Comment

Kinesin Delivers: Identifying Receptors for Motor Proteins

P.J. Hollenbeck

Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907

What is it about movement in cells that commands our attention? What biologist has not enjoyed turning a microscope on a cell, almost any cell, really, and watching all the commotion? When vesicles, mitochondria, chloroplasts, nuclei, or chromosomes move, we are being treated to an elegant, easily observable manifestation of molecular events. Decades of effort to understand intracellular movement have given rise to two of the great thrusts of modern cell biology: the study of which things go where, usually referred to as intracellular trafficking; and the identification of the protein machines that generate movement, the molecular motors. But we have an incomplete picture of how the cell's array of motor proteins gives rise to the variety of journeys that their cargoes make. The kinesin motors that generate movement along microtubule tracks are a case in point (Vale and Fletterick, 1997). As with other motor proteins, the dyneins or myosins, the study of kinesin by the methods of molecular genetics has demonstrated that kinesins are a large family of related motor proteins present across all eukaryotic phyla, and numbering 30–40 members in humans and mice (Kim and Endow, 2000; http://www.blocks.fhcrc.org/~kinesin). Most analysis of this diversity thus far indicates that different kinesins serve to move different cargoes in the cell (Manning and Snyder, 2000). So, where does the trafficking information for the motor proteins reside? What is the cargo "receptor" for kinesin, and what specific protein–protein interactions govern this important matchmaking in the cell? Most work on this question has focused on the first kinesin family member to be discovered, so-called "conventional" kinesin, or kinesin-I. In the case of kinesin-I, the ER membrane protein kinectin has been proposed to be a cargo receptor, but its restricted cellular and phylogenetic distributions (Toyoshima and Sheetz, 1996; Goldstein and Gunwardena, 2000) have prompted some investigators to look further.

This search has recently borne fruit: two groups have reported that kinesin-I binds to cargoes via a set of proteins involved in intracellular signaling (Bowman et al., 2000; Verhey et al., 2001). The proteins, JIP-1, JIP-2, and JIP-3, are thought to serve as scaffolding proteins for the c-Jun NH_2-terminal kinase (JNK)[1] signaling pathway (Davis, 2000). The high affinity and specificity of kinesin binding to the JIP proteins indicates that the complex pairing of motors and cargoes will soon be on the same footing with other protein–protein interactions essential to membrane traffic. Perhaps more exciting, these results connect the organization of organelle traffic with that of cell signaling: whereas the JIP proteins themselves apparently function to hold enzymes of the JNK pathway in proximity to each other, their interaction with kinesin-I may also determine the collective spatial organization of the signaling pathway within the cell.

One potential kinesin receptor was identified by Bowman et al. (2000) when they screened *Drosophila melanogaster* mutants for elements of the machinery of movement other than the motor proteins themselves. They examined larvae with potential axonal transport phenotypes previously seen in kinesin-I mutants (Hurd and Saxton, 1996; Gindhart et al., 1998), and identified a *Drosophila* homologue of the proposed mammalian JNK scaffolding protein, JIP-3. It was clearly essential for transport in *Drosophila*, as mutant larvae had accumulations of vesicles along the axons of their segmental nerves. GFP-tagged JIP-3 protein was expressed in CV-1 cells, where it colocalized with kinesin-I and with Golgi and early secretory vesicles, but not with mitochondria or the ER-to-Golgi intermediate compartment. When they probed the interaction of kinesin-I and JIP-3 by yeast two-hybrid analysis and coprecipitation methods, they found that the NH_2-terminal domain of JIP-3 bound a region of the kinesin light chain (LC) that contains six tetratricopeptide repeat (TPR) motifs (Blatch and Lasle, 1999). They propose that JIP-3 (which they named Sunday Driver) is an organelle membrane protein whose interaction with kinesin is required for transport.

In retrospect, it is not surprising that potential cargo receptors specifically bind the TPR domain of the kinesin LC. Kinesin-I is a heterotetramer comprised of 2 LCs and two heavy chains (HCs; see Fig. 1). It has been thought for some time that the kinesin tail region binds to the motor's cargo (Vale and Fletterick, 1997). Both the HCs and LCs occupy this region of the tetramer, but the preponderance of genetic and biochemical data indicate that the LCs are important or even essential for cargo binding (Yu et al., 1992; Stenoien and Brady, 1997; Gindhart et al., 1998; Tsai et al., 2000). Furthermore, the TPR domain of the LC stands out specifically

Address correspondence to Dr. Peter J Hollenbeck, Department of Biological Sciences, Purdue University, 2237 Lilly Hall, West Lafayette, IN 47907. Tel.: 765-496-3378. Fax: 765-494-0876. E-mail: phollenb@purdue.edu

[1]*Abbreviations used in this paper:* JNK, c-Jun NH_2-terminal kinase; LC, light chain; TPR, tetratricopeptide repeat;

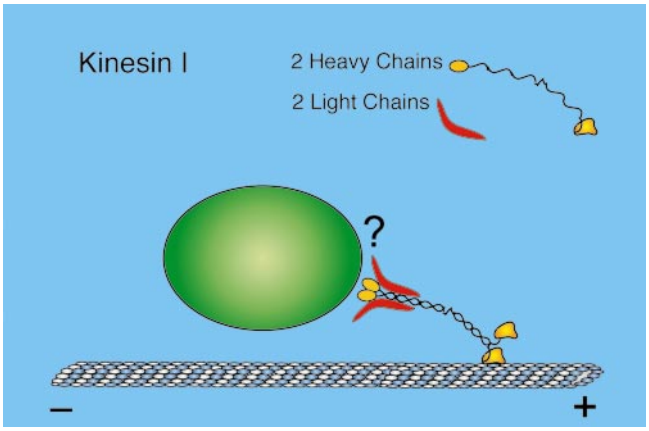

Figure 1. Kinesin-I is a heterotetramer formed by a coiled-coil interaction between two heavy chains (HCs) and the binding of a LC to the COOH-terminal region of each HC. Each HC has an NH_2-terminal catalytic motor domain that interacts with a microtubule during movement. The heterotetramer binds to its cargo at its tail, as shown. However, the exact nature of the interaction, what kinesin is binding to, and how, has not been clear. The LCs have been thought to be essential for this interaction, and the two recent papers discussed here (Bowman et al., 2000; Verhey et al., 2001) identify the TPR domain of the LC as a site of cargo binding. For illustrative purposes, the kinesin molecule is drawn here approximately three times larger than true scale relative to the microtubule and vesicle. (Figure courtesy of W.M. Saxton)

as a likely binding site because antibodies directed against it disrupt kinesin–cargo interactions (Stenoien and Brady, 1997), and it has well characterized, specific protein binding properties (Blatch and Lasle, 1999).

The study by Verhey et al. (2001, this issue) used this view of the LC as a point of departure. They employed the kinesin LC TPR domain as bait in a yeast two-hybrid screen of a mouse brain cDNA library and fished out three binding partners for kinesin: not only JIP-3, but also JIP-1 and JIP-2, which are unrelated to JIP-3, but very similar to each other. Closer examination of kinesin–JIP binding confirmed that the LC TPR domain binds the NH_2-terminal region of JIP-3. But JIP-1 and JIP-2 resembled more closely other TPR-binding proteins, in that they interacted with kinesin via their COOH termini. Verhey et al. (2001) found that the mutation of a single tyrosine three residues from the COOH terminus eliminated JIP-1 binding to kinesin. This surprising result invites comparisons between motor–cargo binding and tyrosine-based sorting signals for protein traffic (Bonifacino and Dell'Angelica, 1999). So kinesin binds, but does it deliver? To address this, they examined the distribution JIP-1 in neuronal cell lines (Fig. 2) and found that the kinesin-I/JIP-1 interaction was necessary for JIP-1 to accumulate in the tips of the neurites. They propose that the transport of JIP-1 to the neurite tip by kinesin-I is important in neuronal development.

But is this really to do with signaling pathways, or is JIP-1 doing double duty as a kinesin receptor? When Verhey et al. (2001) examined whether kinesin also carries any of the signaling proteins that are thought to bind to the JIP-1 scaffold, they identified one kinase in the kinesin/JIP-1 complex that functions upstream of the JNK pathway. Also present was ApoER2, a membrane receptor that may serve as the link between a kinesin/JIP-1 complex and the cargo membrane. They suggest that the link between kinase scaffolding proteins and kinesin motors serves not only to localize membrane proteins and conventional cargoes, but to provide motor-driven spatial regulation of cytoplasmic signaling pathways.

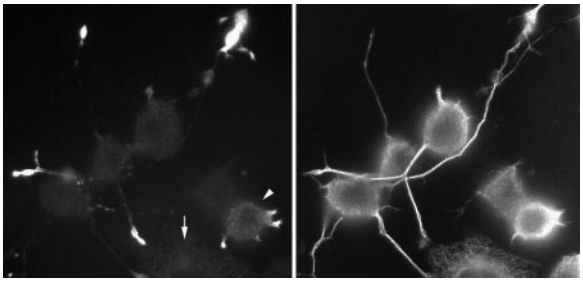

Figure 2. Immunofluorescent staining of differentiating CAD cells shows the expression and localization of endogenous JIP-1 (left) and tubulin (right) proteins. In cells that have not yet begun to extend neurites (arrow at left), JIP-1 expression and localization are not apparent. But, as soon as this neuron-like cell line has established neurites, JIP-1 is localized to their tips via kinesin-I. The cell denoted by an arrowhead at left is just beginning to produce neurites, whereas the two cells near the center of the field have longer neurites and bright JIP-1 staining at the distal ends. (Image courtesy of K.J. Verhey)

So, is it time to start drawing seminar slides of kinesin-based transport centered on these two classes of receptors, JIP-1/2 and JIP-3? Of course not! Already another cargo receptor for kinesin-I has been identified: the amyloid precursor protein, a well-known membrane protein that also interacts with the LC TPR domain (Kamal et al., 2000). In addition, there is good evidence that some cargoes bind the kinesin LC, via other receptors, outside the TPR domain. For example, although several classes of organelles are thought to be moved by kinesin-I, Verhey et al. (2001) found that blocking the binding of the kinesin LC TPR domain to other proteins did not disrupt the organelle distribution in CAD cells. Also, antibody disruption of binding to the LC only displaces about one-third of the kinesin-I from vesicles (Yu et al., 1992), even when the antibodies are directed specifically against the TPR domain (Stenoien and Brady, 1997). And it remains possible that some cargoes bind not the LCs, but the HCs, of kinesin-I at least in some organisms. Not only can the HCs of heterotetrameric kinesin-I bind vesicles in vitro (Skoufias et al., 1994), but *Neurospora crassa* kinesin, which lacks LCs completely, binds its cargo via a site on the HC that is highly conserved among members of the kinesin-I family (Seiler et al., 2000).

The existence of many motor protein receptors, with or without signaling functions, seems not only likely but essential, given the plethora of motors and cargoes in the cell. Indeed, receptors or signaling molecules that bind to other kinesin family members have been reported already (Nagata et al., 1998; Nakagawa et al., 2000; Setou et al., 2000). If you would like to take part in the construction of the phylogenetic tree of motor protein receptors, you had better order your PCR primers soon.

I am grateful to L.S.B. Goldstein and K.J. Verhey for discussions and to an anonymous reviewer for helpful suggestions for the manuscript.

Work in the author's laboratory is supported by a grant from the National Institutes of Health (NS27073).

Submitted: 12 February 2001
Accepted: 12 February 2001

References

Blatch, G.L., and M. Lasle. 1999. The tetratricopeptide repeat: a structural motif mediating protein–protein interactions. *Bioessays.* 21:932–939.

Bonifacino, J.S., and E.C. Dell'Angelica. 1999. Molecular bases for the recognition of tyrosine-based sorting signals. *J. Cell Biol.* 145:923–926.

Bowman, A.B., A. Kamal, B.W. Ritchings, A.V. Philp, M. McGrail, J.G. Gindhart, and L.S.B. Goldstein. 2000. Kinesin-dependent axonal transport is mediated by the Sunday driver (SYD) protein. *Cell*. 103:583–594.

Davis, R.J. 2000. Signal transduction by the JNK kinase group of MAP kinases. *Cell*. 103:239–252.

Gindhart, J.G., C.J. Desai, S. Beushausen, K. Zinn, and L.S.B. Goldstein. 1998. Kinesin light chains are essential for axonal transport in *Drosophila*. *J. Cell Biol.* 141:443–454.

Goldstein, L.S.B., and S. Gunwardena. 2000. Flying through the *Drosophila* cytoskeletal genome. *J. Cell Biol.* 150:F63–F68.

Hurd, D.D., and W.M. Saxton. 1996. Kinesin mutations cause motor neuron disease phenotypes by disrupting fast axonal transport in *Drosophila*. *Genetics*. 144:1075–1085.

Kamal, A., G.B. Stokin, Z.H. Yang, C.H. Xia, and L.S.B. Goldstein. 2000. Axonal transport of amyloid precursor protein is mediated by direct binding to the kinesin light chain subunit of kinesin-I. *Neuron*. 28:449–459.

Kim, A.J., and S.A. Endow. 2000. A kinesin family tree. *J. Cell Sci.* 113:3681–3682.

Manning, B.D., and M. Snyder. 2000. Drivers and passengers wanted! The role of kinesin-associated proteins. *Trends Cell Biol.* 10:281–289.

Nagata, K., A. Puls, C. Futter, P. Aspenstrom, E. Schaefer, T. Nakata, N. Hirokawa, and A. Hall. 1998. The MAP kinase kinase kinase MLK2 colocalizes with activated JNK along microtubules and associates with kinesin superfamily member KIF3. *EMBO (Eur. Mol. Biol. Organ.) J.* 17:149–158.

Nakagawa, T., M. Setou, D.H. Seog, K. Ogasawara, N. Dohmae, K. Takio, and N. Hirokawa. 2000. A novel motor, KIF13A, transports mannose-6-phosphate receptor to plasma membrane through direct interaction with AP-1 complex. *Cell*. 103:569–581.

Seiler, S., J. Kirchner, C. Horn, A. Kallipolitou, G. Woehlke, and M. Schliwa. 2000. Cargo binding and regulatory sites in the tail of fungal conventional kinesin. *Nat. Cell Biol.* 2:333–338.

Setou, M., T. Nakagawa, D.H. Seog, and N. Hirokawa. 2000. Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor-containing vesicle transport. *Science*. 288:1796–1802.

Skoufias, D.A., D.G. Cole, K.P. Wedaman, and J.M. Scholey. 1994. The carboxy-terminal domain of kinesin heavy chain is important for membrane binding. *J. Biol. Chem.* 269:1477–1485.

Stenoien, D.L., and S.T. Brady. 1997. Immunochemical analysis of kinesin light chain function. *Mol. Biol. Cell.* 8:675–689.

Toyoshima, I., and M.P. Sheetz. 1996. Kinectin distribution in chicken nervous system. *Neurosci. Letters.* 211:171–174.

Tsai, M.Y., G. Morfini, G. Szebenyi, and S.T. Brady. 2000. Release of kinesin from vesicles by hsc70 and regulation of fast axonal transport. *Mol. Biol. Cell.* 11:2161–2173.

Vale, R.D., and R.J. Fletterick. 1997. The design plan of kinesin motors. *Annu. Rev. Cell Dev. Biol.* 13:745–777.

Verhey, K.J., D. Meyer, R. Deehan, J. Blenis, B.J. Schnapp, T.A. Rapoport, and B. Margolis. 2001. Cargo of kinesin identified as JIP scaffolding proteins and associated signaling molecules. *J. Cell Biol.* 152:959–970.

Yu, H., I. Toyoshima, E.R. Steuer, and M.P. Sheetz. 1992. Kinesin and cytoplasmic dynein binding to brain microsomes. *J. Biol. Chem.* 267:20457–20464.