Kinesin carries the signal

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Conventional kinesin has long been known to be a molecular motor that transports vesicular cargo, but only recently have we begun to understand how it functions in cells. Regulation of kinesin involves self-inhibition in which a head-to-tail interaction prevents microtubule binding. Although the mechanism of motor activation remains to be clarified, recent progress with respect to cargo binding might provide a clue. Kinesin binds directly to the JIPs (JNK-interacting proteins), identified previously as scaffolding proteins in the JNK (c-Jun NH2-terminal kinase) signaling pathway. The JIPs can allow kinesin to transport many different cargoes and to concentrate and respond to signaling pathways at certain sites within the cell. The use of scaffolding proteins could be a general mechanism by which molecular motors link to their cargoes.

Transport of vesicles and organelles often occurs over long distances. For example, membrane receptors destined for synapses in neuronal cells need to be transported from the cell body down axons that can reach a meter in length. Diffusion would be prohibitively slow and cells have therefore evolved molecular motors that transport vesicle cargoes along microtubule tracks. The first cytoplasmic microtubule-based motor to be discovered is now called conventional kinesin, but it is only one member of a large family of kinesin-related proteins. It is relatively well understood how kinesin uses the energy of ATP to move towards the plus ends of microtubules. By contrast, our understanding of the cell biology lags far behind. We do not even know the cargo of conventional kinesin, although many have been suggested. In addition, we do not know whether the motor connects directly to the cargo, and it is unclear how the motor picks up cargo at one site of the cell and releases it at its destination. There are several reasons why progress has been slow. First, the motors are not very abundant and, at any given time, only a small fraction of them appear to be associated with vesicle cargo. In addition, several closely related motors are found in a single cell and are often not well differentiated by existing antibodies. Finally, the interaction with cargo is often not very strong, and reconstitution of the interaction between purified vesicles and motors has met with only limited success. Despite these obstacles, there has been exciting recent progress on how motors associate with vesicle cargo and how they are regulated. Here, we will review novel functional aspects of conventional kinesin and discuss some generalizations for other motor proteins.

Kinesin is structured in functional domains

In most species, conventional kinesin is a heterotrimer, consisting of two heavy chains (KHCs) and two light chains (KLCs) (Fig. 1). KHC is composed of multiple domains. At the N-terminus is the catalytic motor domain, which contains the sequences necessary for ATP hydrolysis and microtubule binding. The neck linker and neck domains coordinate the movement of the two motor domains and specify the direction of movement along the microtubule polymer. The stalk domain (coils 1 and 2) is responsible for dimerization of the KHCs and association with the light chains. The tail domain plays a role in regulation of motor activity. Most of these domains are separated by hinges that contain helix-breaking residues. KLC contains two notable domains: an α-helical heptad repeat region that is responsible for the interaction with KHC and a series of six tetratricopeptide repeat (TPR) motifs (Fig. 1). TPR motifs are degenerate repeats, often arranged in tandem, that form protein–protein interaction domains.

The structure of kinesin suggests that cargo binds to the stalk and/or tail region. This is consistent with the fact that these non-motor domains are distinct for different kinesin family members that are believed to transport different cargo. Two regions in conventional kinesin have been proposed for interaction with cargo: the TPR motifs in KLC (Ref. 10) and the KHC coiled tail, which is highly conserved across species. Indeed, as discussed on p. 547, the TPR motifs are now known to interact with cargo. However, as some organisms (such as Neurospora) lack KLC proteins, it is likely that KHC can also provide a binding site for cargo.

Keeping kinesin in check

The majority of kinesin in cells is associated with neither microtubules nor vesicles. It is apparently kept in an inactive state when not transporting cargo, preventing its futile movement along microtubules. How is the motor turned off? Kinesin is in a folded conformation such that the KHC globular tail domain interacts with and inhibits the KHC motor domain (Fig. 2). This tail-inhibition model was originally proposed by Hackney on the basis of experiments with purified kinesin, was then supported by studies in intact cells, and has now been confirmed by several recent studies. In contrast to full-length rat KHC, a truncated version binds readily to microtubules in vitro in the presence of the non-hydrolyzable ATP analog AMP-PNP and accumulates in vivo at the periphery of the cell where the plus ends of microtubules are located. Similarity, truncated Neurospora KHC accumulates at the tips of hyphae. In addition, in vitro, truncated KHC molecules bind more frequently to, and move more smoothly and...
Fig. 1. Domain structure of conventional kinesin. Kinesin heavy chain (KHC, red) contains the catalytic motor domain, the neck linker and neck domains, the coil 1 and coil 2 regions of the stalk domain, and the coiled and globular regions of the tail domain. The regulatory IAK region in the globular tail of KHC (Refs 13,21) is indicated. Kinesin light chain (KLC, blue) contains an α-helical heptad repeat region and six tetratricopeptide repeat (TPR) motifs.

Fig. 2. Models for the activation of kinesin motor activity. Soluble kinesin is in a folded conformation and is kept inactive by an interaction of the kinesin heavy chain (KHC, red) tail domain with the KHC motor domain. Kinesin light chain (KLC, blue) is likely to contribute to self-inhibition. (a) Removal of self-inhibition, and thus activation of motor activity, could occur upon binding of cargo to the tail of kinesin. Alternatively, binding of cargo could be insufficient to activate kinesin and further events such as (b) phosphorylation of kinesin, (c) a localized change in pH, or (d) other cellular factors, might be involved.

The folded conformation of kinesin is necessary but not sufficient for tail-inhibition. For example, removal of the hinge region between coils 1 and 2 results in a folded molecule with increased ATPase activity13,19,20 and, although kinesin is folded under physiological salt concentrations at both pH 6.8 and pH 7.2 (Refs 10,16), it is active for microtubule binding only at pH 6.8 (Ref. 10). Thus, it seems that small conformational changes in folded kinesin are sufficient to relieve the tail-mediated inhibition of the motor domain.

The KLC polypeptides can also contribute to self-inhibition of motor activity. The KHC homodimer alone shows low but measurable levels of both microtubule binding in the presence of AMP-PNP and microtubule-stimulated ATPase activity, as well as infrequent microtubule associations and sporadic runs. Each of these activities can be further suppressed by coexpression of KLC (Refs 10,17,19,20). How KLC affects motor activity is unclear, but it is unlikely to be caused by stabilization of the folded conformation of KHC (Ref. 16).

It seems likely that self-inhibition is a general mechanism of motor regulation. More studies need to be done on the kinesin proteins; however, the Kip1 kinesin of Saccharomyces cerevisiae has also been shown to exhibit an ionic-strength dependent transition between extended and compact conformations23. In addition, the activity of the motor domains of several myosin family members has been shown to be regulated by head-to-tail interactions as well as by association with light chains24–26.

How is kinesin activated?

Although it makes biological sense that kinesin would be activated only on demand, the mechanism by which this could occur is unclear. As the tail of kinesin is involved in both self-inhibition of the motor and in cargo binding, the simplest model proposes that cargo binding to the tail of kinesin would release self-inhibition and allow the motor–cargo complex to bind to, and move along, microtubules (Fig. 2a). In other models, the binding of the motor to its cargo might not automatically lead to activation (Fig. 2b–d). For example, a kinase or phosphatase could modify kinesin bound to a vesicle and activate its motility (Fig. 2b). Although there is plenty of evidence that both KHC and KLC can be phosphorylated26, a regulatory role in vivo remains to be established.

Alternatively, a local pH change could be the direct or indirect trigger for kinesin activation (Fig. 2c). Because kinesin in cell extracts can be activated by a pH shift from 7.2 to 6.8 (Ref. 10), proton leakage from
vesicles with a low lumenal pH could create a local acidic environment on the outside of the vesicle that, in turn, could activate the bound kinesin for motility. Finally, other cellular factors such as chaperone proteins could play a role in regulating the activity of bound motors (Fig. 2d).

Any model of motor activation and inactivation has to explain how these events occur at specific sites in the cell. The coupling of cargo binding to motor activation would accomplish this at the site of departure; however, the release of cargo at the destination would remain unclear. If kinesin transports vesicles to the periphery of the cell, one could imagine that upon fusion with the plasma membrane, extracellular signals activate signals inside the cell, for example kinases, that turn off the motor. This would be compatible with the proposed role for phosphorylation in regulating the activity of kinesin. Alternatively, if local pH determines the activity of a bound kinesin motor, this could provide a means of local activation and inactivation. It is possible that several of these pathways lead to motor activation, similar to the case of the myosins in which both phosphorylation and local changes in calcium concentration play a role in regulation.

Also unclear is what happens to the motor after it has been inactivated at its destination. Several studies have suggested that motors are degraded at the synapse, however, removal of the self-inhibitory KHC tail domain results in the accumulation of the motor at the plus ends of microtubules, suggesting that it is normally recycled for further rounds of transport. Clearly, this discussion only shows that much more work is needed to understand the regulation of kinesin in cells.

### The JIP connection

A breakthrough in the field was achieved recently with the identification of the molecules that link kinesin to its cargo. Two entirely different approaches led to the discovery of the same kinesin-binding proteins, the JIPs (JNK interacting proteins), which were previously identified as scaffolding proteins for the kinesin (c-Jun NH2-terminal kinase) signaling pathway. Bowman et al. performed a genetic screen in Drosophila, reasoning that proteins whose mutation results in a phenotype similar to that of the kinesin null mutant would be likely candidates for kinesin–cargo linker proteins. One protein identified in the screen, JIP-3 (called Sunday Driver, SYD), interacts with kinesin through the TPR motifs of KLC. Importantly, mutation of JIP-3 causes organelles to accumulate in axons, as does the kinesin null mutant. Verhey et al. approached the question of kinesin–cargo linker molecules by reasoning that the TPR motifs of KLC are a good candidate domain for cargo binding. This hypothesis was based on structure–function analysis of kinesin domains and the observation that injection of an antibody to this region into squid axoplasm dissociates organelles from microtubules. Using the TPR motifs as the bait in a two-hybrid screen, Verhey et al. identified JIPs 1, 2 and 3 as binding partners and confirmed these interactions by co-immunoprecipitation. A role for JIP-3 in kinesin-mediated transport is also supported by recent experiments in Caenorhabditis elegans demonstrating that loss-of-function mutations in JIP-3 (UNC-16) lead to the mislocalization of synaptic marker proteins.

JIP-1 and -2 are highly related to each other and interact with the KLC TPR motifs through their...
By contrast, JIP-3, which is unrelated to JIP-1 and -2, interacts with the KLC TPR domain through internal sequences. Previous studies on TPR domain-containing proteins demonstrated that TPR domains recognize short sequences at the C-terminus of their partner protein, identical to the mechanism by which the KLC TPRs interact with JIP-1. For example, the TPR domain of the peroxisomal import receptor PEX5 binds the C-terminal SKL peroxisomal targeting signal, and the two TPR domains of Hsp70/Hsp90 organizing protein (Hop) recognize the C-terminal peptides of Hsp70 and Hsp90. The interaction of the KLC TPR motifs with internal sequences in JIP-3 requires further study, but recently it was shown that the TPR domain of the NADPH oxidase subunit p67phox interacts with internal sequences in the partner protein Rac-GTP. In addition, other protein–protein interaction domains, such as PDZ domains, have been demonstrated to bind to C-terminal or internal peptides in their partner proteins. The X-ray structures of TPR domains show that at least three TPR motifs are required to bind the partner protein, so potentially each KLC polypeptide could bind simultaneously to two JIP molecules.

Are the JIPs a cargo for kinesin? The JIPs are found in all organisms and cells that express KLC. They display a highly polarized subcellular localization at the tips of neuronal cell processes and in projections from the surface of insulinoma cells, which places them at the plus ends of microtubules and is consistent with their transport by a plus-end directed motor. In addition to the genetic evidence in Drosophila, two experiments demonstrated that the JIP proteins are indeed a cargo for kinesin in vivo. First, mutations in JIP-1 that abolish its interaction with the KLC TPR motifs also abolish the proper localization of JIP-1 to the nerve terminal. Second, expression of dominant negative kinesin constructs in cultured neuronal cells interfered with the transport of JIP-1 protein to the tips of the neurites.

The JIPs can also serve as adaptors between kinesin and other cargoes. The JIPs were originally identified as organizers of the NK signaling pathway in which they bind to the mitogen activated protein kinase kinase kinase (MAPKKK), MAPKK and MAPK components of the pathway. Bringing the kinases into spatial proximity can facilitate their successive activation while averting their activation by other signaling pathways. In addition, JIP-1 binds to the nucleotide exchange factor of the small G protein Rho (RhoGEF), and JIP-1 and -2 bind to the cytoplasmic domains of transmembrane receptors such as ApoER2, the receptor for the Reelin ligand that controls neuronal migration and is defective in the naturally occurring 'reeler' mouse. Recent evidence indeed shows that kinesin and JIP-1 are in a complex with ApoER2 and a MAPKKK (Ref. 18). These data indicate that JIP-1 and -2 can serve as a link between kinesin and other cargoes (Fig. 3, Table 1).

The situation with JIP-3 is less clear. Bowman et al. proposed that JIP-3 is a membrane protein localized to post-Golgi vesicles, but JIP-3 does not contain an unequivocal transmembrane domain. Although JIP-3 is a soluble scaffolding protein for the JNK signaling pathway, and the endogenous protein is localized at the tips of neurites. Although JIP-3 is

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**Fig. 4.** Model for the regulation of kinesin activity by transported signaling molecules. In the cell body of a nerve cell, scaffolding proteins assemble signaling complexes that include cytoplasmic signaling molecules, such as kinases, and transmembrane (TM) proteins. Kinesin carries the entire complex towards the plus end of the microtubule in the nerve terminal. Upon arrival, the transported vesicles fuse with the plasma membrane (step 1), exposing the TM receptor to its extracellular ligand for binding (step 2). Activation of the TM receptor by ligand binding would then stimulate intracellular signaling kinases (step 3), which would activate other proteins in the cell and inactivate kinesin (step 4).
Thus more likely to serve, similar to IP-1 and -2, as a soluble protein that links kinesin indirectly to its vesicle cargo, the interacting membrane protein(s) remain to be identified (Fig. 3, Table 1). A direct link between kinesin and membrane-bound vesicles has also been suggested for the amyloid precursor protein (APP), the membrane protein that serves as the precursor for peptides that cause Alzheimer’s disease\(^6\). Although the in vitro evidence for APP being a cargo of kinesin is strong, more work is needed to understand how the TPR motifs interact directly with the short cytoplasmic tail of APP, which does not contain the C-terminal recognition sequence of either IP-1 and -2 or any sequence similarity to IP-3.

### Connecting motor proteins to signaling pathways

The indirect linkage of motors and vesicles via scaffolding proteins could be a general mechanism employed by motor proteins, and several examples are listed in Table 1. This general concept has several obvious attractions. First, because scaffolding proteins allow a single motor molecule to associate with several different membrane and soluble cargo proteins, this model would allow motor proteins to transport many different cargos at the same time. Second, scaffolding proteins could assemble multiprotein complexes at great distances from their final locations. Particularly in the case of neuronal cells, it seems advantageous to assemble components of a functional complex in the cell body where the synthesis of cytoplasmic and membrane proteins takes place, load it onto a motor protein and then carry the entire complex to the tips of the neuronal processes (Fig. 4). Third, scaffolding proteins could allow signal transduction pathways to regulate motor activity. For example, the association of kinesin with the JIPs could enable the JNK kinase pathway to phosphorylate either kinesin itself or an associated protein, thereby activating the motor at the point of departure and/or inactivating it at the point of destination (Fig. 4). Recent experiments in C. elegans indeed support a role for the JNK signaling pathway in kinesin-mediated axonal transport (D. Byrd and Y. J in, pers. commun.). Finally, the new concept would allow motor proteins to localize signal transduction pathways in cells. For example, the transport of the ApoER2 receptor in a JIP–kinesin complex to the tip of the axon could allow a localized activation of the Disubstituted Aryl Cysteine (Disaccharide) receptor’s signaling pathways, and perhaps of the JNK signaling pathway, in response to the Reelin protein\(^6\). Likewise, transport of the PDBZ-domain-containing milk proteins by the motor KIF17 localizes neurotransmitter receptors and ion channels at postsynaptic junctions\(^6\). The emerging concept that motors transport vesicle cargo and simultaneously localize signaling pathways brings together previously disparate fields.

### References


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