

Cargo of Kinesin Identified as JIP Scaffolding Proteins and Associated Signaling Molecules

Kristen J. Verhey,* Debra Meyer,[§] Renéé Deehan,* John Blenis,[‡] Bruce J. Schnapp,[‡] Tom A. Rapoport,* and Ben Margolis[§]

*Howard Hughes Medical Institute and [‡]Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115; and [§]Howard Hughes Medical Institute, Department of Internal Medicine, and Department of Biological Chemistry, University of Michigan Medical Center, Ann Arbor, Michigan 48109

Abstract. The cargo that the molecular motor kinesin moves along microtubules has been elusive. We searched for binding partners of the COOH terminus of kinesin light chain, which contains tetratricopeptide repeat (TPR) motifs. Three proteins were found, the c-jun NH₂-terminal kinase (JNK)-interacting proteins (JIPs) JIP-1, JIP-2, and JIP-3, which are scaffolding proteins for the JNK signaling pathway. Concentration of JIPs in nerve terminals requires kinesin, as evident from the analysis of JIP COOH-terminal mutants and dominant negative kinesin constructs. Coprecipitation experiments suggest that kinesin carries the JIP scaffolding

preloaded with cytoplasmic (dual leucine zipper-bearing kinase) and transmembrane signaling molecules (the Reelin receptor, ApoER2). These results demonstrate a direct interaction between conventional kinesin and a cargo, indicate that motor proteins are linked to their membranous cargo via scaffolding proteins, and support a role for motor proteins in spatial regulation of signal transduction pathways.

Key words: kinesin • molecular motors • scaffolding protein • JNK signaling • Reelin

Introduction

Kinesins are a family of motor proteins that use the energy of ATP hydrolysis to move cargoes along microtubules (MTs).¹ The founding member of the family, conventional kinesin, was identified in squid axoplasm over 15 years ago as a motor that could drive fast axonal transport. A large body of biochemical and localization experiments have implicated kinesin in a variety of processes in many cell types (Brendza et al., 2000a,b; Goldstein and Gunawardena, 2000; Goldstein and Yang, 2000; Kamal and Goldstein, 2000; Rogers and Gelfand, 2000; Terada et al., 2000; Yabe et al., 2000). However, evidence linking kinesin directly to any of the proposed cellular roles is lacking; that is, a molecular relationship between kinesin and a cargo has yet to be established.

Genetic studies in *Caenorhabditis elegans*, *Drosophila melanogaster*, and mice have failed to identify the primary

cargo of conventional kinesin although, they have demonstrated that kinesin is required for axonal transport in neuronal cells (Saxton et al., 1991; Gho et al., 1992; Patel et al., 1993; Hurd and Saxton, 1996; Hurd et al., 1996; Gindhart et al., 1998; Tanaka et al., 1998; Rahman et al., 1999). Mammalian conventional kinesin (kinesin family member [KIF]5B) associates with the actin-based motor MyoVA, but neither motor is envisioned as the cargo of the other; rather, this interaction is likely to be important for coordinating the transition of vesicle transport from MTs to actin filaments (Brown, 1999; Huang et al., 1999; Goode et al., 2000). Attempts to identify the kinesin-cargo linker molecule have also met with limited success. The protein kinectin was identified biochemically as a potential kinesin-cargo linker; however, its role has been controversial. No homologue for kinectin exists in the *Drosophila* genome, arguing against its role in kinesin-driven transport (Goldstein and Gunawardena, 2000).

Conventional kinesin is a heterotetramer of two kinesin heavy chains (KHCs) and two kinesin light chains (KLCs). KHC contains three domains: an NH₂-terminal motor domain, a central coiled coil stalk domain for dimerization, and a COOH-terminal globular domain. KLC contains an NH₂-terminal α -helical domain that associates with the KHC stalk and six tetratricopeptide repeat (TPR) motifs (Vale and Fletterick, 1997; Diefenbach et al., 1998; Verhey et al., 1998). The roles of most of the domains of kinesin

K.J. Verhey and D. Meyer contributed equally to this work.

Address correspondence to Kristen Verhey or Bruce Schnapp. K. Verhey, Department of Cell Biology, Harvard Medical School, 240 Longwood Ave., Boston, MA 02115. Tel.: (617) 432-1612. Fax: (617) 432-1190. E-mail: kverhey@hms.harvard.edu

B. Schnapp, Department of Cell Biology, Harvard Medical School, 240 Longwood Ave., Boston, MA 02115. Tel.: (617) 432-3818. E-mail: bschnapp@hms.harvard.edu

¹Abbreviations used in this paper: DLK, dual leucine zipper kinase; HA, hemagglutinin; JIP, JNK-interacting protein; JNK, c-jun NH₂-terminal kinase; KHC, kinesin heavy chain; KIF, kinesin family member; KLC, kinesin light chain; MAP, mitogen-activated kinase; MKK, MAP kinase kinase; MT, microtubule; PP5, protein phosphatase 5; TPR, tetratricopeptide repeat.

have been elucidated, and, in particular, many of the molecular details of how the motor domain of kinesin walks processively along an MT have been clarified (for reviews see Vale and Fletterick, 1997; Manning and Snyder, 2000; Vale and Milligan, 2000; Wade and Kozielski, 2000; Woehlke and Schliwa, 2000).

Given the processive nature of the kinesin motor in vitro, it must be tightly regulated in vivo to prevent its accumulation at the cell periphery. Indeed, cargoless kinesin is inhibited from movement along MTs due to a folded conformation that allows the KHC COOH-terminal domain to associate physically with the NH₂-terminal motor domain and thus inhibit motor activity (Verhey et al., 1998; Coy et al., 1999; Friedman and Vale, 1999; Stock et al., 1999; Manning and Snyder, 2000). KLC stabilizes the inhibitory interaction of the KHC COOH-terminal and motor domains (Verhey et al., 1998). How the motor is activated is unclear. In the simplest scenario, cargo binding alone would activate kinesin, but it is possible that additional activation, such as posttranslational modification (Hollenbeck, 1993; Lee and Hollenbeck, 1995; De Vos et al., 2000), local changes in the cellular environment (Verhey et al., 1998), or chaperone binding (Tsai et al., 2000), is required.

The tail of the kinesin molecule, encompassing the KHC COOH terminus and KLC, is the most likely region to be involved in cargo binding, although it is not established whether regions of both KHC and KLC are required. Several studies point to a role for the KHC COOH terminus in cargo binding, as this region binds membranes (Skoufias et al., 1994; Bi et al., 1997). In addition, several studies in the filamentous fungus *Neurospora crassa* have implied that KHC alone binds to cargo (Steinberg and Schliwa, 1995; Kirchner et al., 1999a; Seiler et al., 2000). However, the results of disruption of KLC in *Drosophila* and mice suggest an essential role for KLC in kinesin function (Gindhart et al., 1998). Our previous work led us to suggest that the TPR motifs of KLC are involved in cargo binding, as all other notable domains of KHC and KLC could be assigned a function either in motor activity or in the interaction between the two chains (Verhey et al., 1998). The KLC TPR motifs are highly conserved across species, and TPR motifs are known to be involved in protein-protein interactions. A role for the KLC TPRs in cargo binding is supported by experiments in which an antibody to this region, when injected into squid axoplasm, dissociates organelles from MTs (Stenoien and Brady, 1997).

TPR motifs consist of degenerate 34-amino acid repeats, often arranged as multiple copies in tandem, and are present in proteins involved in diverse cellular processes such as phosphate transfer, cell cycle control, protein folding, and mitochondrial and peroxisomal import (for reviews see Blatch and Lassel, 1999; Groves and Barford, 1999). In the case of two TPR-containing proteins, the Hsp70/Hsp90 organizing protein Hop and the peroxisomal import receptor PAS8, three or more TPR motifs form a TPR domain that recognizes short stretches of primary amino acid sequence at the COOH termini of their target proteins, -EEVD in Hsp70 and Hsp90 and -SKL in peroxisomal proteins, respectively (Terlecky et al., 1995; Scheufler et al., 2000).

To identify proteins that interact with kinesin, we under-

took a yeast two-hybrid screen using the TPR motifs of KLC as a bait. Three proteins were identified, c-jun NH₂-terminal kinase (JNK)-interacting protein (JIP)-1, JIP-2, and JIP-3. All three have been proposed to act as scaffolding proteins for the JNK kinase signaling pathway. Here, we report that disruption of the kinesin-JIP interaction in neuronal cells results in mislocalization of the JIP-1 scaffolding protein, suggesting that JIPs are a cargo for kinesin. Coprecipitation of kinesin and JIP-1 with the upstream kinase dual leucine zipper kinase (DLK) and the Reelin receptor, ApoER2, suggests that kinesin carries the JIP scaffolding proteins as part of a multiprotein complex. Our results provide the first demonstration of a direct interaction between conventional kinesin and a cargo. In addition, these results suggest a general model in which motor proteins are linked to their membranous cargo via scaffolding proteins. Finally, we discuss a model in which motor proteins localize signal transduction pathways and may in turn be regulated by them.

Materials and Methods

Plasmids

Plasmids encoding Myc-tagged rat KHC and the truncation Myc-KHC-891, as well as HA (hemagglutinin)-tagged rat KLC and the truncation HA-KLC-176, have been described previously (Verhey et al., 1998). To make a construct containing the six TPR motifs of rat KLC in pCDNA3 expression vector, the plasmid pCDNA3-HA-KLC-488 (Verhey et al., 1998) was digested with XhoI, which cuts immediately after the HA tag, and NotI, which cuts in the rat KLC gene preceding the TPR motifs. The linear piece was religated with a linker designed to conserve the reading frame and introduce an NheI site between the HA tag and the TPR motifs. For the two-hybrid system, sequences encoding the TPR motifs of KLC were subcloned from pCDNA3-HA-KLC-TPR6 into vector pGBDU-C1 (James et al., 1996), whereas sequences encoding the TPR motifs of protein phosphatase 5 (PP5) (cDNA gift of D. Barford, University of Oxford, Oxford, UK) were obtained by PCR with primers containing the appropriate restriction sites and were subcloned into pGBDU-C1 (James et al., 1996).

Flag-tagged JIP-1, JIP-2, and JIP-3 in mammalian expression vectors were a gift of Dr. Roger Davis (University of Massachusetts Medical School, Worcester, MA) and have been described previously (Whitmarsh et al., 1998; Yasuda et al., 1999; Kelkar et al., 2000). Myc-tagged JIP-1 (307-711) was described previously (Meyer et al., 1999). Myc-tagged full-length JIP-1 was generated by annealing overlapping oligonucleotides encoding human JIP-1 amino acids 1-120. This sequence was amplified by PCR and then cloned into RK5 Myc-JIP-1 plasmid containing the rest of the human JIP-1 sequence to generate the full-length gene. Myc JIP-1 (307-700) was generated by mutating tyrosine 701 to a stop codon, and Myc JIP-1 (P704A) and (Y709A) were generated by making the appropriate point mutations within the Myc JIP-1 (307-711) plasmid using the QuickChange site-directed mutagenesis kit (Stratagene).

Two-Hybrid Screen

The yeast two-hybrid screen was carried out using a MATCHMAKER GAL4 two-hybrid system 3 (CLONTECH Laboratories, Inc.), and all methods were done according to the manufacturer's protocols. In brief, PJ69-4A cells (James et al., 1996) were transformed with pGBDU-KLC TPRs and selected for growth on media lacking uracil. PJ69-4A KLC TPR yeast were subsequently transformed with a mouse brain MATCHMAKER cDNA library in plasmid pACT2 (CLONTECH Laboratories, Inc.). Positive interacting clones were selected for growth on plates lacking uracil, leucine, and histidine and were supplemented with 10 mM 3-aminotriazole. Positive clones were subsequently tested for growth on plates lacking uracil, leucine, and adenine and for β -galactosidase activity. To confirm the specificity of the interacting plasmids, PJ69-4A yeast expressing only the library plasmid were mated to Y187 yeast expressing the KLC TPRs, the PP5 TPRs, or the bait plasmid pGBDU alone.

Cells, Transfection, Immunofluorescence, and Immunoprecipitation

African green monkey kidney cells (COS) were grown as described (Verhey et al., 1998), except that FuGene 6 (Roche) was used for transfections according to the manufacturer's instructions. Human embryonic kidney HEK293 cells were grown and transfected as described (Meyer et al., 1999). For immunoprecipitation, COS or 293 cells were washed, lysed, and processed for immunoprecipitation with mAbs to the HA, Myc, and Flag tags (12CA5, 9E10, and M2 [Sigma-Aldrich]) as described (Verhey et al., 1998; Meyer et al., 1999). Immunoprecipitates were detected by immunoblotting with polyclonal antibodies to the Myc and HA tags (Santa Cruz Biotechnology, Inc.). A polyclonal antibody to the Flag tag was generated in rabbits against the Flag peptide (DYKDDDDKSHC) and affinity-purified as described (Verhey et al., 1998).

The mouse central nervous system catecholaminergic cell line CAD (Qi et al., 1997) was grown in a 1:1 mixture of F12-DME (Bio-Whittaker) plus 10% FBS (HyClone). Cells were transfected using FuGene 6 (Roche) in Opti-MEM (GIBCO BRL) according to the manufacturer's instructions. The mouse neuroblastoma cell line NIE 115 was maintained as described previously (Meyer et al., 1999) and transfected using Effectene (QIAGEN) according to the manufacturer's instructions. 12 h after transfection, cells were replated on coverslips and switched to differentiation media (F12-DMEM without serum or DME without serum). 48–72 h later, the cells were fixed and processed for indirect immunofluorescence as described (Verhey et al., 1998; Meyer et al., 1999). Localization of endogenous JIP-1 protein was detected using an affinity-purified polyclonal antibody that is specific for JIP-1 (Meyer et al., 1999). Microtubules and neurofilaments were detected with mAbs to tubulin (DM1 α ; Sigma-Aldrich) and neurofilaments H + M (Zymed Laboratories), whereas mitochondria were labeled with MitoTracker (Molecular Probes). Goat anti-mouse fluorescein isothiocyanate-conjugated and goat anti-rabbit rhodamine-conjugated secondary antibodies were used (Molecular Probes).

Immunoprecipitation and MT Binding from Rat Brain

Rat brains were homogenized in buffer A (50 mM Hepes-KOH, pH 7.4, 50 mM potassium acetate, 6 mM magnesium acetate, 1 mM EDTA, 250 mM sucrose, 1 mM DTT, 1 mM PMSF plus protease inhibitors [10 μ g/ml leupeptin, 5 μ g/ml chymostatin, 3 μ g/ml elastatinal, 1 μ g/ml pepstatin]). The homogenate was subjected to a low speed spin (10,000 g, 10 min, 4°C), followed by a high speed spin (>100,000 g, 45 min, 4°C). The resultant supernatant was removed, and the pellet was resuspended in buffer C (50 mM Hepes-KOH, 250 mM sucrose, 1 mM PMSF, protease inhibitors). Aliquots were frozen in liquid nitrogen and stored at -80°C. 1,000–1,500 μ g of high speed supernatant was diluted with an equal volume of buffer B (20 mM Hepes-KOH, pH 7.4, 115 mM potassium acetate, 5 mM sodium acetate, 2 mM MgCl₂, 1 mM EGTA, 10% glycerol, 25 mM β -glycerol phosphate, 10 mM NaF, 1 mM sodium orthovanadate, 1 mM PMSF, protease inhibitors) containing no detergent, 1% Triton X-100, 2% digitonin, or 1% octyl glucoside. After solubilizing by rotation for 20 min at 4°C, the insoluble material was removed by spinning at 14,000 rpm, 15 min, 4°C. The supernatant was used for immunoprecipitation and MT binding experiments.

For immunoprecipitation, 100 μ l of mouse ascites (H2) against KHC (gift of G. Bloom, University of Texas Southwestern Medical Center, Dallas, TX) was added to the supernatant, and immunoprecipitates were isolated by the addition of protein G (Amersham Pharmacia Biotech). The immunoprecipitates were washed three times with buffer B containing no detergent. Associated proteins were detected by immunoblotting the precipitates with rabbit polyclonal antibodies to KHC (peptide khc13; Verhey et al., 1998), to JIP-1/IB1 (number 176; Meyer et al., 1999), to DLK (gift of Lawrence Holzman, University of Michigan Medical School, Ann Arbor, MI; Holzman et al., 1994), to ApoER2 (anti-23, gift of Johannes Nimpf, University of Vienna, Vienna, Austria; Stockinger et al., 2000), to mitogen-activated kinase (MAP) kinase kinase (MKK) 3 (Santa Cruz Biotechnology, Inc.), to TrkA (Upstate Biotechnology), and to the type I sodium channel (Upstate Biotechnology).

MT binding experiments were carried out essentially as described (Verhey et al., 1998). In brief, 2.5 mM ATP or AMP-PNP, 20 μ M taxol, and 2.5 mg/ml taxol-stabilized MTs were added to Triton X-100-solubilized supernatant. After incubation at room temperature, the MTs and associated proteins were isolated by pelleting through a cushion of 10% sucrose in buffer B, with no detergent and 20 μ M taxol. The presence of the polypeptides in the MT pellet was detected by immunoblotting with the indicated antibodies.

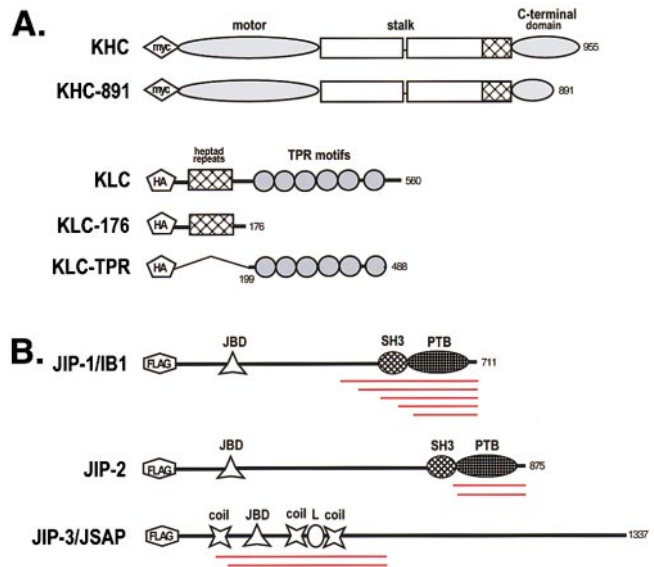


Figure 1. Schematic of protein constructs used in this study. (A) Schematic illustration of the domain structure of KHC, KLC, and their deletion mutants. (B) Schematic illustration of the domain structure of the JIP proteins. The independent overlapping clones isolated in the two-hybrid screen are indicated in red under each protein. JBD, JNK binding domain; coil, predicated coiled coil; L, leucine zipper; SH3, *src* homology domain 3; PTB, phosphotyrosine binding domain.

Results

Direct Interaction of KLC and the JIP Proteins

To identify proteins that interact directly with kinesin, we screened a mouse brain cDNA library using the yeast two-hybrid procedure with the TPR motifs of KLC as a bait. Nine of the clones isolated correspond to overlapping fragments of three different cDNAs encoding JIP-1, JIP-2, and JIP-3 (Fig. 1 B). No interaction of these clones was seen with either of two control bait proteins, the GAL4 DNA binding domain alone, or the TPR motifs of PP5 (data not shown).

JIP-1 (also known as IB1) and JIP-2 are ~50% identical, have a similar domain structure, and can form homodimeric and heterodimeric complexes (Yasuda et al., 1999). JIP-3 (also known as JSAP) can form oligomeric complexes with itself and JIP-2 but has no sequence relationship to JIP-1 or JIP-2 (Ito et al., 1999; Kelkar et al., 2000). The JIP proteins have all been proposed to be scaffolding proteins for the JNK family of MAP kinases (Dickens et al., 1997; Whitmarsh et al., 1998; Ito et al., 1999; Yasuda et al., 1999; Kelkar et al., 2000). They bind simultaneously to the three components of the JNK signaling pathway, the MAP kinase kinase kinase (DLK, MLK3, and others), the MAP kinase kinase (MKK4 and MKK7), and the MAP kinase JNK (for reviews see Davis, 2000; Mielke and Herdegen, 2000). JIP-1 and JIP-2 have additionally been found to interact with rhoGEF (Meyer et al., 1999) and with the cell surface receptor for Reelin, ApoER2 (Stockinger et al., 2000). Scaffolding proteins play a principal role in linking the cytoplasmic domains of transmembrane receptors or ion channels to cytoplasmic signal-

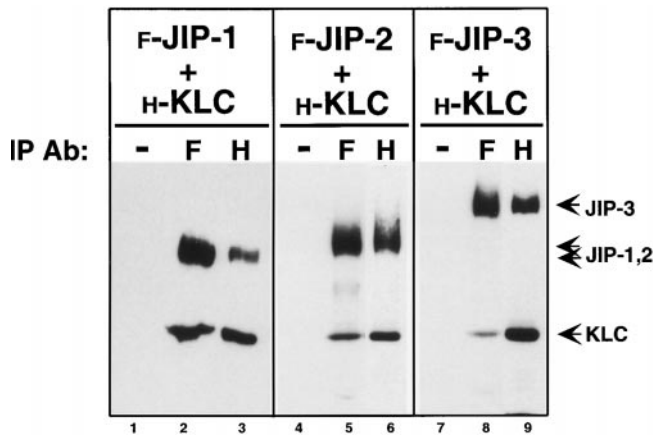


Figure 2. Coimmunoprecipitation of KLC and the JIP proteins. Lysates of COS cells expressing Flag-tagged JIP-1, JIP-2, or JIP-3 together with HA-tagged KLC were immunoprecipitated (IP) with no primary antibody (–), with an anti-Flag mAb (F), or with an anti-HA mAb (H). Precipitates were immunoblotted to detect the expressed proteins using polyclonal antibodies to both epitope tags.

ing molecules thereby maximizing the efficiency of signal propagation and maintaining the specificity of the pathway (for reviews see Garrington and Johnson, 1999; Schillace and Scott, 1999; Burack and Shaw, 2000). An additional function of the JIP scaffolds may be to localize the JNK signaling pathway to a specific area of the cell. The JIPs display a highly polarized subcellular localization at the tips of neuronal cell processes and in extended projections from the surface of insulinoma cells (Meyer et al., 1999; Yasuda et al., 1999; Kelkar et al., 2000). JIPs are also highly enriched at synaptic junctions in adult and developing brains (Pellet et al., 2000). In each case, the JIPs localize to the region of the cell where the plus ends of MTs are found.

Given the two-hybrid interaction between KLC and the JIPs and the requisite transport of the JIPs to the plus ends of MTs, we considered the possibility that the JIPs are a cargo of kinesin. We confirmed the interaction between KLC and the JIP proteins by coimmunoprecipitation of HA-tagged KLC and Flag-tagged JIPs expressed together in COS cells. Using anti-Flag antibodies, precipitation of JIP-1, JIP-2, or JIP-3 resulted in coprecipitation of HA-tagged full-length KLC (Fig. 2, lanes 2, 5, and 8). Conversely, anti-HA antibodies precipitated KLC and coprecipitated the Flag-tagged JIP-1, JIP-2, and JIP-3 proteins (Fig. 2, lanes 3, 6, and 9). Identical results were obtained using the HA-tagged TPR motifs of KLC instead of the full-length protein (data not shown). These data confirm a direct interaction between the TPR motifs of KLC and the JIP proteins.

The JIP-1 COOH-terminal Residues Are Required for Binding to the KLC TPR Motifs

The TPR motifs of several proteins recognize short stretches of primary amino acid sequence at the COOH termini of their binding partners (Terlecky et al., 1995; Scheufler et al., 2000). Interestingly, the extreme COOH-terminal sequences of JIP-1 and JIP-2 (PTEDIYLE) are identical and are completely conserved across species (Fig.

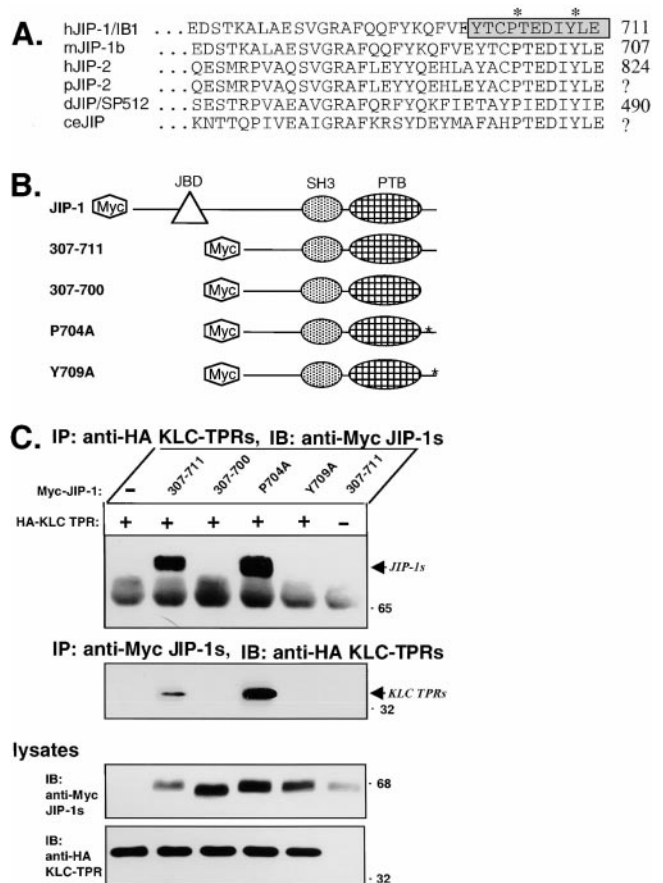


Figure 3. The COOH-terminal residues of JIP-1 are required for interaction with the KLC TPRs. (A) Sequence alignment of the COOH-terminal residues of JIP-1 and JIP-2 across species. The 11 residues deleted in the mutant JIP-1 (307–700) are indicated (boxed and shaded) as are the residues mutated in JIP-1 (P704A) and JIP-1 (Y709A) (*). hJIP-1/IB1, human JIP-1; mJIP-1b, mouse JIP-1b; hJIP-2, human JIP-2; pJIP-2, pig JIP-2 derived from a partial EST (accession AW312953); dJIP/SP512, *Drosophila* JIP-1/2; ceJIP, *C. elegans* JIP sequence derived from cosmid C13A10. (B) Schematic illustration of the Myc-tagged JIP-1 constructs used in this study. (C) Lysates of 293 cells expressing Myc-tagged JIP-1 (307–711), JIP-1 (307–700), JIP-1 (P704A), or JIP-1 (Y709A) together with HA-tagged KLC TPRs were immunoprecipitated (IP) for the KLC TPRs using an anti-HA mAb (top) or for the JIP-1 variants using an anti-Myc mAb (middle). Precipitates were immunoblotted (IB) for associated proteins using anti-Myc (top) or anti-HA polyclonal antibodies (middle). The ~65-kD band in the top panel is the heavy chain of immunoglobulin. Lysates were immunoblotted to detect the expressed proteins (bottom).

3 A). To determine whether these sequences are responsible for binding to the TPR motifs of KLC, we constructed deletion mutants of JIP-1 (Fig. 3 B). We used an NH₂-terminal deletion, JIP-1 (307–711), as the starting point for three additional mutants: deletion of the COOH-terminal 11 amino acids, JIP-1 (307–700), and mutation of P704 or Y709 within those 11 amino acids, JIP-1 (P704A) and JIP-1 (Y709A), respectively. These mutations do not interfere with folding of the COOH-terminal PTB domain as assayed by their ability to bind rhoGEF (data not shown).

The interaction between KLC and the JIP-1 mutant proteins was investigated by coimmunoprecipitation of the

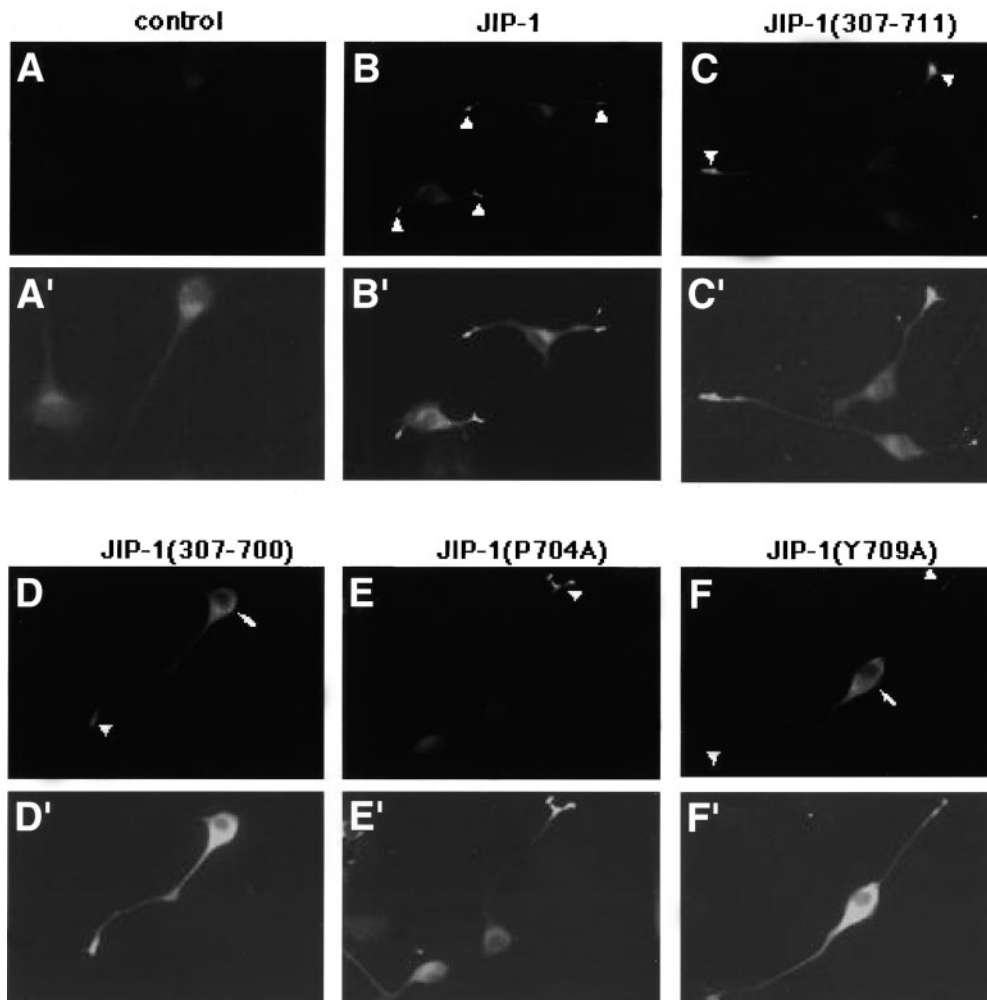


Figure 4. The COOH-terminal residues of JIP-1 are required for proper subcellular localization. N1E 115 cells were transiently transfected with the parental plasmid (control) or with plasmids encoding the indicated JIP-1 variants, differentiated, and the expressed proteins were detected by indirect immunofluorescence microscopy using an anti-Myc mAb. Non-specific background staining is visible in the control cells and is enhanced in A'–F' to aid in visualization of the cells. Myc–JIP-1 variants were scored as positive for correct cellular localization (JIP-1, JIP-1 [307–711], and JIP-1 [P704A]) if fluorescence was more pronounced at the neurite tips (arrowheads), whereas transfected proteins were considered negative for localization (JIP-1 [307–700] and JIP-1 [Y709A]) if fluorescence was observed to be more prominent in the cell body (arrows).

Myc-tagged JIP-1 variants and HA-tagged KLC TPRs expressed together in 293 cells. Immunoprecipitation of the HA-tagged TPR motifs of KLC coprecipitated the NH₂-terminal truncation JIP-1 (307–711), which contains the wild-type tail sequences (Fig. 3 C); this is consistent with the results of the two-hybrid screen where all of the positive JIP-1 and JIP-2 clones encode the extreme COOH termini of the proteins (Fig. 1 B). Immunoprecipitation of the HA-tagged TPR motifs of KLC also coprecipitated the proline to alanine point mutant, JIP-1 (P704A) (Fig. 3 C). In contrast, no coprecipitation of the JIP-1 construct missing the COOH-terminal 11 residues, JIP-1 (307–700), or the tyrosine to alanine mutant, JIP-1 (Y709A), was observed (Fig. 3 C). Conversely, immunoprecipitation of Myc-tagged JIP-1 (307–711) or the JIP-1 (P704A) point mutant, but not the JIP-1 (Y709A) point mutant or the JIP-1 (307–700) deletion mutant, resulted in coprecipitation of the TPR motifs of KLC (Fig. 3 C). Identical results were obtained using full-length HA-tagged KLC instead of the TPR motifs (data not shown). These data demonstrate that the interaction between KLC and JIP-1 requires the last 11 amino acids of JIP-1 and, in particular, the tyrosine residue at position –3 from the end. Thus, the mechanism of interaction between JIP-1 and the TPR motifs of KLC is similar to that of other TPR domain-containing proteins (Terlecky et al., 1995; Scheufler et al., 2000).

JIP-1 Is a Cargo for Kinesin

The demonstration of a direct interaction between kinesin and the JIP proteins, together with the highly polarized subcellular localization of the JIP proteins, raised the question whether JIP localization is dependent on its interaction with kinesin. To address this question, we undertook two approaches.

In the first approach, we asked whether the COOH-terminal residues of JIP-1 that are required for binding to KLC are also important for localization of JIP-1 to the tips of neuronal processes. Myc-tagged full-length JIP-1, the NH₂-terminal truncation JIP-1 (307–711), and the three mutant constructs JIP-1 (307–700), JIP-1 (P704A), and JIP-1 (Y709A) were localized in differentiated N1E 115 cells by indirect immunofluorescence using antibodies to the Myc tag. Untransfected control cells showed nonspecific background staining of the cell body (Fig. 4 A). Correct localization of Myc–JIP-1 fusion proteins was scored as specific staining of the neurite tips compared with the background staining of the cell body. As seen in Fig. 4, B and C, full-length JIP-1 and the NH₂-terminal truncation JIP-1 (307–711) localize to the tips of the neurites (arrowheads indicate tip staining) identical to the staining seen for endogenous JIP-1 (Fig. 5; Meyer et al., 1999; Yasuda et al., 1999; Kelkar et al., 2000; Pellet et al., 2000). Likewise, mutation of the proline residue, JIP-1 (P704A), had no ef-

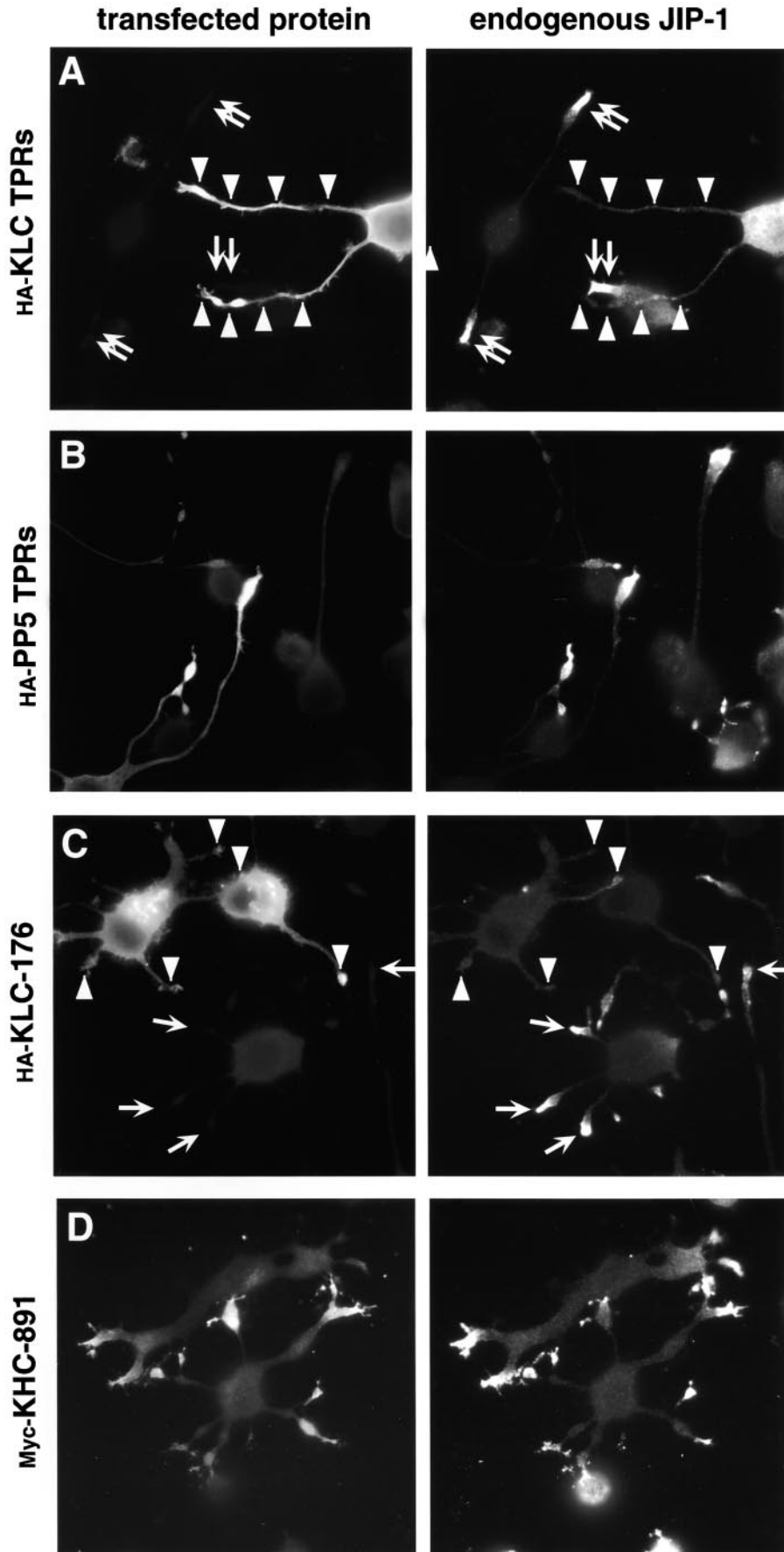


Figure 5. Expression of kinesin dominant negative constructs causes mislocalization of endogenous JIP-1 protein. CAD cells were transiently transfected with plasmids encoding the HA-tagged KLC TPRs (A), HA-tagged PP5 TPRs (B), HA-tagged KLC truncation KLC-176 (C), or Myc-tagged KHC truncation KHC-891 (D). After differentiation, the expressed proteins were detected by indirect immunofluorescence microscopy using mAbs to the epitope tags (left). Endogenous JIP-1 protein was detected with an affinity-purified polyclonal antibody (right). Note that the background fluorescence has been enhanced to show the entire neuronal cell. Arrowheads denote tips of transfected cells; arrows denote tips of untransfected cells.

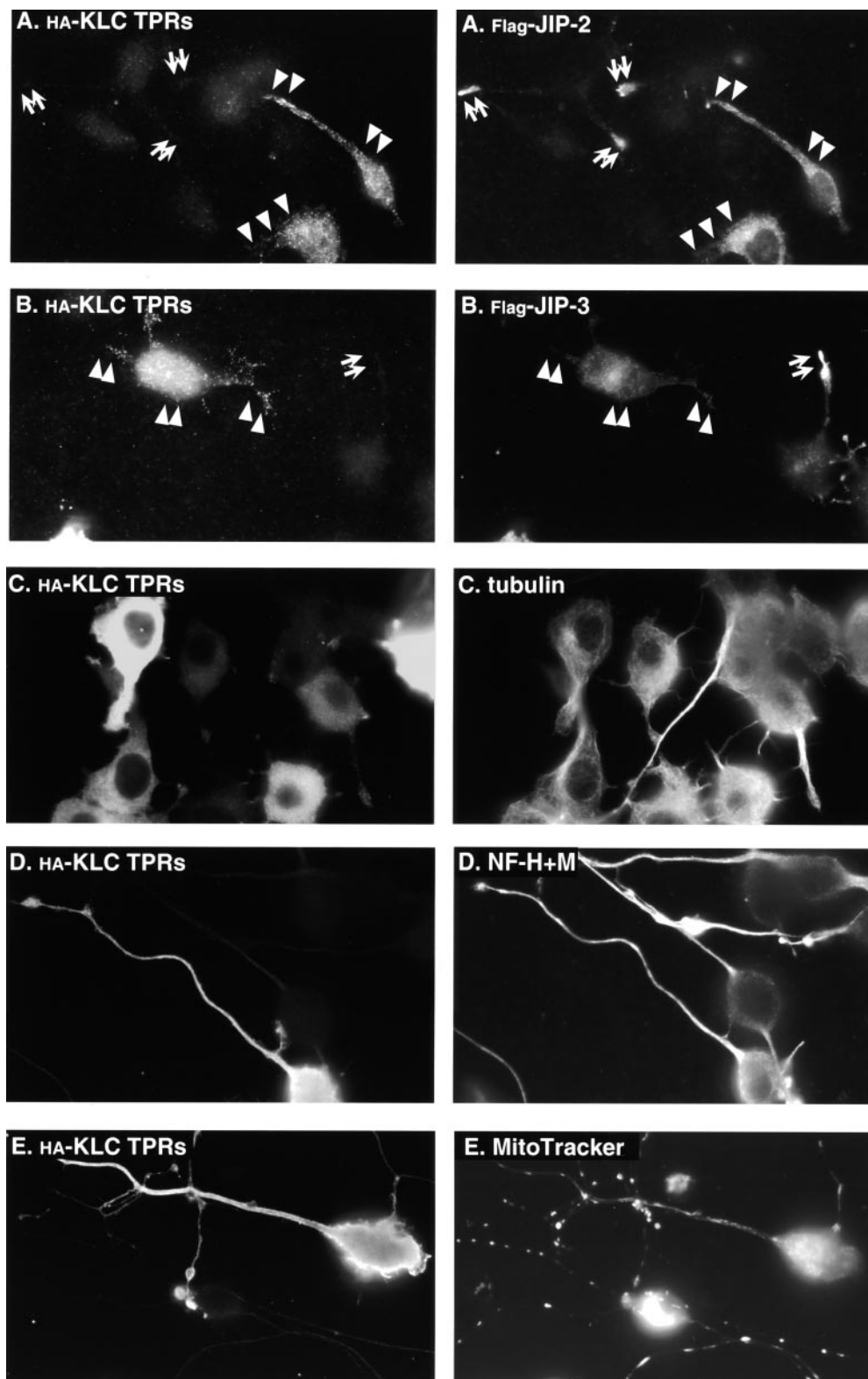


Figure 6. A kinesin dominant negative construct causes mislocalization of expressed JIP-2 and JIP-3 proteins but not endogenous mitochondria, neurofilaments, or MTs. (A and B) CAD cells were transiently transfected with plasmids encoding the HA-tagged KLC TPRs together with Flag-tagged JIP-2 (A) or Flag-tagged JIP-3 (B). After differentiation, the expressed proteins were detected by indirect immunofluorescence microscopy using a mAb to the HA tag (left) and a polyclonal antibody to the Flag tag (right). Arrowheads denote tips of transfected cells; arrows denote tips of untransfected cells. (C–E) CAD cells were transiently transfected with a plasmid encoding the HA-tagged KLC TPRs. After differentiation, the expressed protein was detected by indirect immunofluorescence microscopy using a polyclonal antibody to the HA tag (left). Endogenous MTs, neurofilaments (NF), and mitochondria were detected with mAbs to tubulin (C, right) or neurofilaments H + M (D, right), or with MitoTracker (E, right). Note that in C, the cells were only allowed to differentiate for 12 h, so that MT organization could be assessed before the cell bodies rounded up.

fect on JIP-1 localization (Fig. 4 E). In contrast, JIP-1 with a deletion of the 11 COOH-terminal residues, JIP-1 (307–700), or mutation of the tyrosine residue, JIP-1 (Y709A), exhibited localization throughout the cell with no concentration at the neurite tips (Fig. 4, D and F, arrows indicate staining of the cell body). These data demonstrate that the residues of JIP-1 that are critical for binding to the TPR

motifs of KLC are also required for the proper subcellular localization of JIP-1 and support the model that JIP proteins are a cargo for kinesin.

In the second approach, we asked whether the proper subcellular localization of JIP-1 in cultured neuronal cells could be disrupted by the expression of dominant negative kinesin constructs. One region of kinesin that could act as a

A. IP kinesin

detergent	none		digitonin		
anti-kinesin	-	+	-	+	
					KHC
					JIP-1
					DLK
					ApoER2
					MKK3
					TrkA
					Na ⁺ chnl

B. MT binding

	+	-	-	ATP
	-	+	+	AMP-PNP
	+	+	-	MTs

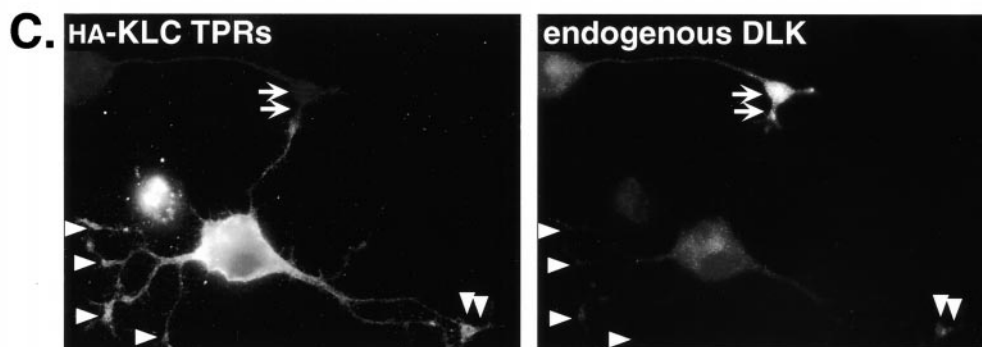


Figure 7. Kinesin and JIP-1 are associated with the upstream kinase DLK and the transmembrane receptor ApoER2. (A) An mAb to KHC (H2) was used to immunoprecipitate (IP) kinesin and associated proteins from a rat brain high speed supernatant in the presence of digitonin or no detergent. The presence of associated polypeptides was detected by immunoblotting the precipitates with polyclonal antibodies to the indicated proteins. (B) Rat brain high speed supernatant was subjected to an MT binding assay in the presence of Triton X-100 by adding ATP, AMP-PNP, and/or MTs as indicated. MTs and bound proteins were sedimented through a sucrose cushion, and the presence of the indicated proteins in the MT pellets was detected by immunoblotting. (C) CAD cells were transiently transfected with a plasmid encoding the HA-tagged KLC TPRs. After differentiation, the expressed protein was detected by indirect immunofluorescence microscopy using a mAb to the HA tag (left). Endogenous DLK kinase was detected with a polyclonal antibody (right). Arrowheads denote tips of transfected cells; arrows denote the tip of an untransfected cell.

dominant negative is that containing the TPR motifs of KLC; this region should bind to kinesin's cargo and prevent cargo transport to the correct location due to the lack of an attached motor domain. Indeed, expression of the HA-tagged TPR motifs of KLC in differentiated CAD cells caused the localization of endogenous JIP-1 protein to shift from the tips of the neuronal processes (Fig. 5 A, arrows indicate untransfected cells) to throughout the entire cell (Fig. 5 A, arrowheads indicate transfected cells). In contrast, localization of endogenous JIP-1 is not disturbed in differentiated CAD cells expressing a control protein, the HA-tagged TPR motifs of PP5 (Fig. 5 B). Identical results were observed upon expression of these constructs in differentiated N1E 115 cells (data not shown).

Another region of kinesin that should act as a dominant negative are the heptad repeats of KLC, which are responsible for linking KLC to KHC (Gauger and Goldstein, 1993; Diefenbach et al., 1998; Verhey et al., 1998). This domain should interact with endogenous KHC and prevent cargo binding due to the absence of the TPR motifs. Indeed, expression of the HA-tagged heptad repeats of KLC (KLC-176; Fig. 1 A) mislocalized endogenous JIP-1 protein in differentiated CAD cells (Fig. 5 C, arrowheads indicate

transfected cells). In contrast, expression of a KHC construct lacking its COOH-terminal 64 amino acids (KHC-891; Fig. 1 A) has no effect on the localization of endogenous JIP-1 (Fig. 5 D), presumably because this construct is lacking only the autoinhibitory domain and still contains the regions necessary for motor function and cargo binding. Indeed, the truncated KHC protein was largely found at the tips of cellular processes (Fig. 5 D), consistent with its uninhibited migration towards the plus end of MTs and in agreement with similar results in COS cells (Verhey et al., 1998) and *Neurospora* (Seiler et al., 2000).

Together, these data demonstrate that a direct interaction with kinesin is required for the correct cellular localization of JIP-1. It should be noted that the dominant negative kinesin mutants had no effect on the rate of axonal outgrowth (data not shown), suggesting that kinesin may not have a crucial role in the differentiation of neuronal cells. We have not tested the effects of expression of the kinesin dominant negatives on the localization of endogenous JIP-2 or JIP-3 due to a lack of appropriate reagents. However, when expressed at low levels in differentiated CAD cells, Flag-tagged JIP-2 and JIP-3 localize to the tips of the neurites (Fig. 6, A and B, arrows indicate untrans-

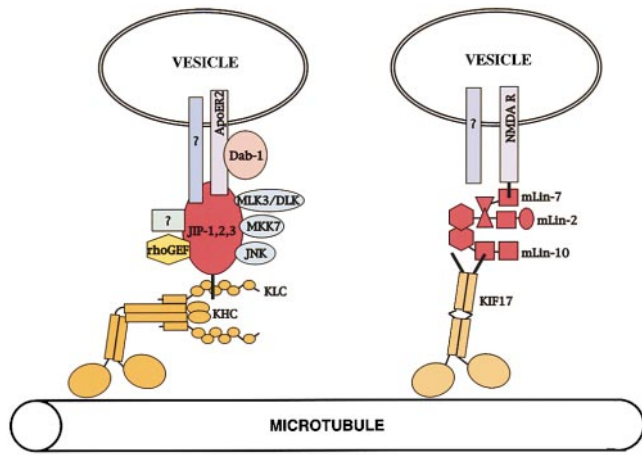


Figure 8. Model for the transport of cargo by kinesins. JIP proteins form a scaffold, on which cytoplasmic as well as plasma membrane proteins are assembled. The entire complex is transported down an axonal process by conventional kinesin. Note that the JIP proteins are known to form homodimers and heterodimers, although only one polypeptide is drawn for clarity. Similarly, LIN-2, -7, and -10 form a scaffold, on which cytoplasmic and transmembrane proteins assemble. The entire complex is transported down a dendritic process by the kinesin superfamily member KIF17.

ected cells), and this localization is abolished by coexpression of the kinesin dominant negative construct (Fig. 6, A and B, arrowheads indicate transfected cells). These data support the assertion that JIP-2 and JIP-3 are also cargoes for kinesin and provide strong evidence that the JIP scaffolding proteins act not only as motor–cargo linkers but are integral components of the cargo as well. Our results argue against the alternative possibility that the JIPs are upstream of kinesin and that their only function is to regulate kinesin’s transport of another cargo.

Importantly, in control experiments, expression of the dominant negative HA-tagged KLC TPRs had no effect on the localization of MTs in CAD cells (Fig. 6 C) or in a variety of other cell lines (data not shown), demonstrating that the effects on JIP localization are not due to a global effect on MT organization. In addition, expression of the HA-tagged KLC TPRs had no effect on the localization of neurofilaments (Fig. 6 D) or mitochondria (Fig. 6 E) in CAD cells, or on the localization of the endoplasmic reticulum, the Golgi complex, endosomes, lysosomes, actin, vimentin, or synaptophysin in a variety of cultured cell lines (data not shown).

Kinesin Associates with Proteins Assembled on the JIP Scaffold

The identification of the JIP proteins as a cargo for kinesin led us to consider whether kinesin carries the JIP proteins alone or together with proteins assembled on the JIP scaffold. To test this, we looked for an interaction *in vivo* between kinesin, the JIPs, and signaling molecules known to interact with JIPs. A high speed supernatant fraction from rat brain was used for immunoprecipitation experiments with an mAb to KHC (H2, gift of G. Bloom). As an additional method of precipitating kinesin and associated pro-

teins, an MT binding assay was performed. The presence of associated proteins was detected by immunoblotting the immunoprecipitates and MT pellets.

We first looked for an association of kinesin with JIP-1 and with a neuronal-specific upstream kinase of the JNK signal transduction pathway, the DLK (Fan et al., 1996). As seen in Fig. 7 A, immunoprecipitation of kinesin resulted in the coprecipitation of JIP-1 and DLK. A complex of these proteins was also detected in the presence of the detergents digitonin (Fig. 7 A), Triton X-100, or octyl glucoside (data not shown). As seen in Fig. 7 B, precipitation of kinesins by binding to MTs in the presence of the non-hydrolyzable ATP analogue AMP-PNP also resulted in the coprecipitation of JIP-1 and DLK. Importantly, the upstream kinase MKK3 of another MAP kinase pathway, the p38 pathway, was not coprecipitated (Fig. 7 A).

Next, we looked for a link between kinesin and a vesicle by probing for the coprecipitation of the transmembrane protein ApoER2, the receptor for the Reelin ligand, which has been shown to interact directly with JIP-1 and JIP-2 (Gotthardt et al., 2000; Stockinger et al., 2000). Although most of the ApoER2 protein is found in a rapidly sedimenting fraction of rat brain homogenates, a small fraction of ApoER2 remains in the high speed supernatant fraction, presumably associated with small vesicles that do not pellet under these conditions (data not shown). As seen in Fig. 7 A, immunoprecipitation of kinesin resulted in the coprecipitation of ApoER2, and this interaction was not disturbed upon solubilization with digitonin (Fig. 7 A), Triton X-100, or octyl glucoside (data not shown). As seen in Fig. 7 B, precipitation of kinesins by binding to MTs in the presence of AMP-PNP also resulted in the coprecipitation of ApoER2. This is a small but significant portion of the ApoER2 found in the high speed supernatant fraction. Importantly, the transmembrane proteins TrkA, a receptor for nerve growth factor, and the type I sodium channel were not coprecipitated (Fig. 7 A), although they were present in the high speed supernatant fraction.

As an additional way of assessing whether kinesin is required for localization of proteins assembled on the JIP scaffold, we looked for an effect of the dominant negative HA-tagged KLC TPRs on the localization of DLK (the antibodies to ApoER2 were not suitable for these experiments). Indeed, localization of endogenous DLK to the tips of the neurites (Fig. 7 C, arrows indicate the tip of an untransfected cell) is abolished by coexpression of the kinesin dominant negative construct (Fig. 7 C, arrowheads indicate transfected cell). Together, these results suggest that the JIP scaffolding complex, which includes DLK and ApoER2, is preassembled before being transported by kinesin, rather than formed by diffusion of its individual components at the site of action.

Discussion

JIPs and Their Associated Proteins Are a Cargo for Kinesin

Our results demonstrate a direct interaction between conventional kinesin and a cargo, the JIP scaffolding proteins. Although kinesin has always been assumed to be an organelle and/or vesicle motor, these results suggest that the

underlying assumption that kinesin links directly to its cargo via a membrane protein may not be true. Instead, our results demonstrate that the kinesin–cargo linker is a soluble scaffolding protein. We postulate that the scaffold assembles other proteins, some of which provide the direct link to a vesicle (Fig. 8). In addition to the proteins known to associate with the JIP scaffolding proteins, there are likely to be others that assemble in this macromolecular complex.

Kinesin and JIP-1 are connected via a direct interaction between the KLC TPR motifs and the JIP-1 COOH-terminal residues. In analogy to other TPR domain-containing proteins (Terlecky et al., 1995; Das et al., 1998; Groves and Barford, 1999; Scheufler et al., 2000), it is likely that three or more TPR motifs of KLC generate an extended surface groove that provides the docking site for the linear JIP-1 (and presumably JIP-2) peptide. The six TPR motifs of KLC could thus bind two JIP-1 and/or JIP-2 molecules simultaneously. Interestingly, the TPR motifs of KLC must bind to internal residues in JIP-3 as the COOH terminus of JIP-3 bears no sequence relation to that of JIP-1 and JIP-2, and the JIP-3 clones isolated in our two-hybrid screen contain only the NH₂-terminal portion of the protein (Fig. 1 B). These results are in agreement with a recent report that demonstrates a direct interaction between the NH₂-terminal half of JIP-3 (Sunday Driver) and the TPR motifs of KLC (Bowman et al., 2000). Our work extends this study by demonstrating that all JIP scaffolding proteins interact with KLC and that this interaction is essential for proper localization of the JIPs. Bowman et al. (2000) claim that JIP-3 is a transmembrane protein that provides a direct link between kinesin and the membrane. However, this is not supported by the sequence of JIP-3, the cytosolic localization of Flag-tagged JIP-3 expressed in CAD cells (Fig. 6 B) or in COS cells (data not shown), or the localization of endogenous JIP-3 in neuronal cells (Kelkar et al., 2000).

Although our results demonstrate that the TPR motifs of KLC interact with a cargo, the JIP scaffolding proteins, they do not exclude that there are regions in kinesin that interact with other cargoes. Additional cargo binding sites could be the variable COOH termini of KLC, which arise from alternative splicing (Khodjakov et al., 1998; Liao and Gundersen, 1998; Gyoeva et al., 2000), or the region of the KHC stalk domain that is highly conserved from fungi to humans (Kirchner et al., 1999a,b; Seiler et al., 2000). The possibility of multiple cargo interactions may explain at least in part the apparently conflicting reports that kinesin transports a large variety of organelles (Goldstein and Philp, 1999; Goldstein and Yang, 2000; Kamal and Goldstein, 2000; Rogers and Gelfand, 2000). Alternatively, kinesin's sole cargo may be the JIPs; it may be via the JNK signaling pathway that kinesin exerts its various effects on cells, as known JNK effectors include tau, neurofilaments, and the p150-Spir actin–regulating protein (Mielke and Herdegen, 2000; Otto et al., 2000).

Role of Kinesin and JNK Signaling in Neurogenesis

The identification of the JIP scaffold proteins as a cargo for kinesin raises intriguing questions on the role of kinesin and the JNK signaling pathway in nerve cell development. Kinesin and JIPs are not necessary for axonal growth because mislocalization of JIP-1 by dominant neg-

ative kinesin constructs does not prevent neurite outgrowth (Fig. 5); rather, kinesin and JIPs may be involved in neuronal migration, pathfinding, and synaptogenesis. This is supported by the fact that, in KHC or KLC mutants in *Drosophila*, the morphology of the larval neuromuscular system remains intact but there are defects in the number of synaptic boutons in action potential propagation and in neurotransmitter release (Gho et al., 1992; Hurd and Saxton, 1996; Hurd et al., 1996; Gindhart et al., 1998). Consistent with the postulated connection between kinesin, JNK signaling, and neurogenesis, mice lacking the ubiquitously expressed conventional kinesin gene *kif5b* and mice lacking the *jnk1* and *jnk2* genes exhibit embryonic lethality with severe defects in early brain development (Kuan et al., 1999). A role for kinesin and JNK signaling in synaptogenesis is also suggested by the developmental expression profile of JIP-1 message and protein, which coincide with the transition from axonal and dendritic outgrowth to a phase in which synaptic contacts are stabilized (Pellet et al., 2000). A possible mechanism may be through the interaction of JIP-1 and/or JIP-2 with ApoER2, the receptor for Reelin. The Reelin protein, which is defective in the naturally occurring *reeler* strain of mouse, has been suggested to function in the maturation of neuronal processes, axonal branching, and synaptogenesis (for reviews see D'Arcangelo and Curran, 1998; Willnow et al., 1999; Bothwell and Giniger, 2000). Together, these results support a model in which localization of kinesin, JIPs, and JNK signaling pathways at the nerve terminal is necessary for neurogenesis.

KIFs and Localization of Signal Transduction Pathways

Kinesins may play a general role in localizing signal transduction cascades. The neuronal-specific KIF17 binds to the first of two PDZ domains in the scaffold protein mLin-10 (Mint1/X11) and thereby transports an associated transmembrane protein, the NMDA receptor, to the ends of dendrites (Fig. 7 A) (Setou et al., 2000). PDZ domain-containing proteins are scaffolding proteins that play a fundamental role in the synaptic localization of neurotransmitter receptors and ion channels and in the assembly of synaptic junctions (for reviews see Fanning and Anderson, 1999; Garner et al., 2000; Sheng and Pak, 2000). Although PDZ domains have no structural relationship to TPR domains, they also bind to short peptide sequences located on the COOH-terminal tails of their target proteins as well as to internal sequences in their binding partners (for reviews see Saras and Heldin, 1996; Cowburn, 1997; Fanning and Anderson, 1999; Oschkinat, 1999). A link between kinesins and signaling pathways was also suggested by the identification of KIF3X, a member of the kinesin II subfamily, in a yeast two-hybrid screen for proteins that interact with MLK2, an upstream kinase that activates the extracellular signal-regulated kinase, JNK, and p38 MAP kinases (Nagata et al., 1998).

Together, these results suggest a general model for kinesins in which the motor binds to a scaffolding protein that then links it to a vesicle (Fig. 7 A). Thus, in addition to their known role in assembling and stabilizing protein complexes, scaffolding proteins can now be seen as a link between motors and membrane trafficking. This linkage would serve to localize not just membrane proteins, but also cytoplasmic signaling molecules, to the appropriate

cellular domain and would provide the targeting mechanism required for spatial regulation of signaling pathways. Indeed, one of the long-standing questions in signal transduction is how cells generate specific responses out of generic signaling pathways (Jordan et al., 2000; Schlessinger, 2000). Spatial concentration of signaling molecules is one mechanism, in addition to regulation of signal strength and timing, by which cells could accomplish this.

A provocative implication raised by our results, and those recently reported for KIF17, is that scaffolding proteins assemble multiprotein complexes at great distances from their final functional locations. Particularly in the case of neuronal cells but applicable to all cells, it seems most advantageous for a cell to assemble the components of a signaling complex in the cell body where synthesis of cytoplasmic and membrane proteins takes place, load it on a motor protein, and thereby carry the entire complex to the tips of the neuronal processes. In addition, since one of the components is a transmembrane receptor, it seems likely that the cargo is not simply soluble signaling molecules but a module of signaling components attached to a vesicle. Further studies are required to address whether these components, and presumably others, are actually associated together on vesicles that move along MTs.

Signal Transduction Pathways May Regulate the Activity of Their Motors

Another provocative implication raised by the model in Fig. 7 is that scaffolds and their associated proteins are required not only for localized signal transduction but also for regulating the motor that delivers them. Regulation of motor activity is required in two cellular contexts: activation of the motor at the site of assembly and/or loading and inactivation of the motor upon delivery of the cargo to its destination. In the case of conventional kinesin, the binding of JIPs to the TPR motifs in the tail of kinesin may unfold the native kinesin protein and activate it for MT binding. In this case, the KLCs would play a role both in directing cargo binding and in regulating motor activity (Verhey et al., 1998). The bound cargo could then stabilize the active state of the motor complex, ensuring that only cargo-bound kinesins are capable of movement along MTs. When kinesin and its cargo reach their destination, the fusion of the transported vesicle with the plasma membrane would expose the receptor, e.g., ApoER2, to its extracellular ligand, e.g., Reelin. This may result in the activation of the JNK signaling cascade, which may in turn inactivate kinesin so it can be recycled back to the cell body or be degraded. This model is consistent with previous reports pointing to a role for phosphorylation of KHC and KLC in regulating kinesin's activity (Sato-Yoshitake et al., 1992; Hollenbeck, 1993; Matthies et al., 1993; McIlvain et al., 1994; Lee and Hollenbeck, 1995; Lindesmith et al., 1997; De Vos et al., 2000). Other potential mechanisms that may play a role in regulating kinesin's activity include local changes in cellular pH (Verhey et al., 1998) and chaperone proteins such as hsc70 (Tsai et al., 2000). Further experiments are required to determine whether JIP binding is necessary and sufficient for activation of the kinesin motor and whether any of the known signal transduction pathways are involved in regulating kinesin activity.

For helpful advice and discussions, we thank Seth Sadis, Eric Olsen, Kathy Buckley, Joachim Herz, and the members of the Margolis and Rap-

oport laboratories, particularly Pascal Stein, Melissa Rolls, and Will Prinz. We are grateful to Drs. Kathy Buckley, Martin Myers, Diane Fingar, Bill Saxton, and Virgil Muresan for critical reading of the manuscript.

The work in the laboratory of T.A. Rapoport by K.J. Verhey and R.M. Deehan was supported by the Howard Hughes Medical Institute. The work in the laboratory of B. Margolis by D. Meyer was supported by the Howard Hughes Medical Institute. B.J. Schnapp and J. Blenis are supported by the National Institutes of Health.

Submitted: 30 November 2000

Revised: 4 January 2001

Accepted: 22 January 2001

References

- Bi, G.Q., R.L. Morris, G. Liao, J.M. Alderton, J.M. Scholey, and R.A. Steinhart. 1997. Kinesin- and myosin-driven steps of vesicle recruitment for Ca^{2+} -regulated exocytosis. *J. Cell Biol.* 138:999–1008.
- Blatch, G.L., and M. Lassle. 1999. The tetratricopeptide repeat: a structural motif mediating protein-protein interactions. *Bioessays.* 21:932–939.
- Bothwell, M., and E. Giniger. 2000. Alzheimer's disease: neurodevelopment converges with neurodegeneration. *Cell.* 102:271–273.
- 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.
- Brendza, R.P., L.R. Serbus, J.B. Duffy, and W.M. Saxton. 2000a. A function for kinesin I in the posterior transport of oskar mRNA and Staufen protein. *Science.* 289:2120–2122.
- Brendza, R.P., K.B. Sheehan, F.R. Turner, and W.M. Saxton. 2000b. Clonal tests of conventional kinesin function during cell proliferation and differentiation. *Mol. Biol. Cell.* 11:1329–1343.
- Brown, S.S. 1999. Cooperation between microtubule- and actin-based motor proteins. *Annu. Rev. Cell Dev. Biol.* 15:63–80.
- Burack, W.R., and A.S. Shaw. 2000. Signal transduction: hanging on a scaffold. *Curr. Opin. Cell Biol.* 12:211–216.
- Cowburn, D. 1997. Peptide recognition by PTB and PDZ domains. *Curr. Opin. Struct. Biol.* 7:835–838.
- Coy, D.L., W.O. Hancock, M. Wagenbach, and J. Howard. 1999. Kinesin's tail domain is an inhibitory regulator of the motor domain. *Nat. Cell Biol.* 1:288–292.
- D'Arcangelo, G., and T. Curran. 1998. Reeler: new tales on an old mutant mouse. *Bioessays.* 20:235–244.
- Das, A.K., P.T.W. Cohen, and D. Barford. 1998. The structure of the tetratricopeptide repeats of protein phosphatase 5: implications for TPR-mediated protein-protein interactions. *EMBO (Eur. Mol. Biol. Organ.) J.* 17:1192–1199.
- Davis, R.J. 2000. Signal transduction by the JNK group of MAP kinases. *Cell.* 103:239–252.
- De Vos, K., F. Severin, F. Van Herreweghe, K. Vancompernelle, V. Goossens, A. Hyman, and J. Grooten. 2000. Tumor necrosis factor induces hyperphosphorylation of kinesin light chain and inhibits kinesin-mediated transport of mitochondria. *J. Cell Biol.* 149:1207–1214.
- Dickens, M., J.S. Rogers, J. Cavanagh, A. Raitano, Z. Xia, J.R. Halpern, M.E. Greenberg, C.L. Sawyers, and R.J. Davis. 1997. A cytoplasmic inhibitor of the JNK signal transduction pathway. *Science.* 277:693–696.
- Diefenbach, R.J., J.P. Mackay, P.J. Armati, and A.L. Cunningham. 1998. The C-terminal region of the stalk domain of ubiquitous human kinesin heavy chain contains the binding site for kinesin light chain. *Biochemistry.* 37:16663–16670.
- Fan, G., S.E. Merritt, M. Kortenjann, P.E. Shaw, and L.B. Holzman. 1996. Dual leucine zipper-bearing kinase (DLK) activates p46SAPK and p38mapk but not ERK2. *J. Biol. Chem.* 271:24788–24793.
- Fanning, A.S., and J.M. Anderson. 1999. PDZ domains: fundamental building blocks in the organization of protein complexes at the plasma membrane. *J. Clin. Invest.* 103:767–772.
- Friedman, D.S., and R.D. Vale. 1999. Single-molecule analysis of kinesin motility reveals regulation by the cargo-binding tail domain. *Nat. Cell Biol.* 1:293–297.
- Garner, C.C., J. Nash, and R.L. Haganir. 2000. PDZ domains in synapse assembly and signalling. *Trends Cell Biol.* 10:274–280.
- Garrington, T.P., and G.L. Johnson. 1999. Organization and regulation of mitogen-activated protein kinase signaling pathways. *Curr. Opin. Cell Biol.* 11:211–218.
- Gauger, A.K., and L.S. Goldstein. 1993. The *Drosophila* kinesin light chain. Primary structure and interaction with kinesin heavy chain. *J. Biol. Chem.* 268:13657–13666.
- Gho, M., K. McDonald, B. Ganetzky, and W.M. Saxton. 1992. Effects of kinesin mutations on neuronal functions. *Science.* 258:313–316.
- Gindhart, J.G., Jr., C.J. Desai, S. Beushausen, K. Zinn, and L.S. Goldstein. 1998. Kinesin light chains are essential for axonal transport in *Drosophila*. *J. Cell Biol.* 141:443–454.
- Goldstein, L.S., and A.V. Philp. 1999. The road less traveled: emerging principles of kinesin motor utilization. *Annu. Rev. Cell Dev. Biol.* 15:141–183.
- Goldstein, L.S.B., and S. Gunawardena. 2000. Flying through the *Drosophila*

- cytoskeletal genome. *J. Cell Biol.* 150:F63–F68.
- Goldstein, L.S., and Z. Yang. 2000. Microtubule-based transport systems in neurons: the roles of kinesins and dyneins. *Annu. Rev. Neurosci.* 23:39–71.
- Goode, B.L., D.G. Drubin, and G. Barnes. 2000. Functional cooperation between the microtubule and actin cytoskeletons. *Curr. Opin. Cell Biol.* 12:63–71.
- Gotthardt, M., M. Trommsdorff, M.F. Nevitt, J. Shelton, J.A. Richardson, W. Stockinger, J. Nimpf, and J. Herz. 2000. Interactions of the low density lipoprotein receptor gene family with cytosolic adaptor and scaffold proteins suggest diverse biological functions in cellular communication and signal transduction. *J. Biol. Chem.* 275:25616–25624.
- Groves, M.R., and D. Barford. 1999. Topological characteristics of helical repeat proteins. *Curr. Opin. Struct. Biol.* 9:383–389.
- Gyoeva, F.K., E.M. Bybikova, and A.A. Minin. 2000. An isoform of kinesin light chain specific for the Golgi complex. *J. Cell Sci.* 113:2047–2054.
- Hollenbeck, P.J. 1993. Phosphorylation of neuronal kinesin heavy and light chains in vivo. *J. Neurochem.* 60:2265–2275.
- Holzman, L.B., S.E. Merritt, and G. Fan. 1994. Identification, molecular cloning, and characterization of dual leucine zipper bearing kinase. A novel serine/threonine protein kinase that defines a second subfamily of mixed lineage kinases. *J. Biol. Chem.* 269:30808–30817.
- Huang, J.D., S.T. Brady, B.W. Richards, D. Stenolen, J.H. Resau, N.G. Copeland, and N.A. Jenkins. 1999. Direct interaction of microtubule- and actin-based transport motors. *Nature.* 397:267–270.
- 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.
- Hurd, D.D., M. Stern, and W.M. Saxton. 1996. Mutation of the axonal transport motor kinesin enhances paralytic and suppresses Shaker in *Drosophila*. *Genetics.* 142:195–204.
- Ito, M., K. Yoshioka, M. Akechi, S. Yamashita, N. Takamatsu, K. Sugiyama, M. Hibi, Y. Nakabeppu, T. Shiba, and K.I. Yamamoto. 1999. JSAP1, a novel jun N-terminal protein kinase (JNK)-binding protein that functions as a Scaffold factor in the JNK signaling pathway. *Mol. Cell Biol.* 19:7539–7548.
- James, P., J. Halladay, and E.A. Craig. 1996. Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. *Genetics.* 144:1425–1436.
- Jordan, J.D., E.M. Landau, and R. Iyengar. 2000. Signaling networks: the origins of cellular multitasking. *Cell.* 103:193–200.
- Kamal, A., and L.S. Goldstein. 2000. Connecting vesicle transport to the cytoskeleton. *Curr. Opin. Cell Biol.* 12:503–508.
- Kelkar, N., S. Gupta, M. Dickens, and R.J. Davis. 2000. Interaction of a mitogen-activated protein kinase signaling module with the neuronal protein JIP3. *Mol. Cell Biol.* 20:1030–1043.
- Khodjakov, A., E.M. Lizunova, A.A. Minin, M.P. Koonce, and F.K. Gyoeva. 1998. A specific light chain of kinesin associates with mitochondria in cultured cells. *Mol. Biol. Cell.* 9:333–343.
- Kirchner, J., S. Seiler, S. Fuchs, and M. Schliwa. 1999a. Functional anatomy of the kinesin molecule in vivo. *EMBO (Eur. Mol. Biol. Organ.) J.* 18:4404–4413.
- Kirchner, J., G. Woehlke, and M. Schliwa. 1999b. Universal and unique features of kinesin motors: insights from a comparison of fungal and animal conventional kinesins. *Biol. Chem.* 380:915–921.
- Kuan, C.Y., D.D. Yang, D.R. Samanta Roy, R.J. Davis, P. Rakic, and R.A. Flavell. 1999. The Jnk1 and Jnk2 protein kinases are required for regional specific apoptosis during early brain development. *Neuron.* 22:667–676.
- Lee, K.D., and P.J. Hollenbeck. 1995. Phosphorylation of kinesin in vivo correlates with organelle association and neurite outgrowth. *J. Biol. Chem.* 270:5600–5605.
- Liao, G., and G.G. Gundersen. 1998. Kinesin is a candidate for cross-bridging microtubules and intermediate filaments. Selective binding of kinesin to detyrosinated tubulin and vimentin. *J. Biol. Chem.* 273:9797–9803.
- Lindesmith, L., J.M. McIlvain, Jr., Y. Argon, and M.P. Sheetz. 1997. Phosphotransferases associated with the regulation of kinesin motor activity. *J. Biol. Chem.* 272:22929–22933.
- Manning, B.D., and M. Snyder. 2000. Drivers and passengers wanted! the role of kinesin-associated proteins. *Trends Cell Biol.* 10:281–289.
- Matthies, H.J., R.J. Miller, and H.C. Palfrey. 1993. Calmodulin binding to and cAMP-dependent phosphorylation of kinesin light chains modulate kinesin ATPase activity. *J. Biol. Chem.* 268:11176–11187.
- McIlvain, J.M., Jr., J.K. Burkhardt, S. Hamm-Alvarez, Y. Argon, and M.P. Sheetz. 1994. Regulation of kinesin activity by phosphorylation of kinesin-associated proteins. *J. Biol. Chem.* 269:19176–19182.
- Meyer, D., A. Liu, and B. Margolis. 1999. Interaction of c-Jun amino-terminal kinase interacting protein-1 with p190 rhoGEF and its localization in differentiated neurons. *J. Biol. Chem.* 274:35113–35118.
- Mielke, K., and T. Herdegen. 2000. JNK and p38 stress kinases—degenerative effectors of signal-transduction-cascades in the nervous system. *Prog. Neurobiol.* 61:45–60.
- Nagata, K., A. Puls, C. Futter, P. Aspenstrom, E. Schaefer, T. Nakata, N. Hirokawa, and A. Hall. 1998. The MAP kinase kinase kinase MLK2 co-localizes with activated JNK along microtubules and associates with kinesin superfamily motor KIF3. *EMBO (Eur. Mol. Biol. Organ.) J.* 17:149–158.
- Oshkinat, H. 1999. A new type of PDZ domain recognition. *Nat. Struct. Biol.* 6:408–410.
- Otto, I.M., T. Raabe, U.E.E. Rennefahrt, P. Bork, U.R. Rapp, and E. Kerkhoff. 2000. The p150-Spir protein provides a link between c-Jun N-terminal kinase function and actin reorganization. *Curr. Biol.* 10:345–348.
- Patel, N., D. Thierry-Mieg, and J.R. Mancillas. 1993. Cloning by insertional mutagenesis of a cDNA encoding *Caenorhabditis elegans* kinesin heavy chain. *Proc. Natl. Acad. Sci. USA.* 90:9181–9185.
- Pellet, J.B., J.A. Haefliger, J.K. Staple, C. Widmann, E. Welker, H. Hirling, C. Bonny, P. Nicod, S. Catsicas, G. Waeber, and B.M. Riederer. 2000. Spatial, temporal and subcellular localization of islet-brain 1 (IB1), a homologue of JIP-1, in mouse brain. *Eur. J. Neurosci.* 12:621–632.
- Qi, Y., J.K.T. Wang, M. McMillian, and D.M. Chikaraishi. 1997. Characterization of a CNS cell line, CAD, in which morphological differentiation is initiated by serum deprivation. *J. Neurosci.* 17:1217–1225.
- Rahman, A., A. Kamal, E.A. Roberts, and L.S. Goldstein. 1999. Defective kinesin heavy chain behavior in mouse kinesin light chain mutants. *J. Cell Biol.* 146:1277–1288.
- Rogers, S.L., and V.I. Gelfand. 2000. Membrane trafficking, organelle transport, and the cytoskeleton. *Curr. Opin. Cell Biol.* 12:57–62.
- Saras, J., and C.H. Heldin. 1996. PDZ domains bind carboxy-terminal sequences of target proteins. *Trends Biochem. Sci.* 21:455–458.
- Sato-Yoshitake, R., H. Yorifuji, M. Inagaki, and N. Hirokawa. 1992. The phosphorylation of kinesin regulates its binding to synaptic vesicles. *J. Biol. Chem.* 267:23930–23936.
- Saxton, W.M., J. Hicks, L.S. Goldstein, and E.C. Raff. 1991. Kinesin heavy chain is essential for viability and neuromuscular functions in *Drosophila*, but mutants show no defects in mitosis. *Cell.* 64:1093–1102.
- Scheuffer, C., A. Brinker, G. Bourenkov, S. Pegoraro, L. Moroder, H. Bartunik, F.U. Hartl, and I. Moarefi. 2000. Structure of TPR domain-peptide complexes: critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine. *Cell.* 101:199–210.
- Schillace, R.V., and J.D. Scott. 1999. Organization of kinases, phosphatases, and receptor signaling complexes. *J. Clin. Invest.* 103:761–765.
- Schlessinger, J. 2000. Cell signaling by receptor tyrosine kinases. *Cell.* 103:211–225.
- 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.
- Sheng, M., and D.T. Pak. 2000. Ligand-gated ion channel interactions with cytoskeletal and signaling proteins. *Annu. Rev. Physiol.* 62:755–778.
- Skoufias, D.A., D.G. Cole, K.P. Wedaman, and J.M. Scholey. 1994. The carboxyl-terminal domain of kinesin heavy chain is important for membrane binding. *J. Biol. Chem.* 269:1477–1485.
- Steinberg, G., and M. Schliwa. 1995. The *Neurospora* organelle motor: a distant relative of conventional kinesin with unconventional properties. *Mol. Biol. Cell.* 6:1605–1618.
- Stenoien, D.L., and S.T. Brady. 1997. Immunocytochemical analysis of kinesin light chain function. *Mol. Biol. Cell.* 8:675–689.
- Stock, M.F., J. Guerrero, B. Cobb, C.T. Eggers, T.G. Huang, X. Li, and D.D. Hackney. 1999. Formation of the compact conformation of kinesin requires a COOH-terminal heavy chain domain and inhibits microtubule-stimulated ATPase activity. *J. Biol. Chem.* 274:14617–14623.
- Stockinger, W., C. Brandes, D. Fasching, M. Hermann, M. Gotthardt, J. Herz, W.J. Schneider, and J. Nimpf. 2000. The reelin receptor ApoER2 recruits JNK-interacting proteins-1 and -2. *J. Biol. Chem.* 275:25625–25632.
- Tanaka, Y., Y. Kanai, Y. Okada, S. Nonaka, S. Takeda, A. Harada, and N. Hirokawa. 1998. Targeted disruption of mouse conventional kinesin heavy chain, kif5B, results in abnormal perinuclear clustering of mitochondria. *Cell.* 93:1147–1158.
- Terada, S., M. Kinjo, and N. Hirokawa. 2000. Oligomeric tubulin in large transporting complex is transported via kinesin in squid giant axons. *Cell.* 103:141–155.
- Terlecky, S.R., W.M. Nuttley, D. McCollum, E. Sock, and S. Subramani. 1995. The *Pichia pastoris* peroxisomal protein PAS8p is the receptor for the C-terminal tripeptide peroxisomal targeting signal. *EMBO (Eur. Mol. Biol. Organ.) J.* 14:3627–3634.
- 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.
- Vale, R.D., and R.A. Milligan. 2000. The way things move: looking under the hood of molecular motor proteins. *Science.* 288:88–95.
- Verhey, K.J., D.L. Lizotte, T. Abramson, L. Barenboim, B.J. Schnapp, and T.A. Rapoport. 1998. Light chain-dependent regulation of kinesin's interaction with microtubules. *J. Cell Biol.* 143:1053–1066.
- Wade, R.H., and F. Kozielski. 2000. Structural links to kinesin directionality and movement. *Nat. Struct. Biol.* 7:456–460.
- Whitmarsh, A.J., J. Cavanagh, C. Tournier, J. Yasuda, and R.J. Davis. 1998. A mammalian scaffold complex that selectively mediates MAP kinase activation. *Science.* 281:1671–1674.
- Willnow, T.E., A. Nykjaer, and J. Herz. 1999. Lipoprotein receptors: new roles for ancient proteins. *Nat. Cell Biol.* 1:E157–E162.
- Woehlke, G., and M. Schliwa. 2000. Directional motility of kinesin motor proteins. *Biochim. Biophys. Acta.* 1496:117–127.
- Yabe, J.T., C. Jung, W.K. Chan, and T.B. Shea. 2000. Phospho-dependent association of neurofilament proteins with kinesin in situ. *Cell Motil. Cytoskeleton.* 45:249–262.
- Yasuda, J., A.J. Whitmarsh, J. Cavanagh, M. Sharma, and R.J. Davis. 1999. The JIP group of mitogen-activated protein kinase scaffold proteins. *Mol. Cell Biol.* 19:7245–7254.