Towards an understanding of kinesin-1 dependent transport pathways through the study of protein–protein interactions

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Abstract
Kinesin-1 is the founding member of a superfamily of motor proteins that transport macromolecules along microtubules in an ATP-dependent manner. Classic studies show that kinesin-1 binds to intracellular cargos through non-covalent interactions with proteins on the cargo surface, that protein–protein interaction domains are present in the cargo-binding tail domain and that phosphorylation-dependent signal transduction pathways regulate kinesin–cargo interactions. A combination of genetics, biochemistry and proteomics has identified processes in which kinesin-1 has an important role, and helped reveal the mechanisms of kinesin-dependent transport events. These approaches have identified more than 35 proteins that bind to kinesin-1, these proteins act as cargos, cargo receptors and regulators of kinesin-1 activity. This review summarizes our current understanding of kinesin-1 associated proteins, and places those protein–protein interactions into the context of kinesin-1 in vivo function.

Keywords: kinesin; microtubule motors; intracellular transport; yeast two-hybrid system; mass spectrometry; genetic interaction screens

INTRODUCTION
Intracellular transport is the process by which motor proteins walk along cytoskeletal filaments to facilitate saltatory movement in an ATP-dependent manner. Motor proteins generally have two functional domains: the head domain containing the ATPase and microtubule-binding motor activity, and the tail domain binding to specific cargos (Figure 1) [1]. Cargos such as chromosomes, vesicles and protein complexes attach to specific motors of the myosin, dynein and kinesin superfamilies. The binding of motor to cargo is followed by movement of the motor–cargo complex along actin filaments or microtubules to the appropriate cellular destination [2, 3]. Motor protein function is essential for many cellular processes, including secretion, muscle contraction, cell division and the positioning of organelles. Eukaryotic genomes encode dozens of motor proteins, and individual cells contain many different motor proteins in the cytoplasm to accommodate cell-specific transport repertoires [4]. Fine-tuning of intracellular transport is accomplished through the regulation of motor–cargo interactions by phosphorylation-dependent signalling pathways, thereby facilitating rapid response to changes in cell transport requirements. It is clear that the specific pairing of motor with cargo and the regulation of motor activity is essential for development, differentiation and maintenance of cell function; how the individual transport events are coordinated within cells is a central question of cell biology.

Microtubule motor proteins fall into two superfamilies, dynein and kinesin, based upon structural features of the mechanochemical head domain. Dynein and kinesin work in concert to mediate bidirectional cargo transport [5]. Cytoplasmic microtubules are typically oriented so that the...
slow-growing (minus) end of the microtubule is located at the microtubule organizing centre (MTOC) near the nucleus, while the fast-growing (plus) end is located at the cell periphery. Kinesins and dyneins recognize this polarity, and walk in a unidirectional manner so that dyneins move toward the microtubule minus end, while most kinesins move toward the microtubule plus end. Current models propose that cargos attach to both kinesin and dynein, and that regulatory molecules, such as kinases, phosphatases and accessory proteins determine whether kinesin or dynein is microtubule-bound, thereby controlling transport direction [6, 7].

In this review, the function of kinesin-1, efforts to identify proteins that can bind the kinesin-1 tail domain and how understanding the function of kinesin-1 binding proteins has improved mechanistic models of how intracellular transport works in the cell are discussed.

Kinesin-1 is evolutionarily conserved and essential
Kinesin-1 was discovered as a protein in squid axoplasm that moves vesicles along microtubules in an ATP-dependent manner [8, 9]. Subsequent studies showed that kinesin-1 is evolutionarily conserved and is found in organisms as diverse as fission yeast and humans (Table 1) [10]. Kinesin-1 is a heterotetramer composed of two heavy chains and two light chains; the heavy chains encode the motor domain, whereas the light chains are required for binding some cargos and negative regulation of motor domain ATPase activity when kinesin-1 is not cargo-bound [11]. Kinesin-1 motors are distinguished from other kinesin superfamily members by structural features of its tail domain [4]. The kinesin-1 tail domain is composed of the C-terminus of the kinesin heavy chains and the light chains, which bind to specific amino acids of the kinesin heavy chain stalk domain (Figure 1) [12]. Initial studies using Drosophila and Caenorhabditis elegans demonstrated unequivocally that kinesin-1 is essential for neuromuscular function and axonal transport of membrane-bound organelles; later efforts in other organisms showed that the function of kinesin-1 is evolutionarily conserved [13–16]. Table 1 shows the results of genetic studies of kinesin-1 function. In general, the observed phenotypes fit with proposed models of kinesin-1 function in the transport of vesicles, mitochondria and messenger RNAs, and are consistent with the results of kinesin-1 inhibition experiments in cultured cells [14]. Mutations in kinesin-1 have been implicated in human disease, as a point mutation in the motor domain of neuron-specific KIF5A has been associated with the autosomal dominant neurodegenerative disorder hereditary spastic paraplegia type 10 (SPG10) [17]. Therefore, studies of kinesin-1 function in model organisms are relevant to understanding intracellular transport in the human nervous system and can potentially lead to the development of therapies for the treatment of SPG10.

Kinesin-1 binding protein identification strategies
A combination of in vitro motility assays, subcellular fractionation experiments and functional tests in cultured cells showed that kinesin-1 is associated with membrane-bound organelles of the endoplasmic reticulum, Golgi complex, mitochondria and lysosomes [11]. Classic experiments showed that kinesin-membrane interactions are salt- and pH-sensitive, and treatment of purified organelles with trypsin blocks the ability of the membranes to bind kinesin-1 in vitro [18, 19]. The identification of conserved protein–protein interaction motifs in the kinesin-1 tail domain provided additional evidence that kinesin-1 binds its cargos through protein–protein interactions [20, 21]. Immunostaining and

![Figure 1: Models of kinesin-1 associated protein (KAP) function. A model of kinesin-1 structure is shown. The head domain binds and moves along microtubules in an ATP-dependent manner, and the tail domain binds to intracellular cargos. Four types of KAPs are shown: cargo-bound receptor that facilitates kinesin–cargo interaction (A), adaptor protein that ‘chaperones’ kinesin–cargo binding (B), KAP that recruits kinesin-1 regulators, such as kinases and phosphatases (C) and KAP proteins that directly regulate kinesin-1 activity by post-translational modification (D). KAPs have been shown to bind both the light chain and heavy chain subunits of the kinesin-1 tail domain.](https://academic.oup.com/bfg/article-abstract/5/1/74/219930/75)
coimmunoprecipitation experiments offered clues as to how kinesin-1 attaches to its cargos. Pharmacological and biochemical experiments showed that kinesin-1 is a phosphoprotein and that phosphorylation can modulate intracellular transport, but the identity of the proteins involved in attaching kinesin-1 to cargos remained elusive until the identification of kinectin in 1992 [22]. Kinectin is a 160 kDa membrane-associated protein found on the surface of the endoplasmic reticulum [23]. It was identified as a kinesin-1 binding protein based upon its copurification with kinesin-1 in detergent extracts of chicken brain microsomes, using a monoclonal antibody to the kinesin-1 head domain as an affinity ligand [22]. The distinctive phenotypes associated with loss of kinesin function in Drosophila and C. elegans, including larval ‘tail flipping’, axonal organelle jams and uncoordinated locomotion provided the basis for genetic interaction screens to identify mutations that enhance or suppress kinesin mutant phenotypes, as well as mutations having phenotypes similar to those observed in kinesin mutants [24–28]. These functional studies, combined with yeast two-hybrid experiments, led to the discovery of sunday driver (SYD), a vesicle-associated protein required for axonal transport [29]. The SYD represented a milestone in the intracellular transport field, as the identity of membrane-bound proteins required for the attachment of kinesin-1 to axonal transports remained elusive for many years, despite being the subject of intense scrutiny. The SYD also provided new insights on the integration of transport and cell signalling pathways, as SYD is a scaffold protein that binds to components of the JNK signalling pathway [29, 30].

Since the discovery of SYD, several kinesin-1 binding proteins have been identified in two-hybrid screens, either using kinesin-1 as bait or by ‘fishing’ kinesin-1 heavy chain or light chain out of a two-hybrid library (Table 2). With the development of mass spectrometry tools for protein identification, several groups have successfully identified kinesin-1 as a constituent of protein complexes purified by coimmunoprecipitation. While mass spectrometry does not reveal which protein or proteins in a complex bind directly to kinesin, these results do provide an excellent starting point for in vitro binding assays and two-hybrid experiments. More than 35 kinesin-1 associated proteins have been identified (Table 2). A detailed description of each protein and the context of its association with kinesin-1 is beyond the scope of this review, but representative examples of each class of kinesin-1 binding protein will be described in detail subsequently.

### Table I: Phenotypes of kinesin-1 mutations

<table>
<thead>
<tr>
<th>Gene</th>
<th>Organism</th>
<th>Phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy chain subunit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KIF5A (neuron-specific)</td>
<td>mouse, human</td>
<td>neonatal lethality; postnatal loss results in neuromuscular phenotype, neurofilament transport defects; hereditary spastic paraplegia (SPG10)</td>
<td>[17, 84, 124]</td>
</tr>
<tr>
<td>KIF5B (ubiquitous)</td>
<td>mouse</td>
<td>neonatal lethality; perinuclear clustering of mitochondria, lysosome dispersion defects</td>
<td>[125]</td>
</tr>
<tr>
<td>KHC</td>
<td>Drosophila</td>
<td>axonal transport defects, abnormal meiotic spindle translocation</td>
<td>[15, 94, 130]</td>
</tr>
<tr>
<td>UNC-I16</td>
<td>C. elegans</td>
<td>axonal transport defects, abnormal meiotic spindle translocation</td>
<td>[15, 94, 130]</td>
</tr>
<tr>
<td>Fungal kinesins (Kin2, Nkin, NhlKIN1, Klp3)</td>
<td>U. maydis, N. crassa, N. haematococca, S. pombe</td>
<td>hyphal extension (cell elongation), vacuole formation, membrane-bound organelle motility</td>
<td>[21, 131–134]</td>
</tr>
<tr>
<td>Light chain subunit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KLC</td>
<td>Drosophila</td>
<td>viable, small body size, motor defects; aggregation of KHC at Golgi membrane; vesicle transport defects</td>
<td>[50, 135]</td>
</tr>
<tr>
<td>KLC-1, KLC-2</td>
<td>C. elegans</td>
<td>axonal transport</td>
<td>[13, 64, 65]</td>
</tr>
<tr>
<td>KLC-1, KLC-2</td>
<td>Drosophila</td>
<td>axonal transport defects, abnormal meiotic spindle translocation</td>
<td>[123, 130]</td>
</tr>
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</table>
Table 2: Kinesin-1 binding proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Organism</th>
<th>Kinesin-1 subunit bound</th>
<th>Identification method</th>
<th>Interaction context</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinectin</td>
<td>chicken, human</td>
<td>KHC</td>
<td>affinity chromatography, immunoscreening of expression library</td>
<td>endoplasmic reticulum membrane</td>
<td>[22, 23, 136]</td>
</tr>
<tr>
<td>Myosin Va</td>
<td>mouse</td>
<td>KHC</td>
<td>yeast two-hybrid</td>
<td>mitochondrial outer membrane</td>
<td>[51, 53]</td>
</tr>
<tr>
<td>Herpes simplex virus US11</td>
<td>human</td>
<td>KHC</td>
<td>affinity chromatography</td>
<td>virus particle transport</td>
<td>[68]</td>
</tr>
<tr>
<td>Milton, GRIF-1/OIP106</td>
<td>Drosophila, human</td>
<td>KHC</td>
<td>immunoprecipitation, mass spectrometry</td>
<td>regulation of kinesin activity (proposed)</td>
<td>[138]</td>
</tr>
<tr>
<td>RanBP2</td>
<td>cattle, mouse</td>
<td>KHC</td>
<td>affinity chromatography, mass spectrometry</td>
<td>regulation of kinesin activity</td>
<td>[98]</td>
</tr>
<tr>
<td>Rab8 ribosome receptor</td>
<td>human</td>
<td>KHC</td>
<td>yeast two-hybrid</td>
<td>dendritic transport of membrane vesicles</td>
<td>[31]</td>
</tr>
<tr>
<td>UNC-76</td>
<td>Drosophila</td>
<td>KHC</td>
<td>yeast two-hybrid</td>
<td>axonal transport of membrane vesicles</td>
<td>[140]</td>
</tr>
<tr>
<td>SNAP23/SNAP25</td>
<td>mouse</td>
<td>KHC</td>
<td>yeast two-hybrid</td>
<td>DPC transport to postsynaptic membrane</td>
<td>[58]</td>
</tr>
<tr>
<td>Syntabulin</td>
<td>rat</td>
<td>KHC</td>
<td>in vivo analysis, immunoprecipitation</td>
<td>axonal transport of syntaxin-bound vesicles</td>
<td>[120]</td>
</tr>
<tr>
<td>Enabled</td>
<td>Drosophila</td>
<td>KHC</td>
<td>yeast two-hybrid, genetic interaction tests</td>
<td>regulation of kinesin activity</td>
<td>[27]</td>
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<tr>
<td>Vaccinia virus A36R</td>
<td>human</td>
<td>KLC</td>
<td>in vivo analysis, yeast two-hybrid</td>
<td>virus particle transport</td>
<td>[67, 70]</td>
</tr>
<tr>
<td>SYD/JIP1–4/UNC-16</td>
<td>Drosophila, rat, C. elegans</td>
<td>KLC</td>
<td>in vivo analysis, yeast two-hybrid, immunoprecipitation, affinity chromatography</td>
<td>axonal transport of Shank, JNK pathway</td>
<td>[29, 60, 94, 123, 141]</td>
</tr>
<tr>
<td>UNC-14</td>
<td>C. elegans</td>
<td>KLC</td>
<td>in vivo analysis, yeast two-hybrid, immunoprecipitation, affinity chromatography</td>
<td>axonal transport of membrane vesicles</td>
<td>[123]</td>
</tr>
<tr>
<td>GBP/FRAT</td>
<td>mouse, Xenopus</td>
<td>KLC</td>
<td>yeast two-hybrid</td>
<td>GSK3 inhibitor at dorsal pole</td>
<td>[90]</td>
</tr>
<tr>
<td>TorsinA</td>
<td>human</td>
<td>KLC</td>
<td>yeast two-hybrid</td>
<td>chaperone of kinesin cargo interactions</td>
<td>[109]</td>
</tr>
<tr>
<td>I4-3-3z</td>
<td>rat, human</td>
<td>KLC</td>
<td>immunoprecipitation, mass spectrometry</td>
<td>regulation of kinesin activity</td>
<td>[112]</td>
</tr>
<tr>
<td>KCA-1</td>
<td>C. elegans</td>
<td>KLC</td>
<td>in vivo analysis, affinity chromatography</td>
<td>meiotic spindle translocation</td>
<td>[130]</td>
</tr>
<tr>
<td>Rootletin</td>
<td>mouse</td>
<td>KLC</td>
<td>yeast two-hybrid</td>
<td>cilial rootlet subunit transport</td>
<td>[142, 143]</td>
</tr>
<tr>
<td>GSK-3p</td>
<td>rat, mouse, squid</td>
<td>KLC</td>
<td>immunoprecipitation, in vitro analysis</td>
<td>regulation of kinesin activity</td>
<td>[91, 144]</td>
</tr>
<tr>
<td>Amyloid precursor protein</td>
<td>mouse, Drosophila</td>
<td>KLC</td>
<td>in vivo analysis, affinity chromatography</td>
<td>axonal transport of membrane vesicles</td>
<td>[50, 145–148]</td>
</tr>
<tr>
<td>Hsc70</td>
<td>hamster, mouse, cattle</td>
<td>KLC</td>
<td>in vitro analysis</td>
<td>axonal transport of kinesin cargo interactions</td>
<td>[149]</td>
</tr>
<tr>
<td>ODFI</td>
<td>mouse</td>
<td>KLC</td>
<td>subcellular fractionation, in vitro analysis, yeast two-hybrid</td>
<td>sperm tail assembly</td>
<td>[150]</td>
</tr>
<tr>
<td>CRMP-2</td>
<td>rat</td>
<td>KLC</td>
<td>immunoprecipitation, affinity chromatography</td>
<td>axonal transport of tubulin subunits</td>
<td>[79]</td>
</tr>
<tr>
<td>YETI</td>
<td>Drosophila</td>
<td>KHC/KLC</td>
<td>yeast two-hybrid</td>
<td>microtubule dynamics (proposed)</td>
<td>[151]</td>
</tr>
<tr>
<td>Staufer-containing mRNPs</td>
<td>Xenopus, human, unknown, but transport in vivo is KHC-dependent [64, 65]</td>
<td>KLC</td>
<td>immunoprecipitation, affinity chromatography, mass spectrometry, subcellular fractionation</td>
<td>mRNP transport</td>
<td>[152–157]</td>
</tr>
<tr>
<td>SKIP</td>
<td>human</td>
<td>unknown</td>
<td>in vivo analysis, affinity chromatography</td>
<td>regulation of Salmonella-containing vacuole (SCV) transport</td>
<td>[158]</td>
</tr>
<tr>
<td>Liprin-α</td>
<td>Drosophila</td>
<td>unknown</td>
<td>affinity chromatography</td>
<td>axonal transport of membrane vesicles</td>
<td>[159]</td>
</tr>
<tr>
<td>Neurofibromin (NFI)</td>
<td>cattle, human</td>
<td>unknown</td>
<td>affinity chromatography, mass spectrometry</td>
<td>transport of membrane-bound NFI</td>
<td>[160]</td>
</tr>
<tr>
<td>Merlin (NF2)</td>
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<td>unknown</td>
<td>affinity chromatography</td>
<td>transport of soluble Merlin complex</td>
<td>[160]</td>
</tr>
<tr>
<td>CPEB-containing mRNPs</td>
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<td>unknown</td>
<td>immunoprecipitation</td>
<td>dendritic transport of mRNPs</td>
<td>[63]</td>
</tr>
<tr>
<td>Peripherin</td>
<td>rat</td>
<td>unknown</td>
<td>immunoprecipitation</td>
<td>intermediate filament subunit transport</td>
<td>[83]</td>
</tr>
<tr>
<td>Neurofilament proteins (NF-L, NF-M, NF-H)</td>
<td>mouse</td>
<td>unknown</td>
<td>in vivo analysis, subcellular fractionation</td>
<td>intermediate filament subunit transport</td>
<td>[84, 161, 162]</td>
</tr>
<tr>
<td>Vimentin</td>
<td>hamster, cattle</td>
<td>unknown</td>
<td>immunostaining, subcellular fractionation, in vitro analysis</td>
<td>intermediate filament subunit transport</td>
<td>[80–82]</td>
</tr>
<tr>
<td>Polyglutamine repeat proteins (Htt, MJD)</td>
<td>Drosophila</td>
<td>unknown</td>
<td>in vivo analysis, subcellular fractionation</td>
<td>axonal transport of polyQ protein complexes</td>
<td>[55]</td>
</tr>
</tbody>
</table>
of signalling molecules to specific locations within the cell, and proteins that regulate kinesin-1 activity.

**Transport of membrane-bound organelles**

The interaction of GRIP1 with kinesin-1 in neurons is important for the transport of kinesin-1 cargos within dendrites of mouse neurons. GRIP1 is a 130 kDa PDZ-domain containing scaffold protein shown to bind mouse KHC [31]. Kinesin-1 function is required for delivery of GRIP1 to dendrites, as reduction of kinesin-1 function in cultured neurons results in perinuclear clustering of GRIP1 [31]. In the context of kinesin–GRIP1 interactions, GRIP1 is required for the delivery of the AMPA and Ephrin-B2 receptors to dendrites [32–37]. It localizes to both glutamatergic and GABAergic synapses, and binds directly to the AMPA receptor [38–40]. GRIP1 also binds to the GABA(A) receptor gamma2 subunit associated protein (GABARAP) [41], members of the Shank family of scaffold proteins [42], the Fraser syndrome protein Fras1 [43], kainate receptor (KAR) subunits GluR5(2b) Glur5(2c) and GluR6 [44], liprin-α [45], glial cell proteoglycan NG2 [46], the prolactin-releasing peptide receptor [47] and the RasGEF GRASP-1 [48]. Loss of GRIP1 function causes defects in dendrite formation in cultured cells [37], embryonic lethality and epidermolysis bullosa in GRIP1 knockout mice [49], and is the genetic basis of the mouse mutation eye-blebs (eb), which is a mouse model of Fraser syndrome [43]. The interaction of kinesin-1 and GRIP1 is interesting because it illustrates the importance of kinesin-mediated transport in development and differentiation. Adaptor proteins such as GRIP1 can increase greatly the number of proteins that may be transported by kinesin, and shows that the destination of cargo–motor complexes is likely governed by the cargo, not the motor protein, as other kinesin-1 cargos, such as amyloid precursor protein, are delivered to axons [50].

Milton is a mitochondria-associated *Drosophila* protein required for the microtubule-dependent transport of mitochondria in axons and photoreceptor cells [51,52]. Mass spectrometry experiments showed that KHC and Milton coimmunoprecipitate in cell extracts [52]. Although a direct association between Milton and KHC has not been demonstrated, the mammalian homologues GRIF-1/ OIP106 interact directly with mammalian KHC in two-hybrid experiments [53]. Milton also has homology to HAP1, which is a mammalian protein that binds to dynein regulator dynactin and huntingtin [54]. These results suggest that Milton and its mammalian homologues provide a docking site for kinesin-1 that enables microtubule-based transport of mitochondria, and has a potential role in linking kinesin-1 to huntingtin, which has been demonstrated in vivo to function in kinesin-1 axonal transport pathways [55].

β-Dystrobrevin is a 71 kDa component of the dystrophin-associated protein complex (DPC) in non-muscle tissues [56, 57]. The DPC is a multi-protein complex that links the actin cytoskeleton to the extracellular matrix [58]. Mouse β-dystrobrevin was used to screen a mouse brain two-hybrid cDNA library; one of the specific interactors was KIF5A [58]. This study also showed that KIF5B can bind specifically to β-dystrobrevin, and that cotransfected KIF5B and β-dystrobrevin colocalize in COS-7 cells. Like other kinesin-binding proteins, β-dystrobrevin contains multiple protein–protein interaction motifs. These motifs bind the DPC components dystrophin, utrophin and syntrophin, which help link cortical actin filaments to the extracellular matrix, and serve as a scaffold for signalling complexes in a manner analogous to SYD (Table 2) [29, 59, 60]. Like GRIP1, β-dystrobrevin is found at the postsynaptic membrane of neurons. Mouse knockouts of the *dtnb* gene encoding β-dystrobrevin appear normal, although localization of specific dystrophin and syntrophin isoforms are disrupted in *dtnb* mutants. These results support a model in which kinesin-1 transports the DPC along microtubules to the plasma membrane through its interaction with β-dystrobrevin.

**mRNP transport**

The directed transport of messenger RNA molecules to discrete locations in the cell has an important role in nervous system development, adaptation and the establishment of the dorsal/ventral and anterior/posterior axes of differentiation during early embryogenesis [61, 62]. Messenger RNAs are transported as part of large protein and RNA macromolecular complexes (mRNPs) analogous to ribosomes, spliceosomes and telomerases. Several groups have identified kinesin-1 as a component of mRNP complexes containing Staufen, fragile X mental retardation protein (FMRP) and other proteins known to be essential for mRNA transport (Table 2). Another set of experiments studying the
function of cytoplasmic polyadenylation element binding protein (CPEB) showed that CPEB copurifies with mRNA, kinesin and dynein in cultured neurons [63]. Interestingly, recent findings show that the heavy chains are required for transport of FMRP in cultured Drosophila cells, but the disruption of KLC function has no effect [64]. Furthermore, localization of posterior mRNAs in Drosophila oocytes requires heavy chain function, but the light chains are not required [65]. These results support biochemical data that some kinesin-1 molecules may lack light chains [66], and open the possibility that kinesin-1 heavy chain can bind to proteins other than KLC with its stalk domain. While the identity of the specific proteins that link kinesin-1 to mRNP is not clear, mRNP transport along microtubules is mediated in some cellular contexts by kinesin-1.

Pathogen transport
Viruses such as herpes simplex virus (HSV) are transported in neurons by anterograde and retrograde transport along microtubules. Infection occurs at the nerve terminus, replication occurs in the nucleus, and new viruses are delivered back to the synapse [67, 68]. Immuno-electron microscopy experiments have shown that kinesin-1 is associated with HSV during anterograde transport from the cell body to the synapse [68]. This interaction was shown to be direct using pulldown and in vitro binding assays, and it is mediated by binding of the US11 viral protein to the C-terminus of KHC [68]. Cultured cell studies show that anterogradely transported HSV particles bind to the kinesin-1 cargo receptor APP [69]; however, the nature of the APP–HSV interaction is not known. It is possible that APP binds to HSV through kinesin-1, or that APP, in some cellular contexts, is recruited to the virus surface and serves as an adaptor for kinesin–virus interactions. A second virus shown to be transported by kinesin-1 is the vaccinia virus; this interaction is mediated by interaction between KLC and the vaccinia A36R envelope protein [70]. This interaction, which was detected using the yeast two-hybrid system, is essential for delivering virus particles to the cell periphery. The interaction between A36R and kinesin-1 is regulated by the Src tyrosine kinase; phosphorylation of A36R disrupts virus–kinesin-1 interactions and facilitates the transition from viral transport along microtubules to actin-based transport [71, 72]. Together, these findings have helped develop a model in which kinesin-1 transports virus particles by binding directly to viral proteins.

Slow axonal transport/transport of cytoskeletal elements
The transport of cytoskeletal elements such as actin, tubulin and intermediate filaments depends upon microtubule motor proteins involved in fast axonal transport [73]. This observation was somewhat surprising, as cytoskeletal elements are associated with slow axonal transport, in which synthesized proteins are transported into the axon 100-fold more slowly than fast axonal transport [73–77]. Therefore, it appears that the motors associated with fast transport in axons also mediate transport of some slow axonal transport cargos. For example, in vitro motility assays using squid giant axons have shown that tubulin undergoes slow axonal transport in a kinesin–dependent manner [78]. Recent experiments have shown that the tubulin binding protein CRMP-2 binds directly to rat kinesin-1 light chains, and that kinesin-1 and CRMP-2 colocalize in cultured neurons. The activity of both kinesin-1 and CRMP-2 are required for neurite elongation and tubulin movement in axons [79]. It is unclear whether CRMP-2 mediates the movement of both monomeric and polymerized tubulin by kinesin along microtubules, or whether kinesin–CRMP-2 interactions mediate both fast and slow axonal transport, but it will be interesting to determine whether CRMP-2 provides a general mechanism for attachment of tubulin to kinesin-1.

Intermediate filament proteins oligomerize to provide cable-like structural support for cells, but they are not a substrate for motor protein translocation. A number of studies have shown that intermediate filament proteins such as neurofilaments, peripherin and vimentin are transported by kinesin-1 along microtubules in cultured cells [76, 80–83] and mouse gene knockout experiments have shown that KIF5A is essential for neurofilament transport [84]. An unresolved issue is how kinesin-1 binds to intermediate filaments, or how kinesin–intermediate filament interactions are regulated. Perhaps there are proteins analogous to CRMP-2 that serve as adaptors between kinesin-1 and specific intermediate filament proteins, or direct coiled-coil interactions between intermediate filaments and the KHC tail domain can occur.
Signal transduction

The GBP/FRAT protein and the Wnt signal transduction pathway play an important role in axis specification during early Xenopus development [85]. During axis specification, the cortex of the egg rotates relative to the inner core cytoplasm in a microtubule- and kinesin-dependent manner [86, 87]. The GBP/FRAT interacts with components of the Wnt signalling pathway; the transport of GBP/FRAT along microtubules to the dorsal side of the embryo negatively regulates the activity of GSK3, which then stabilizes beta-catenin at the dorsal surface [85, 88, 89]. A two-hybrid screen of a mouse brain embryonic cDNA library identified KLC as a FRAT binding protein [90]. Because microtubules at this stage of development are oriented such that the plus ends are located at the dorsal side, it is likely that kinesin-1 transports GBP/FRAT along microtubules to the dorsal side, where localization of its regulatory activity helps establish the dorsal/ventral axis [85]. It is interesting to note that GSK3 has been shown to bind kinesin-1 and negatively regulate its transport activity by disrupting kinesin-cargo interactions [91], but the relevance of this interaction to kinesin-1 transport of GBP/FRAT is unknown.

Activation of the c-Jun NH2-terminal kinase (JNK) signal transduction pathway plays an important role in the regulation of context-dependent responses to extracellular stimuli. In the nervous system, apoptotic pathways are activated by JNKs, which can result in neurodegeneration; however, JNK signal transduction pathways are also essential for nervous system development and differentiation [92]. Efficient JNK signalling is dependent upon the JIP family of scaffold proteins that bind multiple components of the JNK signalling cascade, thereby providing a mechanism for increasing JNK signalling efficiency [93]. Among the binding partners of all known JIP family members is the light chain of kinesin-1, which binds to JIPs through its tetra-tricopeptide repeat domain [29, 30, 94, 95]. Kinesin-1 transports JIPs loaded with signalling proteins along axons, and JNK signalling can be induced by nerve injury during kinesin-dependent anterograde transport [24, 60, 96]. Activation of JNK signalling in axons causes JIP to become associated with the dynein–dynactin retrograde motor complex, presumably to transport the activated JNK signalling complex back to the cell body to facilitate a cellular response to axon injury [96]. An intriguing possibility is that motor proteins transport surveillance machinery into specialized cell structures such as axons to detect changes in cell homoeostasis (such as cell injury), and then transport the activated signal back to the cell body, where transcription-dependent responses can facilitate repair, adaptation or apoptosis.

Regulation of kinesin-1 activity

Several proteins have been shown to regulate the activity of kinesin-1 by binding to its tail domain (Table 2). The UNC-76/FEZ1 is an evolutionarily conserved cytosolic protein required for nervous system function in Drosophila and C. elegans [97, 98]. Using the tail domain of Drosophila KHC as bait, UNC-76 was identified as a specific KHC binding partner [98]. Mutations of the Drosophila Unc-76 gene have a neuromuscular phenotype very similar to that observed in kinesin-1 mutants, whereas FEZ1 overexpression in cultured mammalian cells results in enhanced neurite outgrowth [98, 99]. Other binding partners of UNC-76/FEZ1 include atypical protein kinase C (PKC-ζ) [99], polyomavirus JC virus agnoprotein [100], the mitochondria-associated protein disrupted in schizophrenia 1 (DISC1) [101, 102], Prader–Willi syndrome protein necdin [103], ubiquitin ligase E4B [104] and the next to BRCA1 (NBR1) protein [105]. While the promiscuous interactions of UNC-76/FEZ1 could raise suspicions about binding specificity, in vivo tests using cultured cell models show that FEZ1 interactors enhance (PKC-ζ, E4B, DISC1, necdin) or inhibit (agnoprotein) FEZ1-dependent promotion of neurite outgrowth. A plausible model, based upon the importance of kinesin-1 activity in neurite outgrowth, is that UNC-76/FEZ1 regulation by its binding partners regulates kinesin-1 activity, and the loss of UNC-76/FEZ1 function uncouples kinesin-1 from signals required for neurite outgrowth and axonal transport.

The human protein torsinA belongs to the AAA+ superfamily of chaperone-like proteins [106]. Mutation of the gene DYT1 that encodes torsinA results in the early onset of dystonia, an autosomal dominant neurological disorder with the age of onset between 5 and 25 years [107, 108]. The torsinA protein bound KLC in a yeast two-hybrid screen, and subsequent experiments showed that torsinA and KLC colocalize on membrane vesicles and growth cones of cultured neurons [109]. The interaction of torsinA with membranes may be mediated by its...
interaction with the transmembrane proteins LAP1 and LULL1, which reside on the nuclear envelope and endoplasmic reticulum, respectively [110]. Interestingly, mutant torsinA is found in perinuclear aggregates in the cell body with KLC, suggesting that mutant torsinA could pleiotropically disrupt kinesin-dependent transport pathways by titrating soluble KLC. Based upon the known functions of AAA<sup>+</sup> proteins, torsinA may function to switch kinesin-1 from an inactive to active conformation, or it may help regulate kinesin–cargo interactions [109].

The 14-3-3 family of proteins binds to phospho-serine and phosphothreonine residues on its target proteins, of which over 300 are known to exist [111]. Current models suggest that 14-3-3 proteins may act as adapters to facilitate protein–protein interactions, cause allosteric changes in protein shape upon binding, or block protein–protein interactions through steric interference [111]. Kinesin-1 copurifies with myc-epitope-tagged 14-3-3 in PC12 cultured cells [112]. Subsequent experiments showed that KLC2 binds directly to 14-3-3 in a phosphorylation-dependent manner. Interestingly, 14-3-3 proteins have also been shown to interact with the kinesin-1 superfamily members KIF1C and KIF3 [113, 114], suggesting that phosphorylation-dependent signal transduction pathways may generally regulate motor protein activity by regulating motor–14-3-3 interactions.

**CONCLUSIONS**

The identification of kinesin-1 binding proteins has greatly improved models of kinesin-dependent transport pathways. Transmembrane and peripheral membrane proteins serve as cargo receptors, signaling proteins regulate kinesin–cargo interactions, and molecular chaperones may work by breaking apart kinesin and cargo upon delivery. Most, if not all, kinesin-1 binding proteins have functions distinct from their role in tethering kinesin to cargos. While much is known about the identity of kinesin-1 binding proteins, much remains to be explored. Are there structural features shared by kinesin binding proteins that could help predict whether a novel protein binds kinesin? How are kinesin-1 binding proteins recruited to specific cargos, and how is the subcellular localization of specific kinesin–cargo pairs determined? What is the quaternary structure of mRNPs transported by kinesin? As the spectrum of kinesin-1 interactions in the cell and their importance become better understood, it is likely that those interactions will provide the means to target specific transport events or deliver molecules to specific locations within cells.

A surprising finding is the sheer number of kinesin-1 binding proteins. If one were designing an intracellular transport system de novo, it would seem that only a few kinesin receptors would be required for kinesin-1 transport, or that a conserved domain analogous to the nuclear localization signal or signal peptide would be sufficient for the binding kinesin-1. However, there are many different kinesin-1 binding proteins, and there does not yet appear to be a shared motif sufficient for the binding of the kinesin-1 tail domain. The set of proteins described in Table 2 may be just the beginning, as high-throughput yeast two-hybrid screens, similar functional tests could be performed genetic and molecular tools are available for the validation of interactors conserved in Drosophila and C. elegans. For example, the KHC-binding proteins Ena and UNC-76 exhibit gene dosage–dependent genetic interactions with Drosophila kinesin mutations [27, 98]; similar functional tests could be performed with other kinesin-binding proteins, using either genetic duplications and deletions or transgenes containing overexpression or knockdown constructs. Kinesin function in C. elegans is not essential for viability, so experiments that compare the subcellular localization of kinesin binding proteins in wild type and kinesin mutant backgrounds are relatively straightforward using GFP fusion protein transgenes expressed in the central nervous system [123]. Furthermore, the ability to knockdown gene expression in C. elegans by feeding double-stranded RNA will enable researchers to study transport phenotypes resulting from loss of kinesin-1 binding protein function. The validation of kinesin-1 protein–protein interactions identified in proteome-wide screens has the
potential to increase greatly our understanding of kinesin-dependent intracellular transport, as well as the mechanism by which these transport events occur.

Key Points
- Kinesin-I is an essential motor protein that functions in diverse contexts.
- Kinesin-I binds intracellular cargos through non-covalent interactions with proteins on the cargo surface.
- Binding partners of kinesin-I have other important cellular functions.
- Kinesin-I binding partners have yielded important clues to which transport events are mediated by kinesin-I and how these events may be regulated.
- Proteome-wide interaction screens have identified many putative kinesin-I interactors that await verification.

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