CYTOSKELETAL MOTORS

Kinesin superfamily motor proteins and intracellular transport

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Abstract | Intracellular transport is fundamental for cellular function, survival and morphogenesis. Kinesin superfamily proteins (also known as KIFs) are important molecular motors that directionally transport various cargos, including membranous organelles, protein complexes and mRNAs. The mechanisms by which different kinesins recognize and bind to specific cargos, as well as how kinesins unload cargo and determine the direction of transport, have now been identified. Furthermore, recent molecular genetic experiments have uncovered important and unexpected roles for kinesins in the regulation of such physiological processes as higher brain function, tumour suppression and developmental patterning. These findings open exciting new areas of kinesin research.

Most newly synthesized proteins in the cell are actively transported along directional cytoskeletal filaments to their appropriate destination by molecular motors. Proteins are transported in various membranous organelles and protein complexes, and mRNAs are carried in large protein complexes[1,2]. Directional intracellular transport is most prominent in polarized cells, such as neurons and epithelial cells, and is fundamental for neuronal function and survival because most of the proteins required in the axon and nerve terminals need to be transported from the cell body. Therefore, neurons are a good model system for studying intracellular transport[3].

Among the molecular motors that are involved in intracellular transport, three large superfamilies have been identified — kinesins, dyneins and myosins[3,4]. Kinesins use microtubules as a 'rail' to transport cargo along, and they use the chemical energy of ATP to drive conformational changes that generate motile force[2]. Dyneins use microtubules to drive minus end-directed retrograde transport and the motility of cilia and flagella. Myosins move along actin filaments to drive muscle contraction and short-range transport that typically occurs beneath the plasma membrane. Here, we focus on the role of the kinesin superfamily proteins (also known as KIFs) in the process of intracellular transport in various cell types.

Based on observations made using electron microscopy, five major kinesin families were initially discovered in the mouse brain[5,6]. It is now thought that there are 45 mammalian KIF genes, but there could be twice as many KIF proteins as multiple isoforms can be generated by alternative mRNA splicing[7]. KIFs constitute 15 kinesin families, which are termed kinesin 1 to kinesin 14B according to the results of phylogenetic analyses[1,7,8] (FIG. 1a). These families can be broadly grouped into three types, depending on the position of the motor domain in the molecule: N-kinesins have a motor domain in the amino-terminal region, M-kinesins have a motor domain in the middle and C-kinesins have a motor domain in the carboxy-terminal region. The domain structure of the major KIFs in the various kinesin families is depicted in FIG. 1b. In general, N-kinesins and C-kinesins drive microtubule plus end- and minus end-directed motilities, respectively, and M-kinesins depolymerize microtubules[1,9] (BOX 1).

This Review focuses on several important questions regarding the role of kinesins in intracellular transport. First, we discuss the specific cargos for different KIFs in various cell types, including neurons and ciliated cells, and describe how these KIFs recognize and bind to these cargos. Although kinesins typically use scaffold proteins and adaptor proteins to bind to cargo vesicles, they can sometimes bind to their cargo directly. The cargo–motor relationship has a high level of specificity, although there is redundancy in some cases (TABLE 1). We then address how cargo unloading is controlled by phosphorylation, Rab GTPase activity and Ca^{2+} signalling, and how cell polarity influences and is influenced by intracellular transport. Last, we introduce the emerging role of KIFs in the regulation of several interesting physiological processes in mammals, including higher brain function, left–right body determination and tumour suppression.

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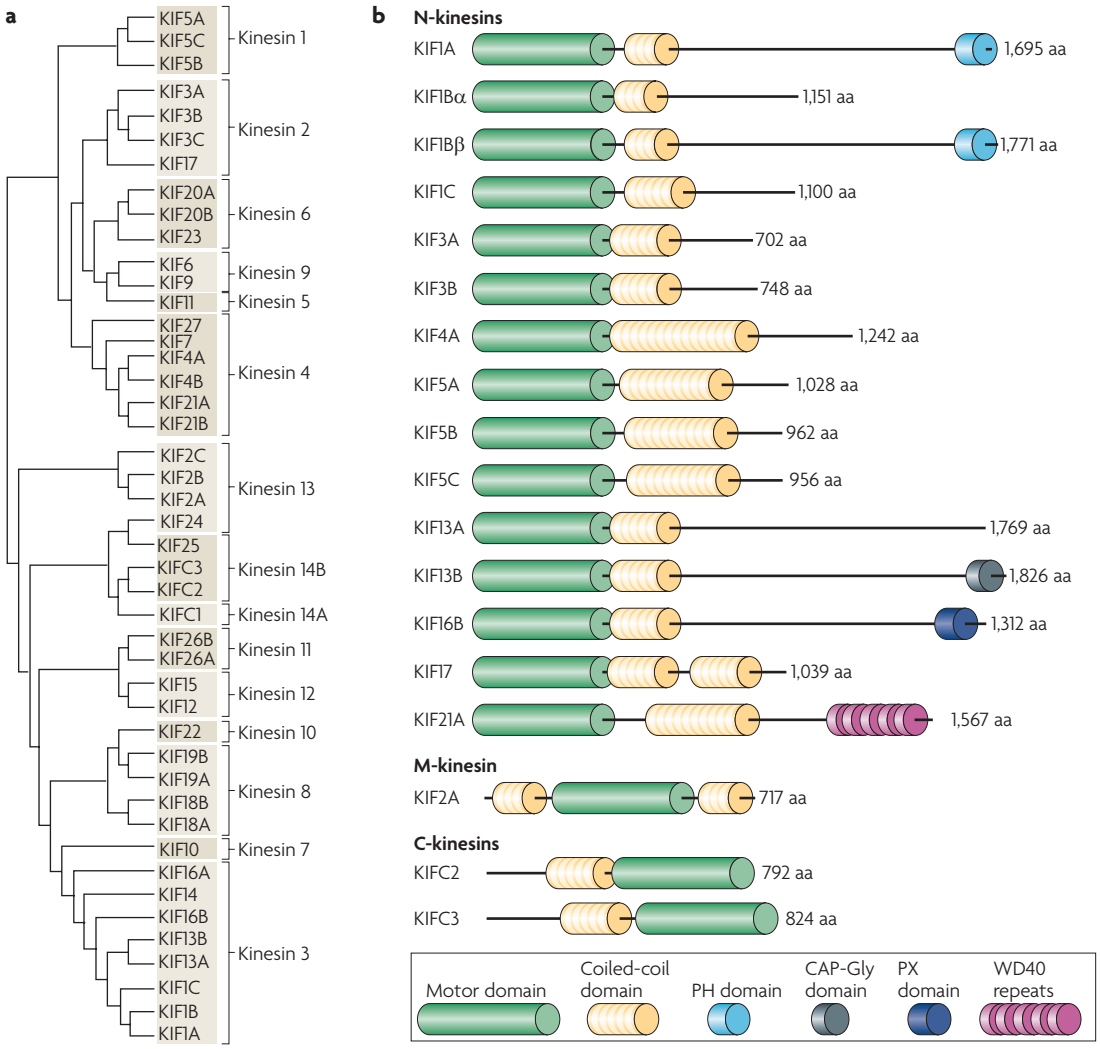


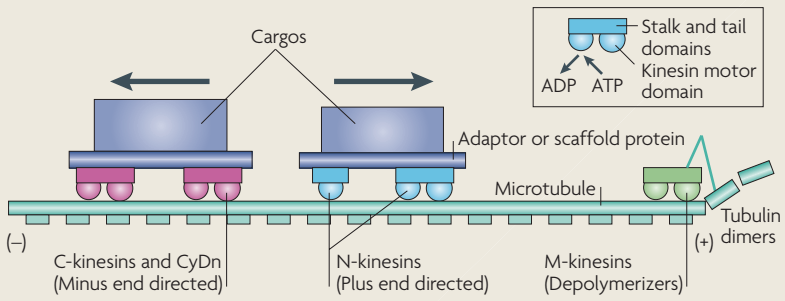
Figure 1 | **The structure and phylogeny of major mouse kinesins. a** | A phylogenetic tree of all 45 kinesin superfamily (also known as KIF) genes in the mouse genome[7], which are classified into 15 families[8,9,15,152–154]. **b** | The domain structure of the major kinesins. In general, kinesins comprise a kinesin motor domain and a coiled-coil domain. There are also gene specific domains, such as the pleckstrin homology (PH) domain of KIF1A and KIF1Bβ, the CAP-Gly domain (a conserved, Gly-rich domain of ~42 residues found in some cytoskeleton-associated proteins) of KIF13B (also known as guanylate kinase-associated kinesin (GAKIN)) and the WD40 repeats of KIF21A. The 15 families of kinesins can be broadly grouped into N-kinesins, M-kinesins and C-kinesins, which contain their motor domain at the amino terminus, in the middle or at the carboxyl terminus, respectively. N-kinesins drive microtubule plus end-directed transport, C-kinesins drive minus end-directed transport and M-kinesins depolymerize microtubules. The three types of kinesin are grouped as indicated. Only the kinesin 13 family contains M-kinesins and only the kinesin 14A and kinesin 14B families contain C-kinesins. All other families consist of N-kinesins. aa, amino acids; PX, Phox homology.

Kinesin-driven transport in axons

In the neuronal axon, various membrane organelles (including synaptic vesicle precursors and mitochondria) and protein complexes are transported bidirectionally along the microtubules to control neuronal functions such as neurite elongation and neuronal polarization. In the axon, microtubule plus ends point to the axon terminal and minus ends point to the cell body. Anterograde transport is powered mainly by N-kinesins, reflecting their microtubule plus end-directed motility. Retrograde transport is powered mainly by cytoplasmic dynein, but some C-kinesins can also help this transport, reflecting their microtubule minus end-directed motility. In some cases, the transport machinery requires an adaptor protein that directly associates with the motor and is transported with it, and that is essential for the transport and association of other components in cargo complexes or vesicles. Membrane organelles are transported by fast axonal transport at a similar speed to kinesin motors *in vitro* (50–200 mm per day), and cytoplasmic proteins, such as tubulin and neurofilament proteins, are transported by slow axonal transport (0.1–3 mm per day)[10]. Although N-kinesins are responsible for fast and slow transport, the mechanism that underlies the speed difference has not been fully elucidated.

WD40 repeat
A protein motif that is composed of a 40 amino acid repeat that forms a blade of the characteristic β-propeller structure. Proteins that contain WD40 repeats participate in G protein-mediated signal transduction, transcriptional regulation, RNA processing and regulation of vesicle formation and trafficking.

Box 1 | **General mechanism of kinesin-mediated cellular transport**



Kinesins transport cargos along microtubules by a standard mechanism (depicted here using a freight train model; see the figure). A kinesin motor generally consists of a kinesin motor domain, which is conserved among kinesin superfamily proteins (KIFs), and unique stalk and tail domains that are used for kinesin dimerization and/or kinesin binding to cargos, adaptors or scaffold proteins. The kinesin motor domain generates force by hydrolysing ATP. Kinesins are largely classified as N-kinesins, M-kinesins or C-kinesins, which contain their motor domain at the amino terminus, in the middle or at the carboxyl terminus, respectively. N-kinesins generally provide plus end-directed motility that is anterograde towards the cell periphery or axon terminals in neurons. Some N-kinesins act as monomers and others act as dimers. C-kinesins, together with cytoplasmic dynein (CyDn), provide minus end-directed motility that is generally retrograde towards the cell centre. M-kinesins depolymerize microtubules. In some cases, adaptors and scaffolds provide a mechanistic link between kinesins and cargos, and they might also have regulatory roles in kinesin-driven intracellular transport, namely in the recognition of specific cargos and the regulation of cargo loading and unloading.

Transport of synaptic proteins. At the axon terminus, neurotransmitter-containing synaptic vesicles are produced by endocytosis. These vesicles contain proteins that have been transported to the plasma membrane in synaptic vesicle precursors. Synaptic vesicles dock and fuse with the synaptic plasma membrane through the soluble *N*-ethylmaleimide-sensitive factor attachment protein (SNAP) receptor (SNARE) complex, the components of which have been transported by multiple kinesin motors. The kinesin 3 family motors KIF1A and KIF1Bβ transport synaptic vesicle precursors that contain synaptic vesicle proteins, such as synaptophysin, synaptotagmin and the small GTPase RAB3A, but they do not transport organelles that contain plasma membrane proteins, such as syntaxin 1A or SNAP25 (REFS 6,11–13) (FIG. 2a; TABLE 1). KIF1Bβ is generated by a splicing variation in the cargo-binding domain of the *KIF1B* gene. Although KIF1A and KIF1Bβ redundantly transport the same cargoes, haploinsufficiency of KIF1Bβ results in abnormal neuronal phenotypes, which suggests that the expression level of these motors is crucial for the transport of synaptic vesicle precursors towards axon termini. KIF1A and KIF1Bβ bind to RAB3 proteins through the adaptor protein mitogen-activated protein kinase (MAPK)-activating death domain (MADD; also known as DENN and hereafter referred to as DENN/MADD), which was first identified as a RAB3 guanine nucleotide exchange factor (GEF; see the section on Rab GTPases below) (FIG. 3).

The KIF5 motor complex consists of two kinesin 1 family KIF5 motors, which are also known as kinesin heavy chains (KHCs). Occasionally, two kinesin light chains (KLCs) associate with the tail domains of the two KIF5 motors. Gene duplication has led to three subtypes of KIF5 in mammals — KIF5A, KIF5B and KIF5C[6,14–17]. Whereas KIF5B is expressed ubiquitously, the expression of KIF5A and KIF5C is specific to neurons[17]. KLC1–4 are cargo adaptors that associate with approximately one-half of KIF5 dimers to produce tetramers that contain two heavy chains and two light chains[17,18]. KIF5 motors transport synaptic vesicle precursors that contain synaptotagmin and synaptobrevin[19,20], and also transport membrane organelles that contain presynaptic plasma membrane proteins, such as syntaxin 1 and SNAP25 (REFS 21,22). SNAP25 directly binds to the KIF5 motors[21]. Syntabulin directly binds to both the KIF5 motors and syntaxin 1, and probably acts as an adaptor protein[22].

Transport of mitochondria. Mitochondria are actively transported in the axon, possibly so that they become distributed along the full length of an axon. The kinesin 3 family motor KIF1Bα and the KIF5 motors are thought to be involved in mitochondrial transport. KIF1Bα is associated with mitochondria and can transport them *in vitro*[23]. Furthermore, the newly identified KIF1Bα-binding protein (KBP) is localized to mitochondria, and its ablation results in mitochondrial aggregation[24]. When the *Kif5b* gene was disrupted in mice, mitochondria accumulated in the centre of cells[25], which suggests that the KIF5 motors are essential for mitochondrial transport. This accumulation could be rescued by exogenous expression of any of the KIF5 motors[17]. The adaptor proteins syntabulin, Ran-binding protein 2 (RanBP2) and the Milton–Miro complex were independently identified as mediators of mitochondrial binding to KIF5 motors. However, the relationship between the many motors and adaptors that regulate mitochondrial distribution is poorly understood[26–29].

Transport for the elongation of neurites. Axonal elongation is an essential process in brain wiring. The kinesin 2 family motors KIF3A and KIF3B generally exist in a heterotrimeric KIF3 motor complex, consisting of KIF3A, KIF3B and KIF-associated protein 3 (KAP3; also known as KIFAP3), and they are essential for axonal elongation[6,30–35]. The armadillo repeats of KAP3 bind to the tail domains of KIF3 and to KIF3 cargos. The KIF3 motors transport fodrin-associated vesicles, which might provide plasma membrane components, to the tips of the neurites[36]. In addition, direct binding of the scaffold protein disrupted in schizophrenia 1 (DISC1) to KIF5 motors enables the axonal transport of a cargo complex that contains nudel and lisencephaly 1 (LIS1; also known as PAFAH1B1), which are essential for axonal elongation[37].

Transport for the polarization of neurons. Neurons are highly polarized cells. Microtubules also have polarity: whereas the plus ends of microtubules point towards the distal end of the axon, the direction of microtubules is mixed in proximal dendrites[38]. Recent studies suggest that the motor domain of KIF5 predominantly recognizes microtubules in axons and that kinesin-based

Synaptophysin
A glycoprotein of 38 kDa that is localized to synaptic vesicle membranes.

Synaptotagmin
One of a group of Ca^{2+}-binding proteins that are involved in the secretion of granules and vesicles, especially in the nervous system.

Haploinsufficiency
Occurs when a diploid organism has only a single functional copy of a gene that does not produce enough of a gene product to bring about a wild-type condition. This leads to an abnormal or diseased state.

Synaptobrevin
An integral synaptic vesicle protein of 18 kDa that is involved in the formation of the SNARE complex in exocytosis. Synaptobrevin is a major target of cleavage by tetanus toxin.

Armadillo repeat
A protein–protein interaction consensus stretch of 40 amino acids.

transport is involved in the specification of a single axon from multiple neurites in neuronal development.

When expressed in neurons, a recombinant KIF5 motor domain specifically localizes to axons[39]. KIF5 recruitment is thought to depend on different microtubule stabilities in the initial segment of the axon (the proximal part of the axon, next to the cell body) because treatment of cells with a low dose of paclitaxel — an inhibitor of microtubule dynamics — causes the mislocalization of the KIF5 motor domain and the missorting of axonal membrane proteins to dendrites[39]. Consistent with this observation, the KIF5 motor domain is a good axon marker in developing neurons[40], and the inhibition of microtubule dynamics in developing neurons affects neuronal polarity[41]. These studies suggest that the association of the KIF5 motor domain with specific microtubules might be an important determinant for directional transport and neuronal polarity.

The KIF3 motor transports the tumour suppressor protein adenomatous polyposis coli (APC), the cell polarity proteins PAR3 and PAR6, and atypical protein kinase C (aPKC) to the tip of nascent axons[42,43]. This transport might have a role in the determination of neuronal polarity.

The kinesin 3 family motor KIF13B (also known as guanylate kinase-associated kinesin (GAKIN)) transports the tumour suppressor protein Discs large (DLG), which is essential for the determination of cortical polarity in *Drosophila melanogaster* neuroblasts[44,45]. KIF13B also binds directly to the adaptor centaurin-α1 (REF. 46), which binds to phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3) and is a GTPase-activating protein (GAP) for the ADP ribosylation factor (ARF) family of small GTPases. KIF13B recruits centaurin-α1 to the plasma membrane at the leading edge of non-neuronal cells and regulates the activity of ARF6. In neurons, KIF13B binds to PtdIns(3,4,5)P_3 through centaurin-α1 and transports PtdIns(3,4,5)P_3-containing vesicles to the tips of axons[47]. The accumulation of PtdIns(3,4,5)P_3 at axonal tips is thought to be essential for axonal specificity by selectively activating the phosphoinositide 3-kinase pathway.

Additional roles of KIF5 motors in axonal transport. In addition to the roles of KIF5 motors in axonal transport that are discussed above, these motors can also transport other cargos in axons. KIF5 motors bind to their cargos through at least two different regions. KIF5 stalk domains can bind to KLCs, which in turn associate with certain cargos[15,18,48], or the specific cargo-binding region of the KIF5 tail domain can directly bind to cargos[49,50]. The tetratricopeptide repeat (TPR) domain of KLCs binds to cargos such as JUN N-terminal kinase (JNK)-interacting proteins (JIPs)[48,51–53]. Although JIPs function primarily as scaffolding proteins to mediate the JNK signalling cascade by directly binding to MAPK, MAPK kinase (MAPKK) and MAPK kinase kinase (MAPKKK)[54], they can also bind to KLC and connect vesicles that contain receptors for apolipoprotein E receptor 2 (APOER2; also known as LRP8) and reelin to the KIF5 complex[52]. β-Amyloid precursor protein (APP) has also been reported to bind to KLC for transport[55], although the molecular mechanism for this is controversial[56]. JIP1 (also known as MAPK8IP1) accelerates the phosphorylation of APP by JNK and assists the transport of phosphorylated forms of APP[57].

Although the KIF5 proteins are generally fast motors, they can also contribute to slow axonal transport[10,58]. In one recent study, they were reported to slowly transport tubulin dimers using the adaptor protein CRMP2 (also known as DPYSL2), which directly binds to the TPR domain of KLC1 (REF. 59). KIF5A is also thought to transport neurofilament proteins[60]. However, the factors that allow KIF5 to drive slow transport, as opposed to fast transport, remain unknown.

Kinesin-driven transport in dendrites

In dendrites, the dynamic transport of chemical transmitter receptors, such as NMDA (*N*-methyl-D-aspartate)- and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)-type glutamate receptors (NMDARs and AMPARs, respectively), and mRNAs is essential for synaptic transmission and plasticity. Much less is known about the role of kinesins in dendrites than in axons, in part owing to the fact that microtubule polarity is mixed in proximal dendrites, unlike the uniform distal polarity of plus ends in axons. However, several examples of kinesin-driven transport in dendrites have been identified.

Local protein synthesis in dendrites is thought to be essential for synapse-specific plasticity. Kinesin 1 family KIF5 motors can directly bind to and transport large RNase-sensitive granules, known as messenger ribonucleoprotein (mRNP) complexes, which contain mRNA and at least 40 kinds of proteins[61]. Fragile X mental retardation protein (FMRP; also known as FMR1) has been implicated in the connection of mRNA to the KIF5-associated KLC[62]. How, when and what mRNAs are assembled into these complexes and transported down the dendrites remain to be discovered.

The cargo-binding domain of KIF5 motors binds to the adaptor glutamate receptor-interacting protein 1 (GRIP1), which binds to the GluR2 subunit of AMPAR[63]. The dendritic transport of GluR2 by KIF5 motors is blocked by the overexpression of the scaffold protein JIP3 (also known as MAPK8IP3), which suggests that adaptor proteins are involved in the determination of cargo distribution. However, the molecular mechanisms for this are still elusive.

The kinesin 2 family motor KIF17 (also known as OSM3) is localized mainly in the cell bodies and dendrites of neurons[64,65]. LIN10 (also known as MINT1), is a direct binding partner of KIF17. KIF17 transports the NMDAR subunit NR2B through the scaffold complex LIN10–LIN2–LIN7 (REFS 65,66). It has also been suggested that KIF17 is required for the distribution of GluR5 to distal dendrites[67] and the voltage-gated potassium channel Kv4.2 to dendrites[68].

Finally, the kinesin 14B family motor KIFC2 is abundantly expressed in the adult brain[64,69,70]. It is localized in cell bodies and dendrites and is thought to transport multivesicular body-like organelles in dendrites[70].

Tetratricopeptide motif
A loosely conserved domain of 30–40 amino acids that is involved in protein–protein interactions.

Voltage-gated potassium channel
A class of transmembrane channel that senses the electrical potential across the plasma membrane to open and admit K^+ flow through the membrane.

Table 1 | **Cargo complexes transported by kinesin superfamily proteins (KIFs)**

Motor*	Light chain involvement	Cargo type	Adaptor or scaffold proteins*	Cargo molecules*	Refs
Axonal transport					
KIF1A or KIF1Bβ	None	Synaptic vesicle precursors	DENN/MADD	Synaptophysin, synaptotagmin and RAB3A	6,11–13, 115
	None	Vesicles	Unknown	PtdIns(4,5)P_2	116
KIF1Bα	None	Mitochondria	KBP	Mitochondrial proteins	23,24
KIF5 motors	None	Mitochondria	Milton, Miro, syntabulin and RanBP2	Mitochondrial proteins	26–29
	Unknown	Lysosomes	Unknown	LAMP2	25,39
	None	Synaptic vesicle precursors	UNC76	UNC51 (ATG1) and synaptotagmin	19,20, 106
	None	Synaptic membrane precursors	Syntabulin	Syntaxin 1	22
	None	Synaptic membrane precursors	None	SNAP25	21
	None	Unknown protein complex	DISC1	Nudel and LIS1	37
	KLC	Vesicles	JIPs	APOER2 (LRP8)	51,52
	KLC	Vesicles	JIP1 (MAPK8IP1)	Phosphorylated APP	57
	KLC	Tubulin dimer	CRMP2	Tubulin	59
KIF5A	Unknown	Neurofilament proteins	Unknown	NF-H, NF-M and NF-L	60
KIF3A or KIF3B (KRP85 and KRP95)	KAP3 (KIFAP3)	Vesicles	Fodrin	Unknown	36
	KAP3	Vesicles	Unknown	APC and PAR3	42,43
	KAP3	Vesicles	Unknown	N-cadherin and β-catenin	134
KIF13B (GAKIN)	None	Vesicles	Centaurin-α1	PtdIns(3,4,5)P_3	46,47
	None	Protein complex	Unknown	DLG	44
KIF21A	None	Unknown protein complex	BIG1	Unknown	148
Dendritic transport					
KIF17 (OSM3)	None	Vesicles	LIN10 (MINT1)–LIN2–LIN7	NR2B	65,66
	None	Vesicles	Unknown	GluR5	67
	None	Vesicles	Unknown	Kv4.2	68
KIF5	None	Vesicles	GRIP1	GluR2	63
	None	mRNP complex	Unknown	hnRNP-U, Pur-α and Pur-β	61
	KLC	mRNP complex	FMRP (also known as FMR1)	Unknown	62
KIFC2	Unknown	Multivesicular body-like organelles	Unknown	Unknown	70
Conventional transport					
KIF5	None	ER	Unknown	Kinectin	71
	Unknown	Golgi–ER vesicles	Unknown	ERGIC58 and p115	73
	Unknown	Endosomes	Unknown	RAB4	88
	Unknown	TGN–plasma membrane vesicles	Unknown	p75	79,82
KIF3A or KIF3B (KLP3 in *Xenopus laevis*)	KAP3	Golgi–ER vesicles	Unknown	KDEL receptor	74
	Unknown	Early and late endosomes	Unknown	RAB4 and RAB7	87–89
	None	Recycling endosomes	RIP11 (RAB11FIP5)	RAB11	90
KIF20A (Rabkinesin 6, Rab6 kinesin and RAB6KIFL)	Unknown	Golgi apparatus	Unknown	RAB6	75

Table 1 (cont.) | **Cargo complexes transported by kinesin superfamily proteins (KIFs)**

Motor*	Light chain involvement	Cargo type	Adaptor or scaffold proteins*	Cargo molecules*	Refs
Conventional transport cont.					
KIFC3	Unknown	Golgi apparatus	Unknown	Unknown	77
	Unknown	TGN–plasma membrane vesicles	Unknown	Annexin XIIIb	80
KIF13A	None	TGN–plasma membrane vesicles	AP-1 complex (β1-adaptin and γ-adaptin)	M6PR	78
KIFC2	Unknown	Early endosomes	Unknown	RAB4	88
KIF16B	None	Early endosomes	Unknown	PtdIns(3,4,5)P_3, EGF, EGF receptor and RAB5	91
KIF4	None	Not applicable	Unknown	PARP1	108
	Unknown	Vesicles	Unknown	L1	150
Intraflagellar transport					
KIF3A or KIF3B	KAP3	IFT complex	Unknown	Complex A, crumbs 3, PAR3, PAR6 and aPKC	93,94
KIF17 (OSM3)	Unknown	IFT complex	Unknown	Complex B and CNGB1b	94,95

* Alternative protein names are provided in brackets. AP-1, adaptor protein complex 1; APC, adenomatous polyposis coli; aPKC, atypical protein kinase C; APOER2, apolipoprotein E receptor 2; APP, β-amyloid precursor protein; ATG1, autophagy-related 1; BIG1, brefeldin A-inhibited guanine nucleotide exchange protein 1; CNGB1b, cyclic nucleotide-gated channel-β1b; CRMP2, collapsin response mediator protein 2; DENN/MADD, mitogen-activated protein kinase-activating death domain; DISC1, disrupted in schizophrenia 1; DLG, Discs large; EGF, epidermal growth factor; ER, endoplasmic reticulum; ERGIC58, ER–Golgi intermediate compartment 58 kDa; FMRP, fragile X mental retardation protein; GAKIN, guanylate kinase-associated kinesin; GluR, AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)-type glutamate receptor; GRIP1, glutamate receptor-interacting protein 1; hnRNP-U, heterogeneous nuclear ribonucleoprotein U; IFT, intraflagellar transport; JIP, JUN amino-terminal kinase (JNK)-interacting protein; KAP3, KIF-associated protein 3; KBP, KIF1-binding protein; KLC, kinesin light chain; Kv4.2, voltage-gated K^+ channel 4.2; LAMP2, lysosome-associated membrane protein 2; LIS1, lisencephaly 1; M6PR, mannose-6-phosphate receptor; mRNP, messenger ribonucleoprotein; NF, neurofilament; NR2B, NMDA (*N*-methyl-D-aspartate)-type glutamate receptor 2B; PAR, partitioning defective; PARP1, poly(ADP-ribose) polymerase 1; PtdIns(3,4,5)P_3, phosphatidylinositol-3,4,5-trisphosphate; PtdIns(4,5)P_2, phosphatidylinositol-4,5-bisphosphate; Pur, purine-rich element binding protein; RanBP2, Ran-binding protein 2; RIP11, RAB11-binding protein; SNAP25, soluble *N*-ethylmaleimide-sensitive factor attachment protein; TGN, *trans*-Golgi network.

Kinesins in conventional transport

In addition to their role in axons and dendrites, kinesins are involved in transport inside the cell body of neurons and in non-neuronal cell types. Specifically, various kinesins transport cargo from the Golgi to the endoplasmic reticulum (ER) and from the *trans*-Golgi network (TGN) to the plasma membrane, and also transport lysosomes and endosomes (FIG. 2b). Kinesins also regulate transport inside specialized processes known as cilia and flagella (FIG. 2c).

Golgi–ER transport. Transport between the ER and the Golgi apparatus is bidirectional. Cytoplasmic dynein is implicated in forward transport from the ER to the Golgi apparatus, towards the microtubule minus ends in the cell centre, where the Golgi apparatus is located. The physiological relevance of KIF5 motors and the 160 kDa isoform of the kinesin-binding protein kinectin in Golgi–ER retrograde transport is debated. Kinectin is concentrated in the integral membrane of the ER of non-neuronal cells and it was thought to function in extending the ER throughout the cytoplasm[71]. However, as the structure of the ER remains normal in *Kif5b*- or kinectin-knockout mice, KIF5 and kinectin might be dispensable for ER extension and Golgi–ER transport[25,72]. But recent work suggests that the light chains of the KIF5 complex, KLC1B and KLC1D, bind to rough ER and Golgi fraction vesicles, respectively, thereby reprising the idea that the KIF5 complex is partly involved in vesicle motility in the ER and Golgi[73]. *Xenopus laevis* kinesin-like protein 3 (Xklp3) motor proteins are responsible for the transport of the KDEL receptor — a membrane protein that retrieves soluble cargo from the Golgi and returns it to the ER[74] — which suggests that the KIF3 complex might also be involved in Golgi–ER traffic. Thus, KIF5 and KIF3 motors might work in synergy to regulate bidirectional ER–Golgi trafficking.

In addition to transport, a tug of war between oppositely directed motors is essential for the correct positioning of membrane organelles in cells. The microtubule plus end-directed kinesin 6 family motor KIF20A (also known as Rabkinesin 6, Rab6 kinesin and RAB6KIFL) and two minus end-directed motors, cytoplasmic dynein and KIFC3 (a kinesin 14B family motor), are thought to contribute to the integrity and positioning of the Golgi apparatus[75–77].

Golgi–plasma membrane transport. The kinesin 3 family motor KIF13A binds directly to β1-adaptin, a subunit of the AP-1 complex that is engaged in vesicular transport from the TGN to the plasma membrane[64,78]. γ-Adaptin and the mannose-6-phosphate receptor are also involved in this cargo complex. KIF5 motors also transport a post-Golgi traffic marker, vesicular stomatitis virus G protein (VSVG), towards the plasma membrane[79].

In polarized epithelial cells, most microtubule minus ends point to the apical side, and an apical transport system is unique to these cells. In polarized MDCK cells,

Apical transport
A mode of organelle transport in polarized cells towards the apical surface of the cell.

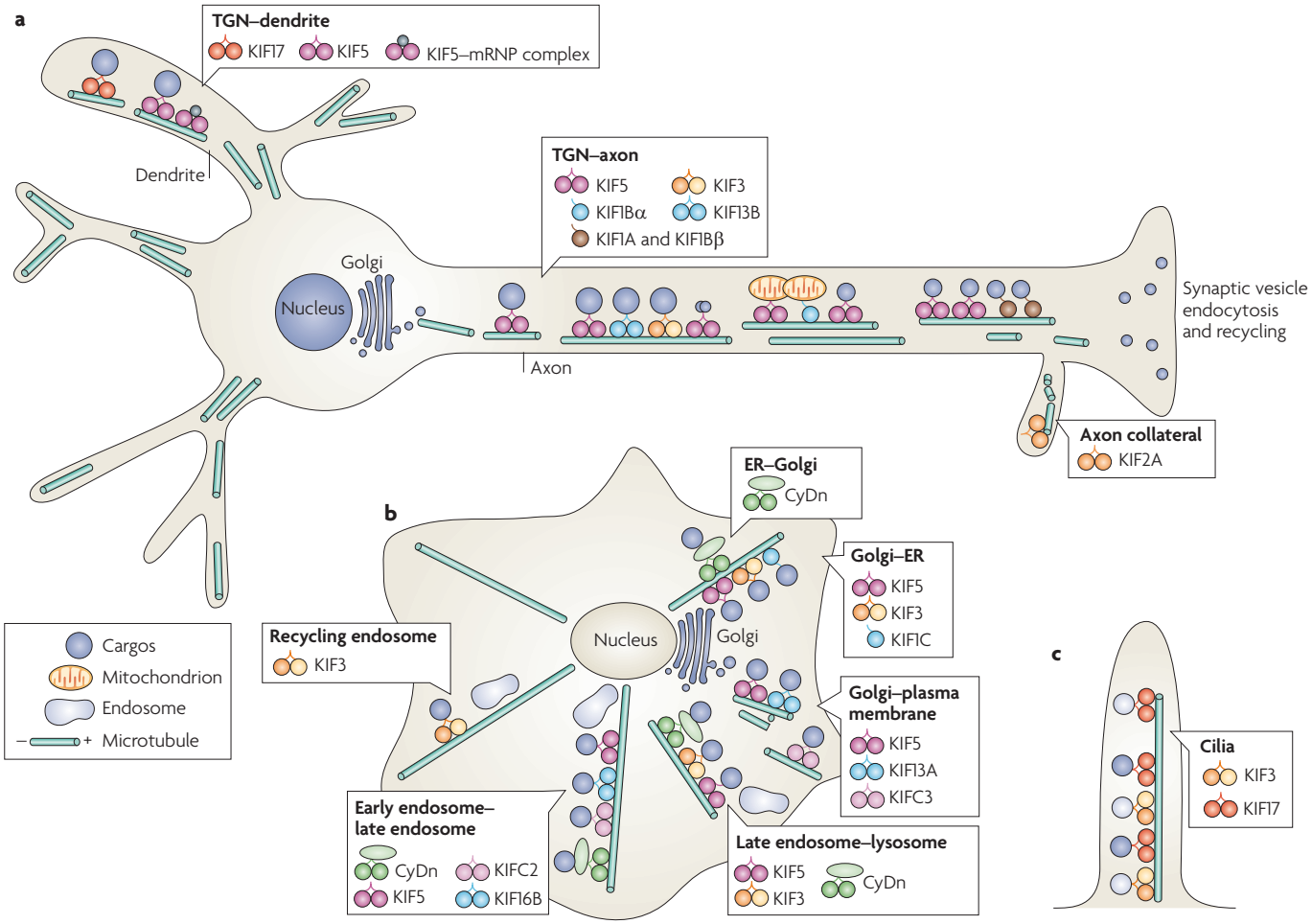


Figure 2 | **Intracellular transport by molecular motors in neurons, non-neuronal cells and cilia. a** | In neuronal axons, microtubules are unipolar and the plus ends always point distally. Therefore, plus end-directed motors transport specific cargos to the cell periphery in axons. These plus end-directed motors include the kinesin 3 family motors KIF1A, KIF1Bβ, KIF1Bα and KIF13B (also known as guanylate kinase-associated kinesin (GAKIN)), the kinesin 1 family KIF5 motors and the kinesin 2 family KIF3 motors. By contrast, the directionality of microtubules is mixed in proximal dendrites. KIF17 (a kinesin 2 family motor; also known as OSM3) and the KIF5 motors are involved in dendritic transport. KIF2A, a kinesin 13 family motor, is involved in microtubule depolymerization. Some KIFs can act as monomers or dimers. KIF5 transports mRNA–protein complexes (messenger ribonucleoprotein complexes (mRNPs)) in dendrites. **b** | In non-neuronal cells, microtubules are usually directed from the microtubule organizing centre to the periphery of the cells. Plus end-directed motors, such as the KIF5 and KIF3 motors and the kinesin 3 family motors KIF1C, KIF13A and KIF16B, tug against the minus end-directed motors, such as cytoplasmic dynein (CyDn), KIFC2 and KIFC3 (members of the kinesin 14B family). This might help to distribute the cargos appropriately. How these motors are differentially involved in the dynamics of intracellular membrane organelles remains unclear. **c** | Bidirectional intraflagellar transport occurs with components of the cilia. The KIF3 motors (both KIF3A and KIF3B; also known as KRP85 and KRP95) and KIF17 are involved in the anterograde transport of at least two different kinds of intraflagellar transport cargo. ER, endoplasmic reticulum; TGN, *trans*-Golgi network.

the kinesin 14B family motor KIFC3 transports TGN-derived vesicles that contain annexin XIIIb and influenza haemagglutinin (HA), an apical membrane marker, to the apical plasma membrane, which lies at the minus end of microtubules[80]. This KIFC3 activity might occur in cooperation with the activity of cytoplasmic dynein. Recent studies have shown that the p120 catenin-binding proteins PLEKHA7 and nezha stabilize apical zonula adherens by anchoring the minus ends of microtubules and recruiting KIFC3 (REF. 81). Additionally, a systemic screen that expressed dominant-negative constructs of KIFs in polarized MDCK cells has revealed that the KIF5B motor transports the neurotrophin receptor p75 to the apical plasma membrane along newly identified plus end, apically directed microtubules in polarized epithelial cells[82].

Zonula adherens
A cell–cell adherens junction that forms a circumferential belt around the apical pole of epithelial cells.

Transport of lysosomes. Lysosomes are dynamic organelles that move between the cell centre and the periphery. The motility of lysosomes is controlled by the pH of the culture medium: treatment with alkaline ringer results in the accumulation of lysosomes in the perinuclear region, whereas reacidification disperses lysosomes into the cytoplasm. This dynamic dispersion of lysosomes is

blocked in mouse *Kif5b*-knockout cells or inhibited by a dominant-negative KIF5 mutant (T93N) that has very low ATPase activity and binds tightly to microtubules[25,83]. This suggests that the KIF5 motor is involved in lysosome transport. Melanosomes are originally lysosome-related organelles. The balance between cytoplasmic dynein and two plus end-directed motors, KIF5B and KIF3, which move along microtubules in opposing directions, regulates the movements of melanosomes[84].

Transport of endosomes. Endomembranes recycle between subcellular compartments and the plasma membrane. The small GTPases of the Rab family regulate the sorting of these membranes by localizing different family members at different compartments throughout their GTP–GDP cycles[85]. Multiple motor proteins have been identified on endosomes. Motors drive cargos in opposite directions, and this determines the localization of endosomes[86]. In the early phase, endosomes that contain RAB5 or RAB4 are driven by cytoplasmic dynein, KIF5, KIF3 and KIFC2 motors. When transferred to late endosomes, these vesicles are labelled with RAB7 bound to cytoplasmic dynein, KIF3 and sometimes KIFC2 (REFS 87,88). KIF3 is detected on late endosomes and lysosomes, as well as on recycling endosomes regulated by the RAB11-binding protein RIP11 (also known as RAB11FIP5)[89,90]. The kinesin 3 family motor KIF16B[64] binds to PtdIns(3,4,5)P_3-positive early endosomes through the PX domain in its tail region[91]. Plus end-directed motility of KIF16B holds endocytosed epidermal growth factor (EGF) and EGF receptors beneath the plasma membrane and protects them from entering the degradation pathway.

Intraflagellar transport. Cilia and flagella are specialized processes composed of microtubule bundles that project from many cell types, including epithelial cells, neurons and fibroblasts. They have multiple functions, such as the generation of motility, fluid flow and mechanochemical sensation. Protein complexes are conveyed in cilia and flagella by intraflagellar transport (IFT)[92], which was first reported to be the bidirectional movement of granule-like particles beneath the flagellar membrane, a necessity for the assembly of cilia and flagella[92]. Motor proteins of the kinesin 2 family have a specific function in IFT[93]. In the sensory cilium of *Caenorhabditis elegans*, all kinesin 2 family motors, namely KIF-3 (also known as KRP-85 and KRP-95) and KIF-17 (also known as OSM-3), are reported to work partially redundantly[94]. In the middle segment of the sensory cilium, the Bardet–Biedl syndrome-related proteins BBS-7 and BBS-8 link KIF-3 and KIF-17 so that they jointly serve as IFT motors, but only KIF-17 serves in the distal segment. In mammals, KIF17 has a role in the targeting of cyclic nucleotide-gated channel β1b (CNGB1b) to the primary cilia of olfactory sensory neurons, to thereby serve in olfaction[95].

Regulation of cargo–motor complexes

For efficient cell function and morphogenesis, cargos need to be delivered to their destinations by specific kinesins and unloaded from these kinesins at the appropriate time and place. Detached kinesins might be inactivated by autoinhibition mechanisms[96–98]. Recent studies have started to reveal the regulatory mechanisms of the spatiotemporal delivery of cargos.

Regulation by phosphorylation. Kinesins are phosphoproteins[99,100]. Therefore, the phosphorylation state of kinesins can regulate their function. Two mechanisms for the phosphorylation-dependent regulation of kinesins can be predicted. First, kinesin phosphorylation might control the association and dissociation of motors with their cargos. Second, kinesin phosphorylation might modulate the binding of kinesins to microtubules.

The function of KIF5 can be regulated by at least two kinases. Protein kinase A-dependent phosphorylation of KIF5–KLC complexes inhibits the association of this motor with synaptic vesicles[99]. Glycogen synthase kinase 3 phosphorylates KLC and inhibits the association of KIF5–KLC complexes with membrane organelles[101].

The Ca^{2+}/calmodulin-dependent protein kinase CaMKII phosphorylates Ser1029 in the tail domain of KIF17, which transports NR2B-containing vesicles[102]. This phosphorylation disrupts binding between KIF17 and the adaptor LIN10, leading to dissociation of KIF17 from NR2B-carrying vesicles (FIG. 4a). The CaMKII-dependent release of NR2B-carrying vesicles from microtubules might account for the activity-dependent insertion of NR2B complexes into postynaptic membranes.

It has been suggested that JNK can phosphorylate KIF5 motors[103,104]. When phosphorylated, the binding of these motors to microtubules becomes weaker. Interestingly, JNK is abnormally activated in spinal and bulbar muscular atrophy models in which a polyglutamine stretch has been inserted into an androgen receptor[103]. Abnormal activation of JNK in these neurons might disturb axonal transport and contribute to the progression of the symptoms. By contrast, genetic experiments have suggested that the JNK pathway upregulates, rather than downregulates, KIF5-dependent axonal transport in *C. elegans* and *D. melanogaster*[19,105]. As well as KIF5 motors, molecules in the JNK signalling pathway are needed for the synaptic vesicle protein synaptobrevin to localize to the axon terminal, although how JNK signalling augments KIF5-dependent axonal transport remains elusive.

In addition to the phosphorylation of KIFs, phosphorylation of adaptors regulates motor–cargo associations. The adaptor protein UNC76 is required for the axonal transport of synaptotagmin-carrying vesicles in *D. melanogaster*: it binds to KIF5 motors *in vitro*, suggesting that these motors transport synaptotagmin vesicles through UNC76 (REF. 106). UNC51, an autophagy-related kinase that is required for axonal elongation[107], binds and phosphorylates UNC76 (REF. 20). Phosphorylated UNC76 then associates with synaptotagmin 1, whereas unphosphorylated UNC76 does not. Furthermore, CaMKII-mediated phosphorylation of poly(ADP-ribose) polymerase 1 (PARP1), a protein that binds to the tail of the kinesin 4 family motor KIF4, causes dissociation of KIF4 from PARP1 (REF. 108).

Melanosome
An organelle that contains melanin, a common light-absorbing pigment.

Endomembrane
An intracellular lipid bilayer membrane that surrounds small spaces, for example to form vesicles and membrane organelles. Endomembranes fuse with and are removed from the plasma membrane by exocytosis and endocytosis, respectively.

PX domain
(Phox homology domain). A lipid and protein interaction domain that consists of 100–130 amino acids and is defined by sequences that are found in two components of the phagocyte NADPH oxidase (phox) complex.

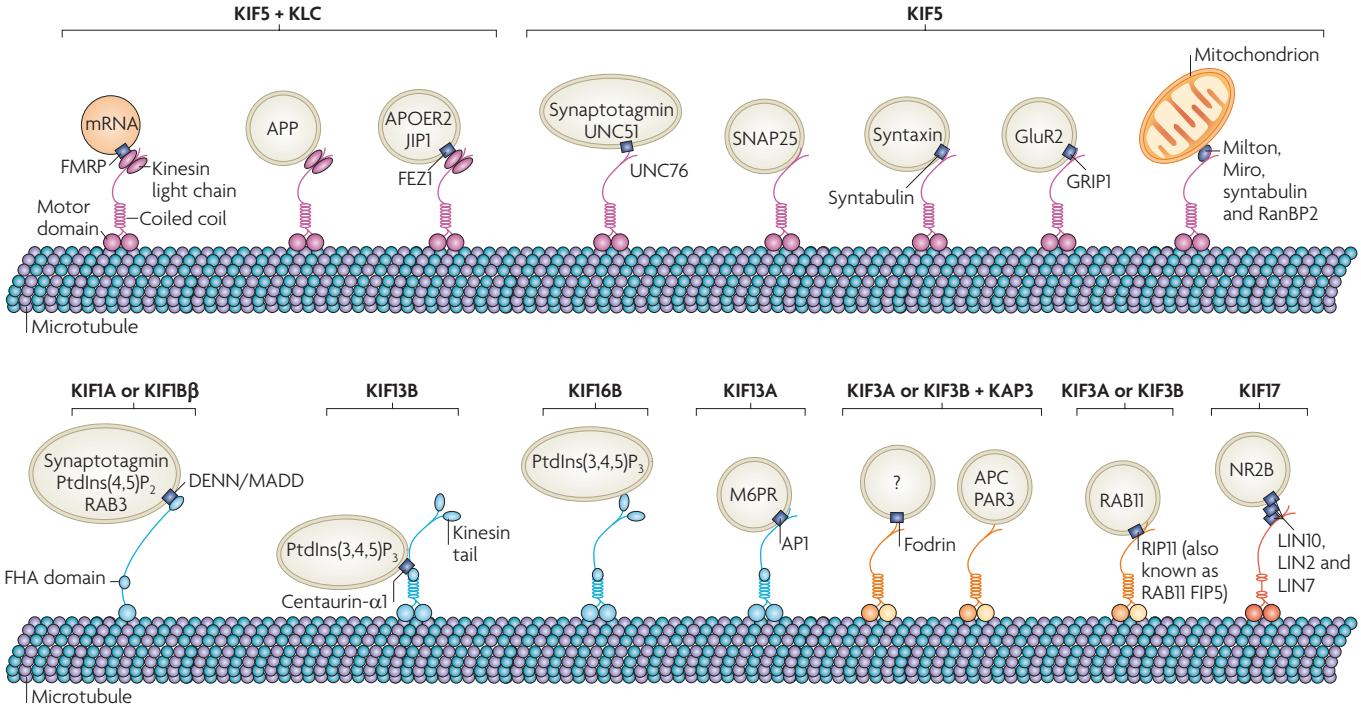


Figure 3 | **Kinesins, cargos and molecules involved in cargo recognition.** Major transport mechanisms that are based on kinesin superfamily proteins (also known as KIFs). Microtubules consist of helically polymerized tubulin molecules. Kinesins associate with microtubules through their head motor domains, either as monomers (one sphere) or dimers (two spheres). Dimerization of KIFs occurs through the coiled-coil domains that are present in their stalks. KIFs associate with their cargos (as indicated) through their tails or occasionally through their light chains or adaptor or scaffold proteins. The kinesin 1 family KIF5 motors can bind to their cargo either through or independently of their kinesin light chains. The kinesin 3 family motors KIF1A and KIF1Bβ redundantly transport synaptic vesicle precursors and GTP-bound RAB3 through the adaptor protein mitogen-activated protein kinase-activating death domain (MADD; also known as DENN and shown as DENN/MADD). KIF13B (also known as guanylate kinase-associated kinesin (GAKIN)), KIF16B and KIF13A are also kinesin 3 family motors. The KIF3 motors and KIF17 (also known as OSM3) are kinesin 2 family motors that have dual functions in cilia, flagella and the cytoplasm. Post-translational modifications of the proteins involved and/or differential constitution of the adaptor complex might determine the motor–cargo pairing in each case. AP1, adaptor protein complex 1; APC, adenomatous polyposis coli; APP, β-Amyloid precursor protein; FEZ1, fasciculation and elongation protein-ζ1; FHA, Forkhead associated; FMRP, fragile X mental retardation protein (also known as FMR1); GluR2, AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)-type glutamate receptor 2; GRIP1, glutamate receptor-interacting protein 1; JIP1, JUN amino-terminal kinase (JNK)-interacting protein 1 (also known as MAPK8IP1); KAP3, kinesin superfamily-associated protein 3 (also known as KIFAP3); M6PR, mannose-6-phosphate receptor, cation dependent; NR2B, NMDA (*N*-methyl-D-aspartate)-type glutamate receptor 2B; PAR3, partitioning defective 3; $PtdIns(3,4,5)P_3$, phosphatidylinositol3,4,5-trisphosphate; $PtdIns(4,5)P_2$, phosphatidylinositol 4,5-bisphosphate; RanBP2, Ran-binding protein 2; RIP11, RAB11 family-interacting protein 3 (also known as RAB11FIP5); SNAP25, soluble *N*-ethylmaleimide-sensitive factor attachment protein 25.

Regulation by Rab GTPases. Members of the Rab family of small GTPases control organelle localization in a GTP–GDP-dependent manner[109]. The nucleotide states of Rab GTPases are controlled by GAPs and GEFs. When GAPs are activated, Rab GTPases exist in a GDP-bound form. When GEFs are activated, Rab GTPases exist in a GTP-bound form. Each Rab binds to a specific class of organelle (see below). The GTP-bound forms of Rab proteins specifically bind to 'Rab effector' proteins, whereas the GDP-bound forms do not. If Rab proteins on organelles recruit motor proteins in the GTP-bound form and release them in the GDP-bound form, this could explain why the nucleotide switch of Rab GTPases can control the distribution of organelles. Thus, Rab GTPases are also good candidates for a role in regulating the association and dissociation between kinesins and organelles.

RAB6 is thought to control intra-Golgi transport[110]. The GTP-bound form of RAB6 localizes to the Golgi apparatus but the GDP-bound form does not. KIF20A was identified as a kinesin that can specifically bind to the GTP-bound form of RAB6 (REF. 75). When RAB6 is converted to its GDP-bound form, KIF20A dissociates from the Golgi. This could control the motility and localization of the Golgi apparatus. Other work suggests a role for KIF20A in cytokinesis in mitotic cells, rather than in Golgi transport in interphase cells[111,112].

RAB5 controls endocytosis[113]. The GTP-bound form of RAB5 locally recruits human VPS34, a phosphoinositide 3-kinase, to endosomes. VPS34 locally synthesizes PtdIns-3-phosphate in endosomes and recruits KIF16B[91]. It has also been suggested that cytoplasmic dynein is recruited to endosomes by the GTP-bound form of RAB5 (REF. 114). Thus, RAB5–GTP can recruit

motors for the transport of endosomes in both directions along microtubules. How the GTPase cycle of RAB5 determines the direction of endosome movement along microtubules remains unclear.

RAB3 is a synaptic vesicle protein that controls the exocytosis of synaptic vesicles. To do this, RAB3 must be transported the long distance to the axon terminal from the cell body. KIF1A and KIF1Bβ transport RAB3-carrying vesicles[6,12,13,115], and the pleckstrin homology (PH) domain of KIF1A and KIF1Bβ is necessary, but not sufficient, to transport cargo to axon terminals[116117]. Recently, it has been revealed that DENN/MADD binds to both the GTP-bound form of RAB3 and the stalk domain of KIF1A and KIF1Bβ[115] (FIG. 4b). Because DENN/MADD is thought to be a GEF for RAB3, this might help to maintain RAB3 in the GTP-bound form during the long-range axonal transport of RAB3-carrying vesicles. In fact, the GTP-bound form of RAB3 is transported into axons, whereas the GDP-bound form of RAB3 is not.

Regulation by Ca^{2+} signalling. Mitochondria are bidirectionally transported in neurons and other cells by kinesin 1 family (KIF5) and kinesin 3 family (KIF1Bα) members[23,25]. It has long been known that mitochondria are concentrated to regions with high energy requirements, such as presynaptic and postsynaptic regions[118], and that high Ca^{2+} influx occurs in these regions. Indeed, several studies have established that Ca^{2+} significantly arrests mitochondrial motility[119–122]. The recent discovery of the adaptors Milton and Miro, which bridge KIF5 motors and mitochondria, has shed some light on this phenomenon[27,123,124], and Miro might control mitochondrial motility in a Ca^{2+}-dependent manner by one of two mechanisms. Miro contains two EF hand motifs, which are modulated by Ca^{2+}. One model suggests that the EF hand motifs of Miro can bind to the motor domain of KIF5 proteins and inhibit motility when high Ca^{2+} is added[29] (FIG. 4c). The other model suggests that high Ca^{2+} disrupts the binding between KIF5 proteins and the Milton–Miro adaptor complex, although how the EF hand motif is involved remains unclear[125]. Further evidence is needed to completely understand the mechanism of Ca^{2+}-dependent regulation of mitochondrial motility.

Physiological relevance of kinesins

In the past decade, several lines of knockout and conditional knockout mice for various KIF genes have shown unexpected phenotypes[13,25,108,126–138], which has revealed several essential roles for kinesins in physiology (FIG. 5). These suggest that molecular motors are not just effectors of signal transduction cascades, but that they transport and/or bind to important signal transduction molecules to actively modulate their function.

KIF3, cilia and left–right determination. *Kif3a*- or *Kif3b*-knockout mice die at the mid-gestation stage and show systemic symptoms, including randomization of left–right body determination[126,128,129] (FIG. 5a). As a result, expression levels of master regulatory genes of the left side — *Nodal*, *Lefty2* and *Pitx2* — are also disturbed.

Pleckstrin homology (PH) domain
A sequence of 100 amino acids that is present in many signalling molecules and binds to lipid products of phosphoinositide kinases.

EF hand
A protein motif that might bind to Ca^{2+}.

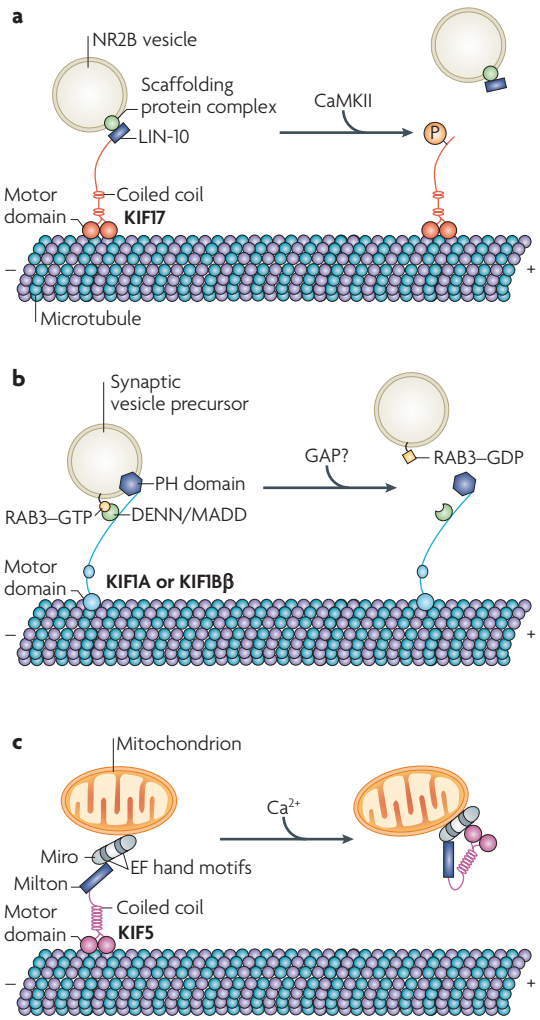


Figure 4 | **Regulation of kinesin–cargo binding by three different mechanisms. a** | Phosphorylation. The kinesin 2 family motor KIF17 (also known as OSM3) transports vesicles that carry NMDA (*N*-methyl-D-aspartate) receptor subunit 2B (NR2B) towards the plus end of microtubules through a scaffolding protein complex[65]. Phosphorylation of KIF17 by the Ca^{2+}/calmodulin-dependent protein kinase CaMKII disrupts the direct interaction between KIF17 and its interacting partner LIN10 (the mouse homologue of *Caenorhabditis elegans* LIN-10), leading to the unloading of NR2B-carrying vesicles[102]. **b** | The Rab GTPase cycle. The kinesin 3 family motors KIF1A and KIF1Bβ transport RAB3-carrying synaptic vesicle precursors through the adaptor protein mitogen-activated protein kinase-activating death domain (MADD; also known as DENN and shown as DENN/MADD)[115]. DENN/MADD recognizes the GTP-bound form of RAB3 on vesicles. Because the membrane-binding capacity of the pleckstrin homology (PH) domain of KIF1A and KIF1Bβ is insufficient to transport cargo[116] when RAB3 is inactivated by GTPase-activating proteins at the axon terminus, RAB3–GDP-carrying vesicles are released. **c** | Calcium. The kinesin 1 family motor KIF5 transports mitochondria through interaction with the Milton–Miro complex[29]. Miro has two EF hand motifs, which sense intracellular Ca^{2+} levels. In response to high Ca^{2+} influx, these motifs bind to the motor domain of KIF5 to inhibit its activity. As a result, mitochondria motility is inhibited.

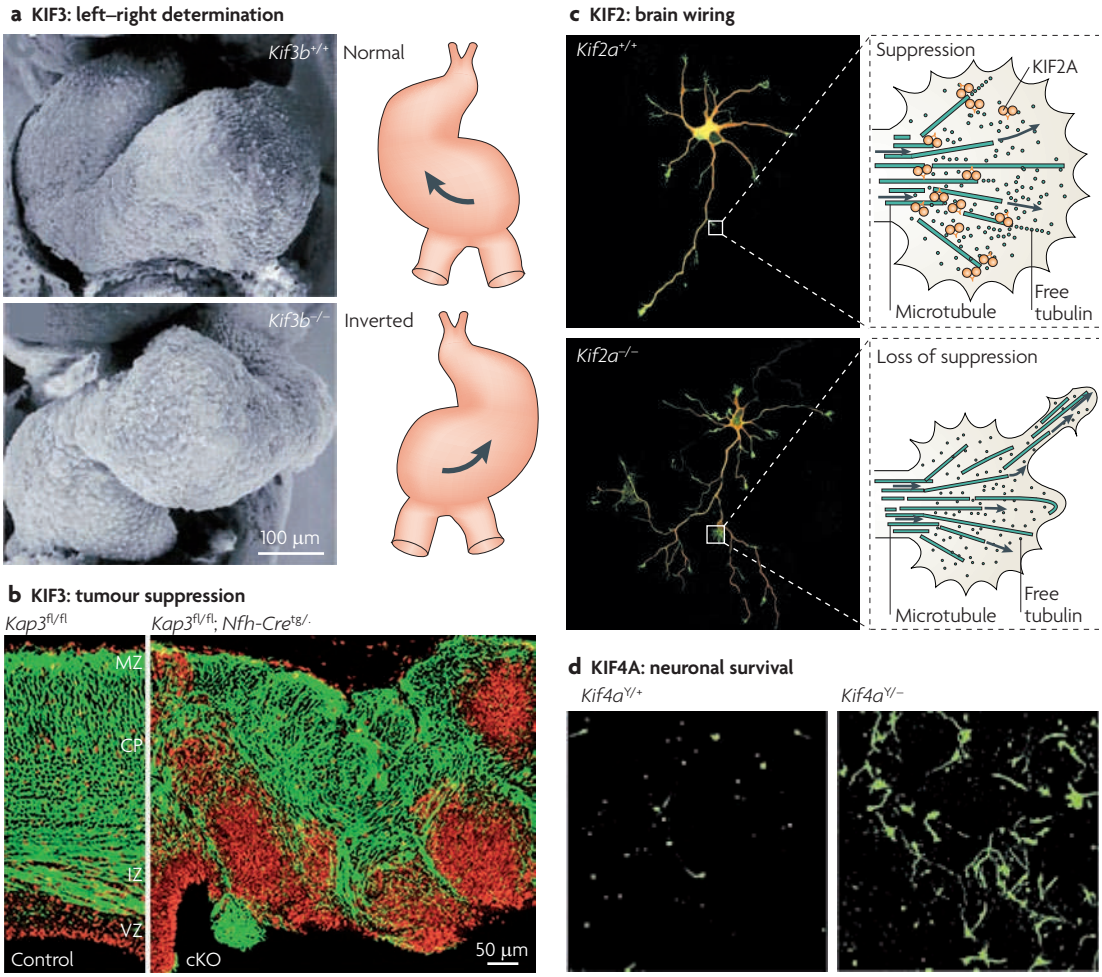


Figure 5 | **The physiological relevance of kinesins in mice.** Mouse models have revealed significant roles for kinesins in mammalian physiology. **a** | The kinesin 2 family motor KIF3 is required for proper left–right body determination. These electron micrographs show normal (*Kif3b*$^{+/+}$) and abnormal (inverted; *Kif3b*$^{-/-}$) heart loops. **b** | KIF3 can suppress tumorigenesis. These confocal images show normal brain histology (homozygous *Kap3* (also known as *Kifap3*)-floxed (*Kap3*$^{fl/fl}$) mice) next to a section from mice with neuron-specific conditional knockout (cKO) of *Kap3* (*Kap3*$^{fl/fl}$; *Nfh-Cre*$^{tg/-}$) that have brain tumours. In the cKO mouse brain, neuronal progenitor cells (red) proliferate extensively, invade the upper layer and form tumour-like abnormal focal nodules. Green staining represents differentiated neurons. **c** | *Kif2a*-knockout neurons (*Kif2a*$^{-/-}$) exhibit abnormal axon branching compared with control neurons (*Kif2a*$^{+/+}$), as shown in these confocal images. The cartoons show how the kinesin 13 family motor KIF2A suppresses the unnecessary extension of axon collaterals by regulating the dynamic equilibrium of microtubules. In the absence of KIF2A, this collateral suppression is impaired and abnormal extension of microtubules is observed. **d** | The kinesin 4 family motor KIF4A is required for activity-dependent neuronal survival. Fluorescent microscopy images show that *Kif4a*-null neurons (neurons with X-linked *Kif4a* knocked out from the male chromosome (*Kif4a*$^{Y/-}$)) have a better survival rate than control *Kif4a*$^{Y/+}$ neurons. CP, cortical plate; IZ, intermediate zone; MZ, marginal zone; VZ, ventricular zone. Images in part **a** are reproduced, with permission, from REF. 126 © (1998) Elsevier. Cartoons in part **a** are reproduced, with permission, from REF. 155 © (2009) Annual Reviews. Part **b** is reproduced, with permission, from *Nature Cell Biology* REF. 134 © (2005) Macmillan Publishers Ltd. All rights reserved. Part **c** is reproduced, with permission, from REF. 132 © (2003) Elsevier and Part **d** is reproduced, with permission, from REF. 108 © (2006) Elsevier.

The following mechanism of left–right body determination, which involves these KIF3 motors, has been proposed[126,128,129,139–142]. KIF3 generates cilia in the ventral node, a crucial region for left–right determination. The monocilia in the ventral node rotate in a clockwise direction. Meanwhile, planar cell polarity of node cells along the anterior–posterior axis might account for the tilt of the rotation axis towards the posterior side. The resulting clockwise rotation around a posteriorly tilted axis reveals a leftward flow of the extraembryonic fluid (nodal flow). This rotation transports extracellular signalling complexes (called nodal vesicular parcels) that are released from node cells to the left. The contents of these nodal vesicular parcels elevates the intracellular Ca^{2+} concentration specifically on the left side, providing an initial cue for left-specific developmental pathways. This mechanism seems to be evolutionarily conserved in fish, *X. laevis*, mice and humans.

Planar cell polarity
A one-dimensional polarity on a cell sheet that is essential for many aspects of the development of tissues.

Kif3 conditional knockout mice also provide experimental models of human ciliopathies, such as Kartagener syndrome, polycystic kidney disease and Joubert syndrome. In these diseases, a lack of cilia gives rise to severe symptoms, including retinal degeneration[130], polydactyly[135], skeletal development defects[136,137], polycystic kidneys[133] and obesity[138]. These symptoms occur through the perturbation of essential signalling cascades, such as the hedgehog pathway[143].

KIF3 and the suppression of tumorigenesis. The KIF3 motor complex can also function as a tumour suppressor. Conditional inactivation of the KAP3 subunit of the KIF3 complex in neural progenitor cells results in embryonic brain tumours[134] (FIG. 5b). This phenocopies N-cadherin-ablated animals, and N-cadherin and β-catenin abnormally accumulate in the cytoplasm of *Kap3*-knockout cells. Further studies suggest that KIF3 normally transports vesicles that contain N-cadherin and β-catenin towards the plasma membrane. This contributes to the stabilization of cell–cell adhesions and to the downregulation of canonical Wnt signalling, which promotes cell proliferation. Thus, the activity of β-catenin and N-cadherin can be altered by their localization, which seems to be under the control of the molecular motor KIF3. This is one of the first examples of a molecular motor directly modulating the location and activity of signalling molecules to contribute to tumour suppression.

KIF1A, KIF1Bβ, KIF5A and KIF21A in neuropathies. Axonal transport is a focus of neurodegenerative disease research[144]. KIF1A and KIF1Bβ have high similarity in their primary sequences and are reported to transport synaptic vesicle precursors[12,13,115]. Knockout of either gene in mice is lethal in the perinatal period and causes severe neuronal degeneration and synaptic dysfunction[13,127]. Mice that are heterozygous for the *Kif1b* gene are viable, but they suffer from progressive peripheral neuropathy. These heterozygote mice could serve as a good model system for studying the pathogenesis of peripheral neuropathy. A mutation in the motor domain of KIF5A is responsible for hereditary spastic paraplegia, a familial neuropathy[145]. KIF21A, a kinesin 4 family motor, is highly enriched in axons and dendrites[146]. Heterozygous missense mutations in *KIF21A* exons are responsible for CFEOM1 (congenital fibrosis of the extraocular muscle type 1) in humans[147]. Recently, brefeldin A-inhibited guanine nucleotide exchange protein 1 (BIG1) was reported to bind to KIF21A, but its relation to the disease remains unknown[148].

KIF2A and brain wiring. The kinesin 13 family motor KIF2A directly regulates the morphology of neurons[132]. *Kif2a*-knockout mice are born alive but die within one day of birth. Their brains show multiple abnormalities, including laminary defects and defects of nerve nuclei. Abnormally elongated and abnormally branched axon collaterals were identified in the hippocampus and cerebral cortex (FIG. 5c). In knockout neurons, axon collaterals are significantly elongated and neuronal migration is severely inhibited. Furthermore, KIF2A has been shown to cause the ATP-dependent depolymerization of microtubules. Thus, KIF2A might destabilize microtubules in certain axon collaterals to suppress unnecessary elongation, thereby having a fundamental role in brain wiring.

KIF4A and activity-dependent neuronal survival. KIF4A is expressed in the nucleus and growth cones of juvenile neurons[149]. It has been reported to transport an adhesion molecule called L1 in the axon[150], and more recently a gene targeting study has revealed a new role for this motor[108]. Intriguingly, KIF4A-null neurons exhibit extraordinarily high survival rates even without stimulation and are resistant to apoptosis (FIG. 5d). KIF4A was found to associate with PARP1. Under basal conditions, the KIF4A–PARP1 complex localizes to the nucleus and KIF4A inhibits PARP1 activity. If the neuron stays inactive, inactive PARP1 will lead to apoptosis. However, upon membrane depolarization, PARP1 is phosphorylated by CaMKII and dissociates from KIF4A, which causes PARP1 activation. Activated PARP1 prevents the neuron from undergoing apoptosis and KIF4A is released to the cytoplasm to transport its cargo. This mechanism might be essential for activity-dependent survival of developing neurons.

KIF17 and KIF5 motors in higher brain function. A transgenic mouse strain that overexpresses KIF17 was used to study the role of this motor *in vivo*[131]. The mouse strain showed a significant improvement in working and spatial memories. Intriguingly, in the transgenic mouse brain, the transcription of intrinsic *Nr2b* and translation of intrinsic *Kif17* were upregulated. This occurred in parallel to enhanced phosphorylation of the cyclic AMP-responsive element-binding protein (CREB), which has putative binding sites in the enhancer regions of *Nr2b* and *Kif17* and therefore might increase the transcription levels of these proteins further. Thus, KIF17 overexpression might enhance the trafficking of NR2B to synapses, which increases intracellular Ca^{2+} levels and CREB phosphorylation, and might serve as a positive-feedback loop that enhances learning and memory. This is the first indication that a molecular motor could be directly involved in higher brain function.

This positive-feedback loop between a kinesin motor and the CREB system has been further suggested in the gill withdrawal reflex of *Aplysia mollusca*[151]. Repeated application of serotonin induced a form of neuronal plasticity called long-term facilitation (LTF), and a homologue of KIF5 — *A. mollusca* KHC1 — was upregulated. This upregulation led to an increase in vesicle traffic carrying the synaptic scaffold protein Piccolo, which is essential for LTF induction. Overexpression of KHC1 in the 24 hour phase required a positive-feedback loop that involved CREB activation. This study suggests a role for KIF5-mediated transport machinery in the induction of long-term memory.

Hereditary spastic paraplegia
A human progressive neuronal disease of hereditary origin that is characterized by increasing weakness and stiffness of the legs.

Laminary defect
A developmental defect of the brain that disorganizes the laminary structure of the cortex.

Long-term facilitation
A mode of synaptic plasticity in which stimulation results in a persistent increase in synaptic transmission.

Conclusions and perspectives

Intracellular transport is fundamental for important cellular functions in both polarized and non-polarized cells. Kinesins have a major role in this process by directionally transporting various cargos, albeit sometimes redundantly. Such cargos include synaptic vesicle precursors (transported by KIF5 motors and KIF1A or KIF1Bβ), plasma membrane precursors (KIF5 motors and KIF3), mitochondria (KIF1Bα and KIF5 motors), lysosomes (KIF5 motors), NMDARs (KIF17), AMPARs (KIF5 motors), N-cadherin- or β-catenin-containing vesicles (KIF3), and mRNAs (KIF5 motors). KIF3 and KIF17 also transport protein complexes in cilia and flagella. Although the cargo for many kinesins has been identified, the function of a number of kinesins needs to be further addressed.

Kinesins often recognize scaffold proteins or adaptor proteins and bind to cargo membrane proteins indirectly as part of a protein complex. In other cases, kinesins directly bind to membrane proteins in the cargo. Three mechanisms have been implicated in the regulation of the unloading of cargos from kinesins: phosphorylation of kinesins or cargos[102], control by small G proteins[91,115] and Ca^{2+} signalling[29]. Although several kinases (protein kinase A and glycogen synthase kinase 3 for KIF5 motors and CaMKII for KIF17) have been identified in the regulation of motor–cargo unloading, more work is needed to elucidate how spatial information inside cells and signals outside of cells regulate kinesins through these kinases. Furthermore, based on the possibility that one kinesin is regulated by several kinases, crosstalk between multiple signals is expected and might be required to accomplish signal-dependent regulation of intracellular transport. As for the regulation by small G proteins, the nucleotide state of GTPases is controlled by a GAP and a GEF. Future studies are needed to determine what signals activate GAPs and GEFs and what signals control the association and dissociation of kinesins and cargos, thereby controlling intracellular transport.

Important information on how the motor domain of KIF5 can specifically recognize axons, by a mechanism that involves regionally different microtubule dynamics within a neuron, has been revealed[39], but the basis for this phenomenon needs to be identified. In short, a number of key questions regarding the mechanism of cargo recognition, the regulation of binding between kinesins and cargos and the control of directional transport need to be answered. For example, in neurons, does the motor or the cargo provide the selectivity for axonal rather than dendritic transport? Why are certain kinds of cargos transported by more than one type of kinesin? Is this a limitation of the research methods used to date or a real reflection of redundancy in the cell? How does a single type of kinesin, for example KIF5, bind to multiple partner proteins that allow it to carry so many different cargos? How many motors are needed to transport a cargo and how do multiple motors coordinate their activities? How is slow axonal transport accomplished and regulated?

Last, unexpected phenotypes of knockout and transgenic mice have revealed that molecular motors have significant roles in physiological processes, such as the development of the body axis (KIF3)[126,128,129], brain wiring and development (KIF2 and KIF4)[108,132] and higher brain function (KIF17)[131]. In addition, kinesins can protect against cancer (KIF3)[134] and neuropathy (KIF1A, KIF1Bβ, KIF5A and KIF21A)[13,127,145,147]. Future studies will further reveal how kinesins have a role in disease, higher brain function and development by modulating the local communication of essential proteins.

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DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
KIF1B
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
CFEOM1 | Joubert syndrome | Kartagener syndrome | polycystic kidney | spinal and bulbar muscular atrophy
UniProtKB: http://www.uniprot.org
JIP1 | JIP3 | KAP3 | KIF1A | KIF2A | KIF3A | KIF3B | KIF4 | KIF5A | KIF5B | KIF5C | KIF13A | KIF13B | KIF17 | KIF20A | KIF21A | KIF2 | KIF3 | MADD | MIRO | RAB3A | RAB6 | RanBP2 | SNAP25 | syntaxin 1A | Xklp3

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