35 DHC can detach and diffuse to its next binding site. ATP hydrolysis induces
36 remodeling of the linker domain and allows the motor to complete the step
37 [105]. The amino terminus of the DHC mediates dimerization and binding to
38 DICs, DLICs and DLCs to form the cargo-binding domain. DICs contain the
39 dynactin-binding region. Dynactin consists of eight core subunits: p150^{Glued},
40 dynamitin/p50, Arp1, Arp11, p62, p24, p25 and p27 [106]. A dimer of p150^{Glued}
41 forms a bridge between the dynein intermediate chain and microtubules that
42 is believed to increase motor processivity. Dynamitin/p50 overexpression
43 inhibits dynein-mediated axonal transport by causing the release of p150^{Glued}
44 and p24, thus disrupting the dynein-dynactin complex [107]. BICD2 is required
45 to stabilize the interaction between dynein and dynactin, allowing the
46 processive movement of the complex [108].
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Glossary

Cargo: Organelles, vesicles, RNA or proteins that are carried by molecular motor complexes along microtubules.

Microtubules: polymers of tubulin that extend throughout axons and dendrites and form the tracks along which molecular motors move. Microtubules are polarized in axons, conferring directionality to axonal transport.

Neurofilaments: a type of intermediate filament found specifically in neurons. Neurofilaments are composed of three major subunits: light (NF-L), medium (NF-M) and heavy (NF-H). They are thought to provide structural support for axons, regulate axon diameter and act as a scaffold for various axonal components.

Protein kinase: an ATP-dependent enzyme (phosphotransferase) that adds a phosphate group to its protein substrate. Phosphorylation typically causes a functional change in the substrate, such as altering its activity, cellular location or association with other proteins.

Squid axoplasm: extruded cytoplasm of the giant axons of the squid *Loligo pealeii*. Differential interference contrast (DIC) microscopy is used to observe the bidirectional movement of membrane-bound organelles in real time. Axoplasm can be perfused with recombinant proteins and small molecular inhibitors in order to determine their effects on axonal transport.

Figure legends

Figure 1. Cargo-specific regulation of anterograde axonal transport by protein kinases.

Examples of cargoes whose anterograde axonal transport is regulated by phosphorylation. Protein kinases that have been shown to regulate the anterograde transport of specific cargoes are listed. Their molecular targets are indicated in parentheses. For many of the molecular targets, the phosphorylation site(s) have been identified (see **Table 1**). Question marks indicate that contradicting results have been reported on the effect of the specific protein kinase on axonal transport.

Figure 2. Cargo-specific regulation of retrograde axonal transport by protein kinases.

Examples of cargoes whose retrograde axonal transport is regulated by phosphorylation. Protein kinases that have been shown to regulate the retrograde transport of specific cargoes are listed, together with their molecular targets, if known. For many of the molecular targets, the phosphorylation site(s) have been identified (see **Table 1**).

Table 1. Protein kinases that directly regulate axonal transport ^a

Kinase	Target	Cargo affected	Effect on AT		Type of neuron	Ref
			aAT	rAT		
GSK3 β	KLC2	Membrane-bound organelles	-	-	Squid axoplasm	[23, 26]
	DIC1B (Ser87/Thr88); DIC2C (Ser88/Thr89)	Acidic organelles	NE	-	Dorsal root ganglia	[28]
	?	Mitochondria, APP-containing vesicles, lipid droplets, synaptic vesicles (?), BDNF-containing secretory vesicles	-	-	Hippocampal neurons, <i>Drosophila</i> segmental nerves	[24, 25, 27, 29]
	Tau	Tau	+		Cortical neurons	[35]
	NF-H	Neurofilaments	-		NB2a/d1 cells	[36]
ERK1/2	DIC1B (Ser80); DIC2C (Ser81)	Signaling endosomes		+	Hippocampal neurons	[42]
	KLC1 (Ser460)	Calsyntenin-associated vesicles (APP)	-	+	Cortical neurons	[41]
	GSK3 β (Ser9)	Neurofilaments	+		NB2a/d1 cells, dorsal root ganglia	[36]
p38 MAPK	KIF5C (Ser175/176)	Membrane-bound organelles	-	NE	Squid axoplasm	[48]
	?	Mitochondria	-	-	Hippocampal neurons	[49]
	?	Synaptic vesicles	-		<i>Drosophila</i> segmental nerves	[50]
	NF-M and -H	Neurofilaments	-		Cortical neurons	[51, 53]
JNK	JIP1 (Ser421)	APP-containing vesicles	+	-	Dorsal root ganglia	[60]
	KIF5C (Ser176)	Membrane-bound organelles	-	-	Squid axoplasm	[55, 56]
	KIF5B	Mitochondria, synaptic vesicles	-	-	Hippocampal neurons	[55-57]
	?	Neurofilaments	-		NB2a/d1 cells, dorsal root ganglia	[64]
Cdk5	LMTK2/PP1	Membrane-bound organelles	+	+	Squid axoplasm, dorsal root ganglia	[70, 109]
	?	Synaptic vesicles	+		<i>C.elegans</i> motor neurons	[110]
	Ndel1	Acidic organelles	+	+	Dorsal root ganglia	[72]
	MEK	Neurofilaments	-		NB2a/d1 cells	[46]
	NF-M and -H	Neurofilaments	-		Dorsal root ganglia, SH-SY5Y	[36, 76, 77, 79]
Akt	htt (Ser421)	BDNF-containing secretory vesicles, APP-containing vesicles, synaptic vesicles	+	-	Cortical neurons	[80]
	GSK3 β (Ser9)	Mitochondria	+	+	Hippocampal neurons	[24, 81]
PKA	Kinesin	Small vesicles (not mitochondria)	-	NE	Crayfish walking leg giant axon	[19]
	?	Mitochondria	+	+	Dorsal root ganglia	[83]
CK2	KLC1 and 2	Membrane-bound organelles	-	-	Squid axoplasm	[82]
PKC	?	Membrane-bound organelles	-	-	Sympathetic and sensory neurons, squid axoplasm	[84, 85]
PINK1	Miro (Ser156)	Mitochondria	-	-	Cortical neurons	[86]

a. rAT, retrograde axonal transport. aAT, anterograde axonal transport. +, enhanced; -, inhibited; NE, no effect.

Table 2. Protein kinases implicated in the deregulation of axonal transport in neurological diseases ^a

Kinase	Target	Cargo affected	Effect on AT		Type of neuron	Disease association	Ref
			aAT	rAT			
p38 MAPK	KIF5C (Ser175/176)	Membrane-bound organelles	-	NE	Squid axoplasm	Amyotrophic lateral sclerosis	[48]
	?	Signalling endosomes (CTB)	-		Optic nerve	Glaucoma	[111]
	?	Mitochondria	-	-	Hippocampal neurons	Alzheimer's disease	[49]
	NF-M and -H	Neurofilaments	-		Cortical and motor neurons	Amyotrophic lateral sclerosis	[51, 53, 93]
Cdk5	NF-M and -H	Neurofilaments	-		SH-SY5Y, N2A	Charcot-Marie-Tooth type 2F, Alzheimer's disease	[36, 76, 77, 79]
CK2	KLC1 and 2	Membrane-bound organelles	-	-	Squid axoplasm	Alzheimer's disease	[82]
GSK3 β	?	Synaptic vesicles, mitochondria, APP-containing vesicles, BDNF-containing secretory vesicles	-	-	Hippocampal neurons, <i>Drosophila</i> segmental nerves, squid axoplasm	Alzheimer's disease	[23, 25, 27, 29, 96-98]
	KLC	Mitochondria	-	-	Cortical neurons	Krabbe disease	[23]
JNK3	KIF5C (Ser176)	Membrane-bound organelles	-	-	Squid axoplasm	Huntington's disease	[56]
	KIF5	Membrane-bound organelles	-	-	Squid axoplasm	Spinal bulbar muscular atrophy	[55]
PKC	?	Membrane-bound organelles	-	NE	Squid axoplasm	Neurotoxin-induced Parkinson's disease	[85]

a. rAT, retrograde axonal transport. aAT, anterograde axonal transport. +, enhanced; -, inhibited; NE, no effect.

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KG and GS conceived the layout of the review, KG drafted the manuscript, and KG and GS assembled the figures. All authors amended the manuscript and approved the submitted version.

Competing financial interests

The authors declare no competing financial interests.

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Outstanding Questions

- Do kinases other than ERK1/2 and GSK3 β directly phosphorylate cytoplasmic dynein? What effects does this process have on axonal transport?
- Do protein kinases phosphorylate the dynactin complex and, if so, how does this affect the activity and processivity of the dynein motor?
- Are kinesin-2 and -3 regulated by direct phosphorylation as seen for kinesin-1?
- Is the axonal transport of RNA also regulated by protein kinases? Which aspects of the regulatory network are shared between the different classes of organelles and how is specificity achieved?
- What are the molecular mechanisms underlying the deregulation of protein kinases in neurodegenerative diseases and how is axonal transport affected?
- Can we specifically target protein kinases and axonal transport as a therapeutic strategy for neurodegenerative diseases and other disorders of the nervous system?

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Figure 1
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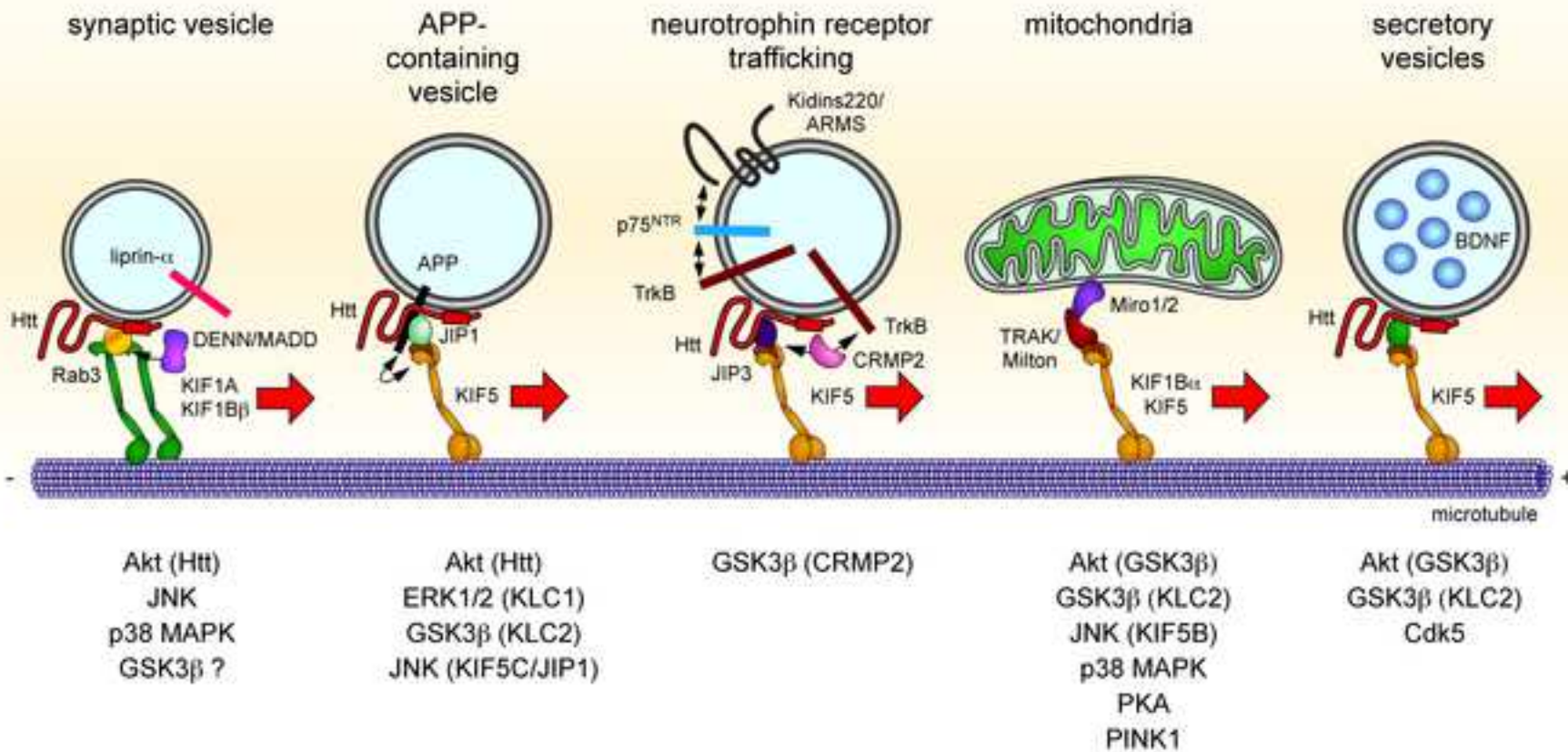
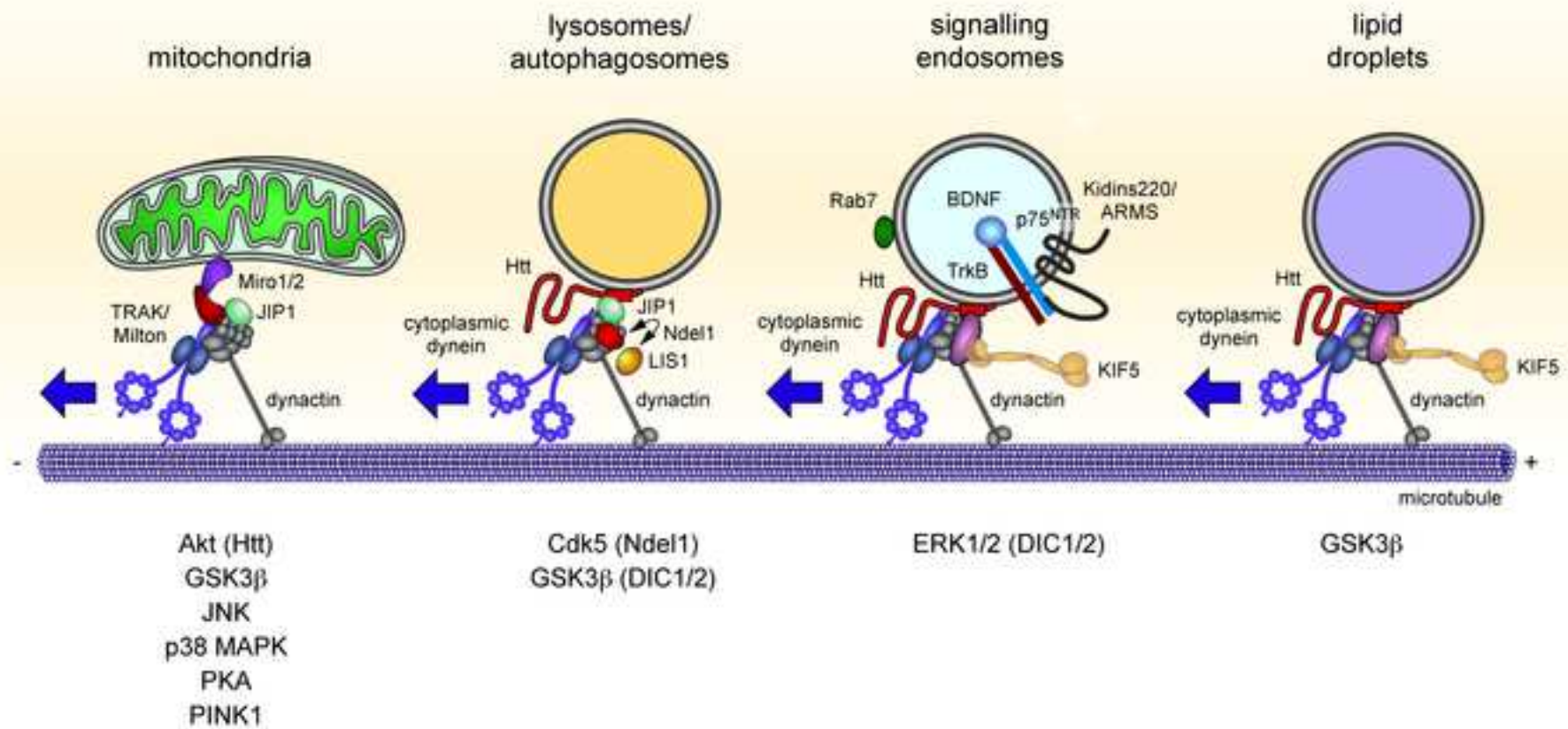
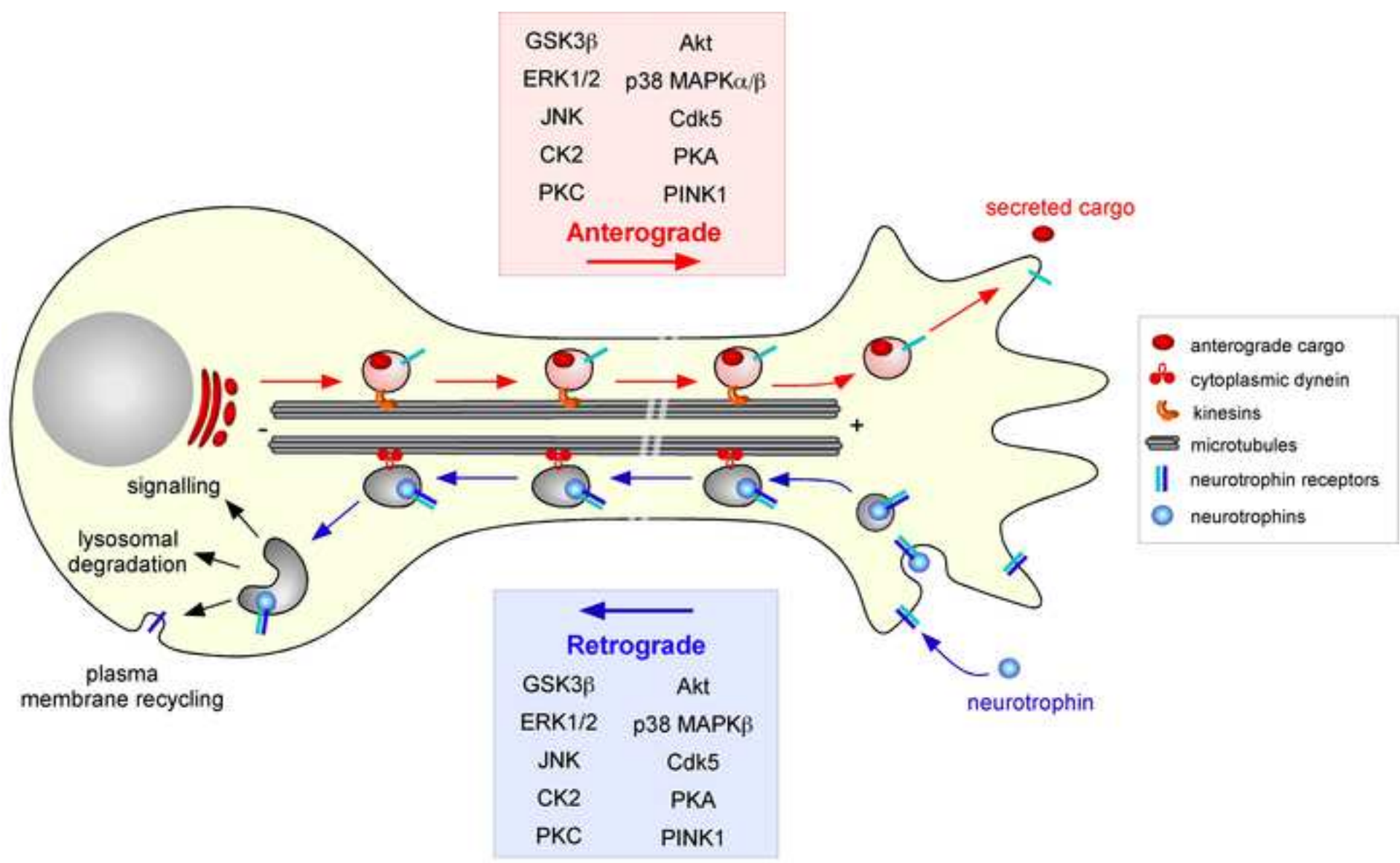


Figure 2

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← Retrograde transport





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