THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 279, No. 18, Issue of April 30, pp. 19201–19208, 2004
Printed in U.S.A.
© 2004 by The American Society for Biochemistry and Molecular Biology, Inc.

A Direct Interaction between Cytoplasmic Dynein and Kinesin I May Coordinate Motor Activity*

Received for publication, December 9, 2003, and in revised form, February 18, 2004
Published, JBC Papers in Press, February 24, 2004, DOI 10.1074/jbc.M313472200

Lee A. Ligon, Mariko Tokito, Jeffrey M. Finklestein, Francesca E. Grossman, and Erika L. F. Holzbaur‡

From the Department of Physiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-6085

Cytoplasmic dynein and kinesin I are both unidirectional intracellular motors. Dynein moves cargo toward the cell center, and kinesin moves cargo toward the cell periphery. There is growing evidence that bi-directional motility is regulated in the cell, potentially through direct interactions between oppositely oriented motors. We have identified a direct interaction between cytoplasmic dynein and kinesin I. Using the yeast two-hybrid assay and affinity chromatography, we demonstrate that the intermediate chain of dynein binds to kinesin light chains 1 and 2. The interaction is both direct and specific. Co-immunoprecipitation experiments demonstrate an interaction between endogenous proteins in rat brain cytosol. Double-label immunocytochemistry reveals a partial co-localization of vesicle-associated motor proteins. Together these observations suggest that soluble motors can interact, potentially allowing kinesin I to actively localize dynein to cellular sites of function. There is also a vesicle population with both dynein and kinesin I bound that may be capable of bi-directional motility along cellular microtubules.

Intracellular transport along microtubules is driven by the motors kinesin and cytoplasmic dynein. Kinesin I is a plus end-directed motor, consisting of two heavy chains and two light chains (1). Cytoplasmic dynein is a minus end-directed motor, composed of two globular heads each formed from a single dynein heavy chain and a base formed from intermediate, light intermediate, and light chains (1). The accessory complex dynactin binds to dynein and may be required to increase the processivity of dynein-driven movement (2, 3).

In the cell, kinesin powers anterograde transport, moving vesicles and organelles toward the cell periphery. Cytoplasmic dynein drives the retrograde transport of organelles and proteins toward microtubule minus ends. In addition to its role in increasing the processivity of dynein-driven motility, dynactin has been shown to mediate interactions of cytoplasmic dynein with some of its intracellular cargos (4, 5). The mechanisms of cargo coupling to kinesin are yet to be fully understood, but at least some cargos have been shown to bind directly to kinesin light chains (6).

Although *in vitro* motility assays clearly indicate that kinesin and cytoplasmic dynein can function independently to produce motility in opposite directions along microtubules, multiple studies have suggested that the activities of these two motors are coordinately coupled in the cell. This coordination may be most clear in axonal transport. Studies in extruded squid axoplasm have indicated that the specific inhibition of either kinesin (7, 8) or dynein/dynactin (9) results in a bi-directional block in the transport of vesicles along microtubules. Interdependence of anterograde and retrograde motors has also been noted genetically. Martin *et al.* (10) identified dominant genetic interactions between the kinesin, cytoplasmic dynein, and dynactin genes in *Drosophila*. Mutations in either motor result in an inhibition of axonal transport and the accumulation of organelles in axonal swellings that stain for markers of both anterograde and retrograde motility.

Here we provide biochemical evidence for a direct interaction between conventional kinesin and cytoplasmic dynein, mediated by an interaction between dynein intermediate chain (DIC)[1] and kinesin light chains (KLCs). Immunoprecipitation studies indicate that this interaction is physiologically relevant. Although the interaction is most robust between soluble motors, co-localization studies indicate that some vesicles have both motors bound, allowing for bi-directional motility. The results reported here provide further evidence for a key role of KLCs in mediating interactions between motors and cargo in the cell and suggest that cytoplasmic dynein may represent one of the cellular cargos of kinesin I.

EXPERIMENTAL PROCEDURES

Yeast Two-hybrid Interaction Screen—We used the LexA yeast two-hybrid system to screen an oligo(T)-primed human fetal brain library (Stratagene) for proteins interacting with DIC. The majority of clones isolated were found to encode $p150^{Glued}$, so we further screened positives by Southern blot to identify novel clones, one of which was identified by data base searching as encoding a TPR protein. Binding and domain mapping studies between kinesin and dynein were performed by subcloning full-length or partial sequences, as noted, of DIC, KLC1, and KLC2 into either the pB42Ad or pJK202 vectors (Stratagene) and scoring for growth on medium lacking adenine in comparison with the appropriate empty vector, as previously described (4).

Affinity Chromatography and Immunoprecipitations—Mouse cDNA clones encoding kinesin light chains 1 and 2 were used to generate GST fusion constructs, which were expressed in *Escherichia coli* and purified (4). Purified recombinant DIC or rat brain cytosolic extracts were fractionated over affinity columns generated from either GST fusion proteins or GST alone bound to glutathione-Sepharose, followed by elution of bound proteins with glutathione (4). The binding of kinesin to DIC was examined by fractionating either purified recombinant KLCs, *in vitro* translated truncation constructs, or rat brain cytosol over affinity

* This work was supported by National Institutes of Health Grant GM48661 (to E. L. F. H.) and a National Institutes of Health postdoctoral fellowship (to L. A. L.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Dept. of Physiology, University of Pennsylvania School of Medicine, D400 Richards Bldg., 3700 Hamilton Walk, Philadelphia, PA 19104-6085. Tel.: 215-573-3257; Fax: 215-573-5851; E-mail: holzbaur@mail.med.upenn.edu.

[1] The abbreviations used are: DIC, dynein intermediate chain; KLC, kinesin light chain; GST, glutathione *S*-transferase; BSA, bovine serum albumin; PIPES, 1,4-piperazinediethanesulfonic acid; TPR, tetratricopeptide repeat; MgAMP-PNP, Mg-5′-adenylyl imidodiphosphate; KHC, kinesin heavy chain.

This is an Open Access article under the CC BY license.

columns generated from either purified recombinant DIC or BSA covalently linked to a Sepharose matrix, followed by elution of bound proteins with 1 M NaCl (2). Western blots were probed with antibodies to: kinesin heavy chain (Chemicon monoclonal antibodies 1613 and 1614), KLCs 1 and 2 (Chemicon monoclonal antibodies 1616 and 1617 and monoclonal antibody 63-90 (generously provided by Dr. Scott Brady of the University of Illinois at Chicago), cytoplasmic dynein (monoclonal antibody MAB1618 to DIC from Chemicon, affinity-purified polyclonal antibodies UP1467 and UP1468 to DIC generated in our laboratory, and polyclonal antibodies to heavy chain and the Tctex-1 light chain generously provided by Drs. Richard Vallee of Columbia University and Stephen King of the University of Connecticut), and dynactin (affinity-purified polyclonal antibody UP235 to $p150^{Glued}$ generated in our laboratory).

Immunoprecipitations were performed from rat brain cytosol (the supernatant from a 100,000 × *g* centrifugation of rat brain homogenate) in 50 mM PIPES, 50 mM HEPES, 1 mM EDTA, 2 mM $MgSO_4$, pH 7.0, in the absence or the presence of 1% Triton X-100 and 0.5% Igepal. Immunoprecipitations were also performed from the 100,000 × *g* pellet resuspended in 50 mM Tris, 50 mM KCl, with 1% Triton X-100, and 0.5% Igepal. Immunoprecipitates were washed eight times in 50 mM Tris, 50 mM KCl with 1% Triton X-100 (4). Control immunoprecipitations were performed in parallel with protein A beads alone. Further purification of rat brain vesicles was performed by fractionating the membranes from the 100,000 × *g* pellet by flotation upward though a sucrose step gradient, as described (4). Soluble proteins were retained in the bottom layer of 2 M sucrose, and the vesicles were isolated from the 1.5/0.6 M sucrose interface. The vesicles were solubilized in 1% Triton X-100 for immunoprecipitation experiments.

The vesicles purified by flotation were incubated with microtubules polymerized from purified tubulin (Cytoskeleton) in the absence or presence of 5 mM MgATP or 10 mM MgAMP-PNP and then centrifuged through a cushion of 40% sucrose at 8,000 × *g*. Gel samples made from the supernatant and the pellet fractions were analyzed by SDS-PAGE and Western blot.

Immunocytochemistry and Immunofluorescence Microscopy—Cytoplasmic dynein and kinesin were localized in cultured PtK2, Rat2, and Cos7 cells by immunocytochemistry (11), using all the antibodies to kinesin and dynein described above in all possible combinations. The cells were counterstained with antibodies to tubulin (clone DM1A from Sigma or YL1/2 from Serotec) and visualized with Alexa 350-, 488-, and 594-conjugated secondary antibodies (Molecular Probes). The vesicles purified by flotation were bound to taxol-stabilized microtubules, fixed with glutaraldehyde, pelleted onto poly-L-lysine-coated coverslips (12), and processed for immunocytochemistry with antibodies to kinesin, dynein, and tubulin. The images were acquired on a Leica DMIRBE microscope with a 63× or 100× Planapo objective using OpenLab software (Improvision) and an Orca ER CCD camera (Hamamatsu).

RESULTS

We performed a yeast two-hybrid screen for proteins interacting with DIC and isolated a clone encoding TPR1, a human protein with three tetratricopeptide repeats (TPR). The TPR motif is a 34-residue degenerate sequence that forms two antiparallel α-helices; tandem arrays of TPRs form an amphipathic channel that mediates protein-protein interactions (13). The function of TPR1 is not yet known; therefore, the significance of this interaction is not clear. However, the demonstration of an interaction between DIC and a TPR protein, coupled with previous observations of functional and genetic interactions between dynein and kinesin, led us to test for an interaction between DIC and kinesin light chains. KLC1 and KLC2 each have six TPR motifs that are involved in motor-cargo interactions (6). KLC1 is a 61-kDa protein primarily expressed in neuronal tissues, and KLC2 is a 67-kDa protein that is more ubiquitously expressed (14).

We first tested for interactions between DIC and the kinesin light chains using the yeast two-hybrid assay. Both KLC1 and KLC2 were observed to interact with DIC in this assay, but the interaction between DIC and KLC2 was qualitatively more robust (Fig. 1*A*). To further investigate the interaction, we generated GST-KLC1 and GST-KLC2 fusion proteins and used affinity chromatography to probe for the binding of DIC. Recombinant DIC bound to both the KLC1 and KLC2 affinity

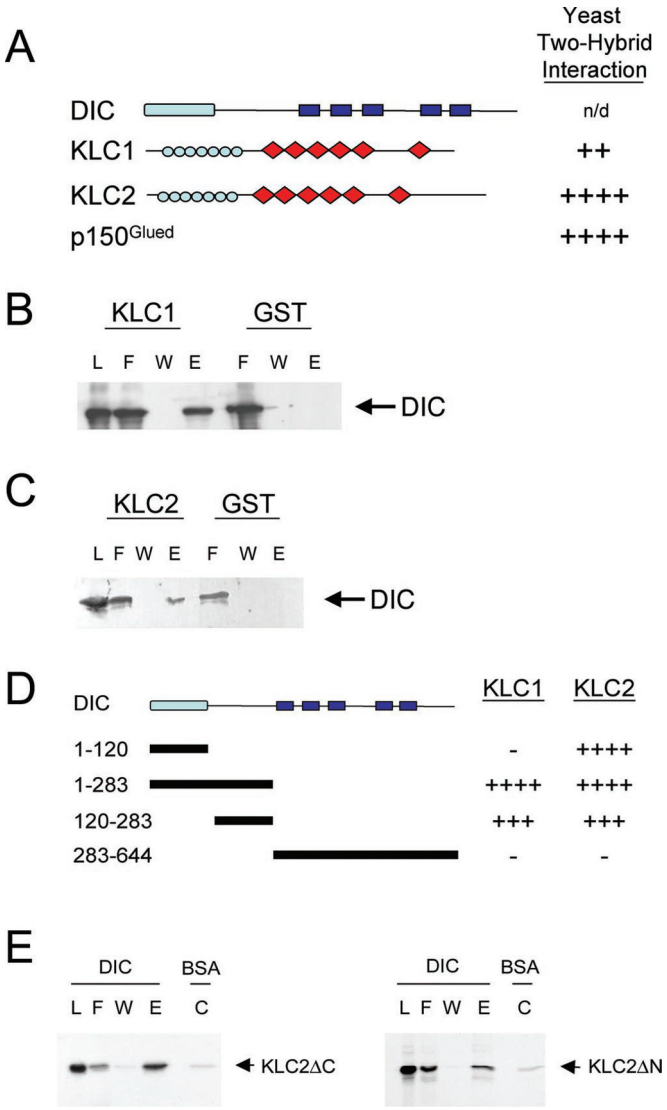

FIG. 1. **Direct interactions between dynein intermediate chain and kinesin light chains 1 and 2.** *A*, schematics of DIC and KLC1 and KLC2. The $p150^{Glued}$-binding site on DIC is indicated by a *light blue rectangle*, and the WD40 repeats (15) are indicated by *dark blue rectangles*. KLC1 and KLC2 share similar structures, with coiled coil domains at the N terminus (*light blue circles*) and six consensus TPR motifs toward the C terminus (*red diamonds*) (14). The yeast two-hybrid assay was used to test for interactions between DIC and either KLC1 or KLC2. The interaction between DIC and the $p150^{Glued}$ subunit of dynactin was examined in parallel as a positive control, and vector only was used as a negative control. Interactions were scored qualitatively by colony size and number. *B* and *C*, the binding of DIC to KLC1 and KLC2 was examined by affinity chromatography. Purified recombinant DIC was loaded onto GST-KLC1, GST-KLC2, or GST only columns, and following extensive washes, bound proteins were eluted with glutathione. The resulting load (*L*), flow-through (*F*), wash (*W*), and elution (*E*) fractions were analyzed by SDS-PAGE and Western blot probed with antibody to DIC. DIC was specifically retained on the GST-KLC1 (*B*) and GST-KLC2 columns (*C*) but not on control GST columns. *D*, mapping of the KLC-binding site in DIC was performed using the yeast two-hybrid assay and scored as above. *E*, we examined the binding of *in vitro* translated constructs of KLC2 lacking either the C-terminal TPR motifs (KLC2ΔC) or the N-terminal domain (KLCΔN) to DIC affinity columns and BSA control columns. Both the KLC2ΔC and KLCΔN fragments bound significantly to the DIC affinity matrix (*lane E*) but not to the BSA control column (*lane C*), indicating that the interactions of these polypeptides are not solely mediated by the TPR motifs of kinesin light chains. The construct KLC2ΔC spans residues 1–179, and the construct KLC2ΔN spans from residue 161 to the C terminus of the polypeptide. Shown are the load (*L*), flow-through (*F*), wash (*W*), and eluate (*E*) fractions from the DIC affinity columns and the eluate fractions (*C*) from the BSA control columns.

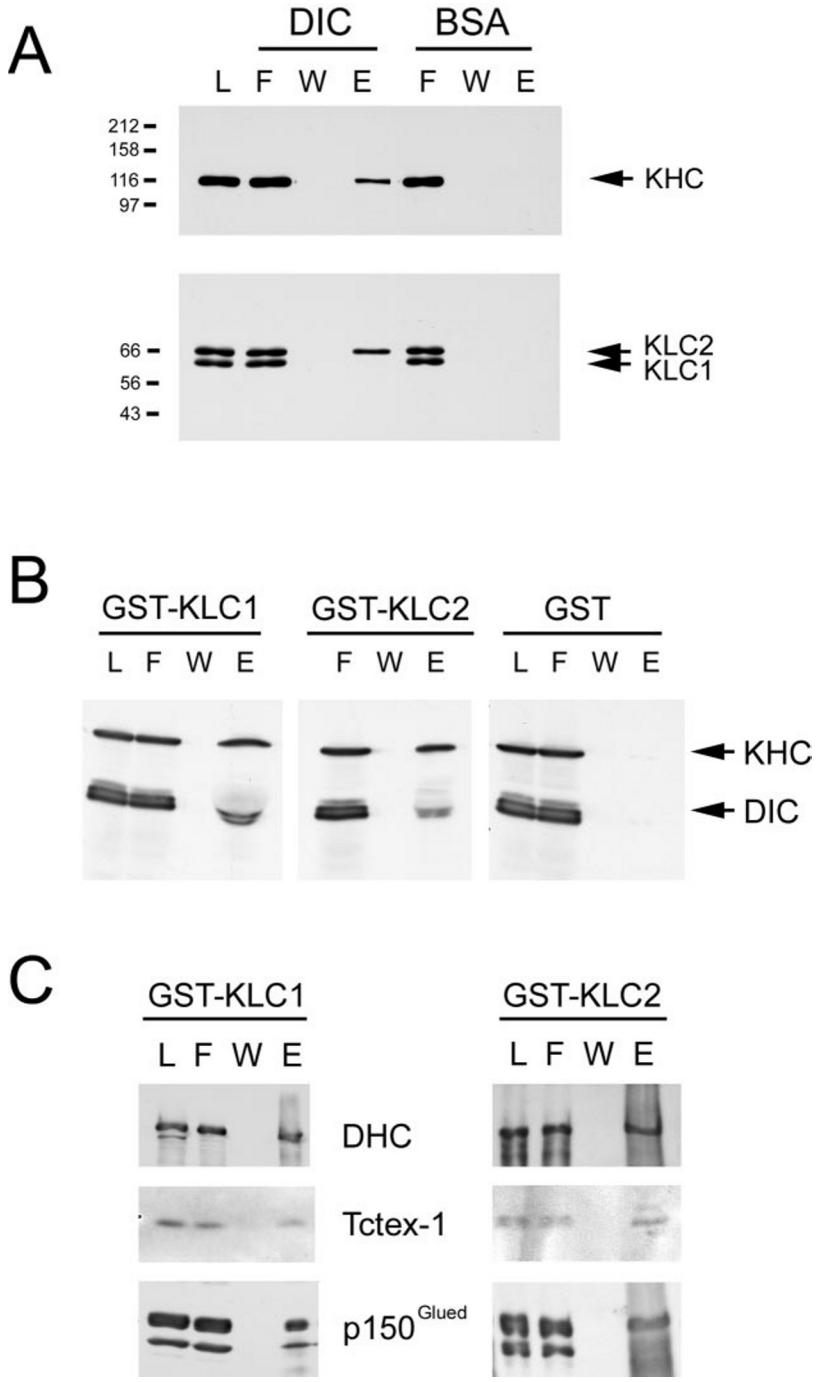

FIG. 2. **Binding studies with endogenous proteins suggest that dynein may interact specifically with kinesin containing KLC2.** *A*, rat brain cytosol was loaded onto a DIC affinity column, as well as a BSA control column. After extensive washes, specifically bound proteins were eluted with 1 M NaCl. The resulting load (*L*), flow-through (*F*), wash (*W*), and elution (*E*) fractions were fractionated by SDS-PAGE and analyzed by probing Western blots with antibodies to KHC and to kinesin light chains (KLC1 and KLC2). Kinesin heavy chain and kinesin light chain 2 were both specifically retained by the DIC affinity column but not the BSA control column. KLC1 was not retained by either column, suggesting that the interaction between endogenous proteins may be relatively specific for KLC2. *B*, dynein from rat brain cytosol was specifically retained by both GST-KLC1 and GST-KLC2 but not by GST only. *C*, fractions from the GST-KLC1 and GST-KLC2 columns were analyzed by Western blots probed with antibodies to dynein heavy chain (*DHC*), dynein light chain Tctex-1, and the $p150^{Glued}$ subunit of dynactin.

columns but not the control column (Fig. 1, *B* and *C*), suggesting that the dynein intermediate chain can bind directly to kinesin light chains 1 and 2. In the reciprocal experiment, KLC2 bound to the DIC column and not to a control BSA column. In contrast, KLC1 did not bind significantly to the DIC column (data not shown). This observation supports the qualitative analysis of the yeast two-hybrid assay, suggesting that KLC2 binds more strongly to DIC than does KLC1.

We mapped the binding site for KLCs within the dynein intermediate chain polypeptide using the yeast two-hybrid assay to test constructs spanning residues 1–120, 1–283, 120–283, and 283–644 of DIC (Fig. 1*D*). No significant interaction was observed between KLC1 or KLC2 and the DIC fragment 283–644, indicating that the association between these polypeptides does not involve the C-terminal WD40 repeat region of DIC. The DIC constructs spanning residues 1–283 or 120–283 were consistently most positive in this assay, suggesting that the primary KLC-binding site in dynein spans residues 120–283. Residues 1–120 of DIC did not interact significantly with KLC1 but did demonstrate consistent interactions with KLC2, suggesting that there might be a secondary site of association between dynein and KLC2 that may explain the apparently higher affinity of DIC for this isoform. The inverse experiment was also performed to map the binding site for dynein within the KLC polypeptides. Affinity chromatography experiments revealed that the C-terminal TPR repeat domain of KLC2 bound significantly to a DIC affinity column and not to a BSA control column (Fig. 1*E*). However, we also noted binding of the N-terminal domain of KLC2 to a DIC column but not to a BSA control column (Fig. 1*E*). A similar result was observed with KLC1. These observations suggest that the interaction of DIC with kinesin light chains is not limited to the TPR

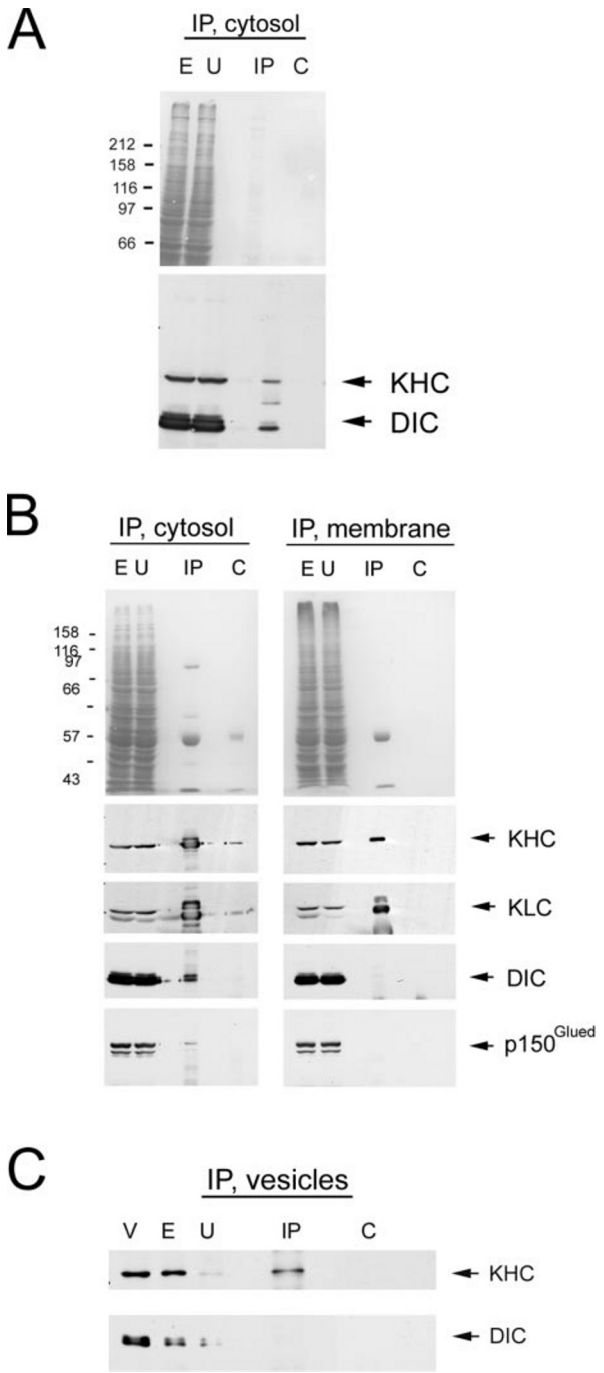

FIG. 3. **Cytoplasmic dynein and kinesin interact in rat brain cytosol.** *A*, co-immunoprecipitation of cytoplasmic dynein and kinesin from cytosolic fractions was observed with a monoclonal antibody to kinesin heavy chain. The *upper panel* is stained for total protein, and the *bottom panel* shows immunoblots probed with antibodies to KHC and dynein (*DIC*). *E*, extract; *U*, unbound; *IP*, immunoprecipitate; *C*, control. *B*, cytoplasmic dynein co-precipitates with kinesin from cytosol but not from a membrane-enriched fraction. Immunoprecipitation experiments were performed from rat brain cytosolic extract and from a membrane-enriched fraction from rat brain using a monoclonal antibody to kinesin light chain. The *upper panel* shows the extract (*E*), unbound (*U*), immunoprecipitate (*IP*), and control (*C*) fractions stained with Coomassie Blue for total protein. The *lower panels* are immunoblots probed with antibodies to KHC, KLC, DIC, and the $p150^{Glued}$ subunit of dynactin ($p150^{Glued}$). Cytoplasmic dynein was co-immunoprecipitated with kinesin from the cytosolic fraction but not from the membrane-enriched fraction. *C*, rat brain vesicles isolated by flotation through a sucrose gradient (*V*) were extracted with Triton X-100 (*E*) and incubated with antibody to kinesin light chain. The unbound fraction (*U*) shows depletion of kinesin. The immunoprecipitate (*IP*) fraction was positive for kinesin but not for dynein. The control precipitate (*C*) showed no nonspecific precipitation of either kinesin or dynein in this assay.

motifs of the light chains but also involves determinants in the N terminus.

These *in vitro* binding studies with purified proteins indicate that DIC can bind directly to kinesin light chains. To test whether these interactions occur with endogenous proteins, we loaded a rat brain cytosolic extract onto a DIC affinity column. Although both KLC1 and KLC2 were present in the cytosolic extract, only KLC2 was retained on the DIC affinity column (Fig. 2*A*). No significant binding of either KLC1 or KLC2 to a BSA control column was observed. Kinesin heavy chain was also specifically retained by the DIC affinity column (Fig. 2*A*), indicating that intact kinesin interacts with DIC.

We then performed the reciprocal experiment and fractionated rat brain cytosolic extract over GST-KLC1, GST-KLC2, or GST control columns. Dynein intermediate chain, dynein heavy chain, and a dynein light chain (Tctex-1) were all specifically retained by both the KLC1 and KLC2 columns but not by the control GST column (Fig. 2, *B* and *C*), indicating that the intact dynein complex interacts with kinesin light chains. Dynactin was also retained on both the KLC1 and KLC2 columns (Fig. 2*C*); however, dynactin binds to dynein (2), and thus the association with kinesin may be indirect.

These experiments indicate that the interaction between kinesin and dynein is both direct, because binding is observed between purified recombinant proteins, and specific, because dynein is specifically retained from brain cytosol by a KLC affinity column, and kinesin is specifically retained on a DIC affinity column. To test the physiological relevance of the association, we looked for the co-immunoprecipitation of cytoplasmic dynein and kinesin from rat brain cytosol. We observed that a fraction of cytoplasmic dynein was co-precipitated using two different monoclonal antibodies to KHC (Fig. 3*A* and data not shown). We also observed the co-immunoprecipitation of dynein and kinesin from cytosol using two independent monoclonal antibodies to KLC (Fig. 3*B*, *left panel*, and data not shown). Most of the dynein did not co-precipitate with kinesin, indicating that only a fraction of the total cytosolic pools of the motors are associated under these conditions. We observed only a very limited co-immunoprecipitation of dynactin, not significantly above background (Fig. 3*B*).

There are significant pools of both soluble and vesicle-associated kinesin and dynein in the cell. Therefore, we probed for an interaction between cytoplasmic dynein and kinesin in a vesicle-enriched fraction from rat brain. As shown in Fig. 3*B*, immunoprecipitation of kinesin from membrane-enriched fractions using a monoclonal antibody to KLC led to the robust co-immunoprecipitation of kinesin heavy and light chains as expected (Fig. 3*B*, *right panel*). In contrast, neither dynein nor dynactin was consistently observed to co-immunoprecipitate with kinesin from membrane-enriched fractions. Control experiments indicate that the co-immunoprecipitation of dynein and kinesin from the soluble fraction was not disrupted by the low levels of detergent required to solubilize membrane-associated proteins from the vesicular fraction. We further purified membrane fractions by flotation upward through a sucrose step gradient, and immunoprecipitations were performed using monoclonal antibodies to either kinesin heavy chain or light chain. We did not observe significant co-immunoprecipitation of dynein from these fractions (Fig. 3*C* and data not shown).

These immunoprecipitation data indicate that the interaction between dynein and kinesin occurs in the cell. This interaction may be more robust between soluble rather than vesicle-associated motor proteins or alternatively may be disrupted during the solubilization of vesicles prior to immunoprecipitation. To follow up on these biochemical observations, we performed immunocytochemistry in several epithelial and fibro-

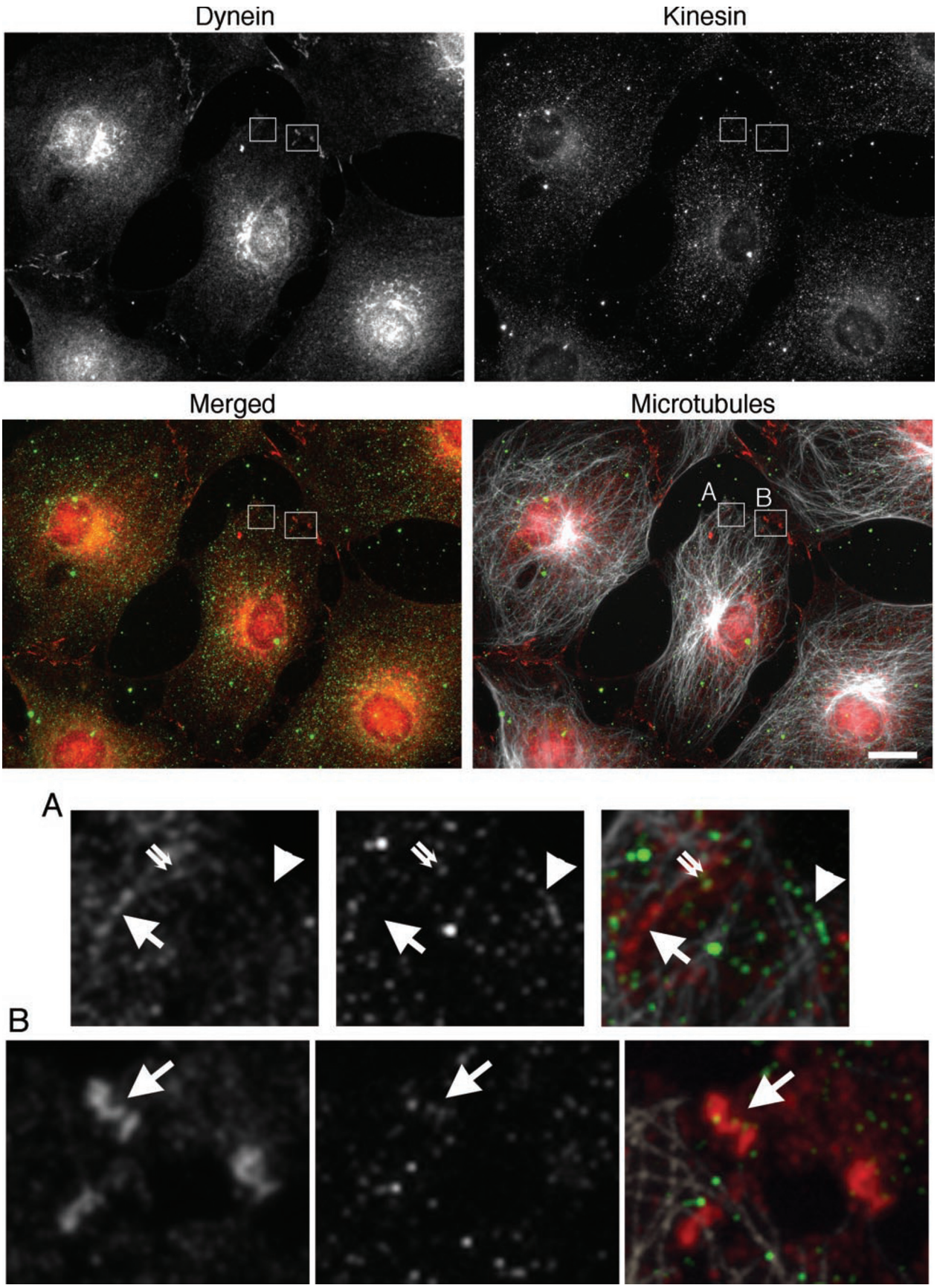

FIG. 4. **Cytoplasmic dynein and kinesin are on partially overlapping populations of vesicles in cells.** PtK2 epithelial cells in culture were labeled with antibodies to dynein intermediate chain (*red*, UP1467), kinesin heavy chain (*green*, MAB1614), and tubulin (YL1/2). *Insets A* and *B* are shown in magnified view. *Inset A* shows a region at the cell periphery where individual organelles and vesicles may be distinguished. Here, some vesicles are clearly labeled primarily with dynein antibodies (*arrow*), some are labeled primarily with kinesin antibodies (*arrowhead*), and some are labeled with both (*double arrow*). *Inset B* shows a cell-cell adherens junction with dynein localized to the junction (*arrow*). Kinesin is localized to bright puncta along this junction. *Scale bar*, 10 μm.

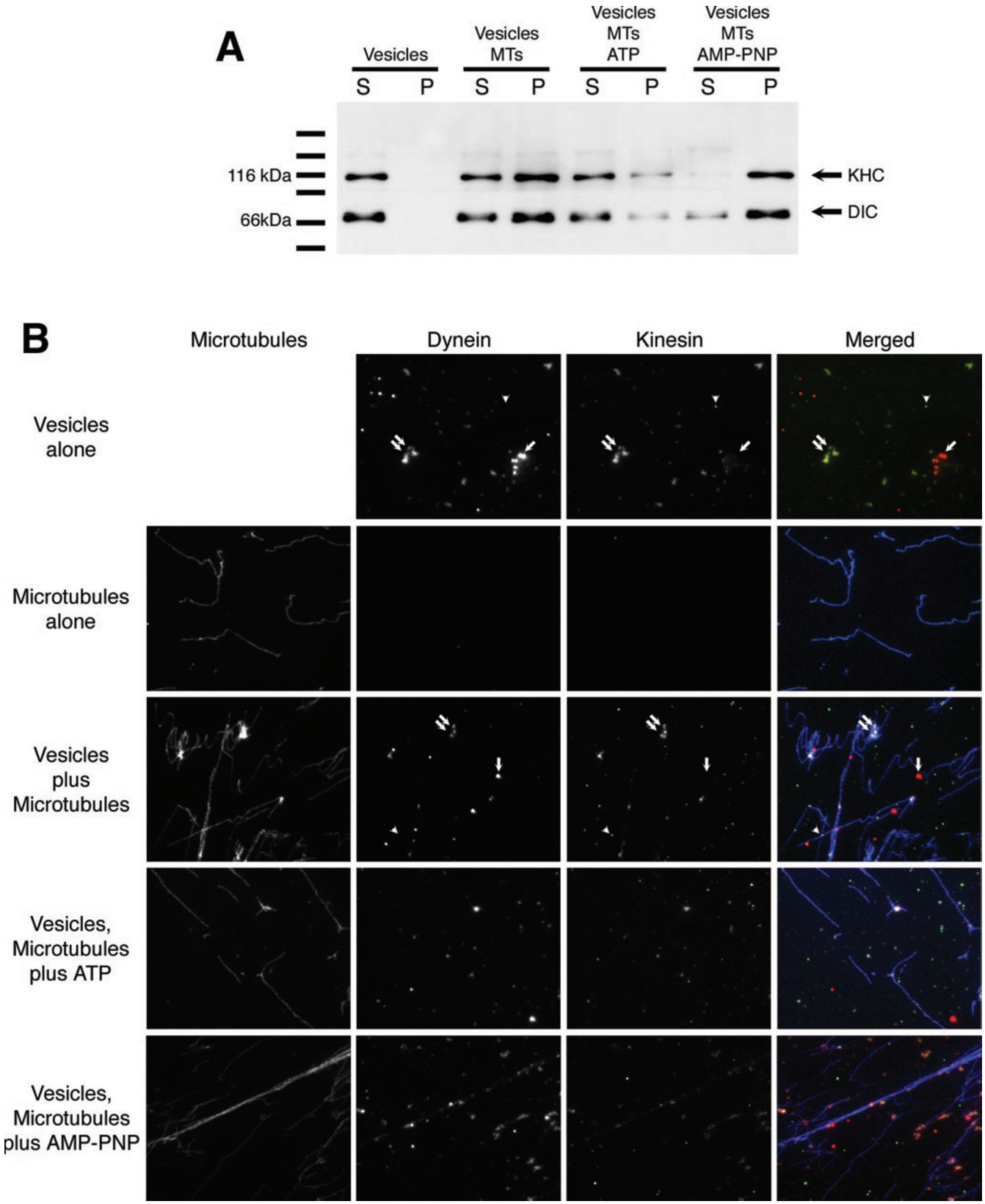

FIG. 5. **Cytoplasmic dynein and kinesin are on partially overlapping populations of vesicles isolated from rat brain.** *A*, vesicles purified by flotation through a sucrose step gradient were incubated in the absence or presence of microtubules, MgATP, and MgAMP-PNP. Vesicles bound to microtubules were then pelleted by centrifugation through a sucrose cushion. The fractions were separated by SDS-PAGE, and the resultant blots were probed with antibodies to KHC and DIC. In the absence of microtubules, the vesicles did not pellet. But when microtubules were added, the vesicles containing both kinesin and dynein pelleted. The addition of MgATP decreased the amount of motors in the pellet, and the addition of the nonhydrolyzable ATP analog AMP-PNP increased the amount that pelleted. *B*, purified vesicles were examined by immunocytochemistry with antibodies to KHC (*green*, MAB1614), DIC (*red*, UP1467), and tubulin (*blue*, DM1A). Some vesicles were clearly labeled primarily by dynein antibodies (*arrows*), some were labeled by kinesin antibodies (*arrowhead*), and some were labeled by both antibodies (*double arrows*). When vesicles were incubated with microtubules, most of the vesicles appeared to be associated with the microtubules. The addition of ATP released many but not all of the vesicles, and the addition of AMP-PNP appeared to augment the binding of vesicles to microtubules.

blast cell lines using a battery of monoclonal and polyclonal antibodies to dynein and kinesin. Both motors are densely distributed in the perinuclear region, and therefore the true extent of co-localization is difficult to assess in this part of the cell. At the cell periphery, where individual organelles and vesicles can more readily be discerned, we saw partial co-localization of the two motor proteins to vesicular puncta (Fig. 4, *double arrows*). However, many of the vesicles and organelles appeared to be predominantly labeled with either kinesin (*arrowhead*) or dynein (*arrow*) antibodies, similar to previous observations with a more limited set of antibodies (16). Interestingly, we often noted rows of these singly labeled vesicles arrayed along microtubules (Fig. 4, *inset A*).

We also observed partial co-localization of the two motors at points of cell-cell contact in epithelial cells (Fig. 4, *inset B*). Dynein is localized to adherens junctions in epithelial cells (11); here, we see kinesin puncta decorating these dynein patches. This localization of kinesin to sites of cell-cell contact may reflect a role for the motor in the biogenesis of cadherin-dependent cell-cell contacts (17).

To further examine the interaction of vesicle-associated dynein and kinesin, we incubated vesicles with microtubules in the absence or presence of either MgATP or MgAMP-PNP. We isolated the microtubule-bound vesicles by sedimentation and analyzed the results by both immunoblot and immunofluorescence. Both dynein and kinesin co-sedimented with microtubules when the vesicular fractions were incubated with microtubules. The degree of co-sedimentation was substantially reduced in the presence of ATP, indicating that the observed linkages were motor-dependent. Conversely, the nonhydrolyzable ATP analog AMP-PNP increased the amount of motors that co-pelleted with microtubules in this assay (Fig. 5*A*).

Immunofluorescence analysis showed that many dispersed vesicles were clearly stained with antibodies to either dynein or kinesin (Fig. 5*B*). However, approximately one-third of the vesicles were labeled with antibodies to both motors (Fig. 5*B*, *double arrows*). Dynein-bound and kinesin-bound vesicles, as well as vesicles with both motors bound were all observed to associate with microtubules. Association of vesicles with microtubules was more pronounced in the presence of AMP-PNP, and the addition of MgATP induced significant dissociation. These localization studies are consistent with results from immunocytochemistry, indicating that although the most robust association between dynein and kinesin occurs in the soluble pool of motors, there is a population of vesicles with both motors bound.

DISCUSSION

Together, these observations identify a direct interaction between cytoplasmic dynein and kinesin, mediated by kinesin light chains. Although light chains may not be necessary for all kinesin-cargo interactions, some motor-cargo interactions appear to be mediated by direct binding to KLCs (18, 19), and the TPR motifs of KLCs have been shown to interact with vesicular proteins such as amyloid precursor protein and components of the c-Jun N-terminal kinase signaling pathway (Ref. 20; reviewed in Ref. 6). Our results suggest that kinesin I may also interact with a novel cargo, the motor cytoplasmic dynein, via KLCs. Although both KLCs can bind to DIC, the most pronounced interaction was between KLC2 and DIC. Although the differential roles of KLC1 and KLC2 have yet to be fully established, several studies have suggested that KLCs are involved in determining the specificity of kinesin-cargo interactions in the cell.

There are two possible models for the role of these direct interactions between plus and minus end-directed motor complexes. The first is that the interaction serves a transport role. The most robust interaction between dynein and kinesin was observed in the soluble pools of these motors, suggesting that one motor serves as a cargo for the other. Kinesin may be required to transport dynein to the periphery of the cell, the end of the axon, or to the plus end of the microtubule. When axonal transport is blocked by ligature, kinesin accumulates proximally, and dynein accumulates both proximally and distally, consistent with the known directionalities of the motors, as well as with the fact that dynein must be transported down the axon prior to initiating active transport back to the cell body (22, 23). Further, dynein was observed to accumulate in large aggregates in axons in kinesin light chain mutants in *Drosophila* (24). Kinesin-mediated transport of dynein may also explain the kinesin dependence of the robust localization of dynein to microtubule plus ends observed in *Aspergillus* (25), although the lack of kinesin lights chains in this species suggests that distinct interaction mechanisms may be involved.

However, we also noted some co-localization of cytoplasmic dynein and kinesin to vesicles, suggesting that there may be cargo-associated motor platforms that coordinate bi-directional organelle motility. The direct interaction between kinesin I and cytoplasmic dynein that we have described may thus be similar to the observation that kinesin II can bind to dynactin through its nonmotor subunit KAP, leading to the hypothesis that dynactin may coordinate the bi-directional motility of melanosomes (21). Although kinesins I and II are distinct cellular motors, it is interesting that in both cases specific interactions with the minus end-directed motor dynein have been observed, in one case via a direct interaction with dynein (this study) and in the other case via an interaction with dynactin (21).

The interaction we have observed between kinesin I and dynein may also have a regulatory role. Kinesin light chains have been reported to affect kinesin motor function, stabilizing an inactive form of the motor in which the tail of kinesin folds back and inhibits the motor domain in the head (26). Binding of light chains to cargo may induce a conformational change that releases this inhibition. It will be of interest to determine whether the binding of dynein to the kinesin light chain provides a similar release from inhibition. The binding of kinesin to dynein may also have some regulatory effect on dynein motor function. Such a model would provide for a carefully regulated interaction between oppositely oriented motors that would allow for the efficient transport of cytoplasmic dynein down the axon in its inactive state. Activation of dynein at the axon terminus might induce dissociation from kinesin, freeing dynein to catalyze the motility of cargo back to the cell body. Together the direct coupling of the motors would allow for the efficient localization of dynein to its site of function, which is an important problem in cells with axonal projections that can reach a meter in length. The demonstration of biochemical interactions between oppositely oriented microtubule-based motors provides a mechanistic explanation for their observed interdependency and provides testable hypotheses for further investigations.

Acknowledgments—We gratefully acknowledge the contributions of Spencer Shelly and Penney Gilbert of the University of Pennsylvania. We also thank Scott Brady, Richard Vallee, and Stephen King, who provided antibodies to kinesin and dynein, and Larry Goldstein, who provided cDNA clones of KLCs 1 and 2.

REFERENCES

1. Vale, R. D. (2003) *Cell* **112,** 467–480
2. Karki, S., and Holzbaur, E. L. F. (1995) *J. Biol. Chem.* **270,** 28806–28811
3. King, S. J., and Schroer, T. A. (2000) *Nat. Cell Biol.* **2,** 20–24
4. Holleran, E. A., Ligon, L. A., Tokito, M. K., Stankewich, M. K., Morrow, J. S., and Holzbaur, E. L. F. (2001) *J. Biol. Chem.* **276,** 36598–36605
5. Muresan, V., Stankewich, M. C., Steffen, W., Morrow, J. S., Holzbaur, E. L. F., and Schnapp, B. J. (2001) *Mol. Cell* **7,** 173–183
6. Hollenbeck, P. J. (2001) *J. Cell Biol.* **152,** 25–28
7. Brady, S. T., Pfister, K. K, and Bloom, G. S. (1990) *Proc. Natl. Acad. Sci* .

U. S. A. **87,** 1061–1065
8. Stenoien, D. L., and Brady, S. T. (1997) *Mol. Biol. Cell* **8,** 675–689
9. Waterman-Storer, C. M., Kuznetsov, S. A., Karki, S., Tabb, J. S., Weiss, D. G., Langford, G. M., and Holzbaur, E. L. F. (1997) *Proc. Natl. Acad. Sci . U. S. A.* **94,** 12180–12185
10. Martin, A. A., Iyaduri, S. J., Gassman, A., Gindhart, J. G., Hays, T. S., and Saxton, W. M. (1999) *Mol. Biol. Cell* **10,** 3717–3728
11. Ligon, L. A., Karki, S., Tokito, M. K., and Holzbaur, E. L. F. (2001) *Nat. Cell Biol.* **3,** 913–917
12. Ligon, L. A., Shelly, S. S., Tokito, M., and Holzbaur, E. L. F. (2003) *Mol. Biol. Cell* **14,** 1405–1417
13. Blatch, G. L., and Lassle, M. (1999) *Bioessays* **21,** 932–939
14. Rahman, A., Friedman, D. S., and Goldstein, L. S. (1998) *J. Biol. Chem.* **273,** 15395–15403
15. Wilkerson, C. G., King, S. M., Koutoulis, A., Pazour, G. J., and Witman, G. B. (1995) *J. Cell Biol.* **129,** 169–178
16. Lin, S. X., Pfister, K. K., and Collins, C. A. (1996) *Cell Motil. Cytoskeleton* **34,** 299–312
17. Mary, S., Charrasse, S., Meriane, M., Comunale, F., Travo, P, Blangy, A., and Gauthier-Rouveiere, C. (2002) *Mol. Biol. Cell* **13,** 285–301
18. Gyoeva, F. K., Bybikova, E. M, and Minin, A. A. (2000) *J. Cell Sci.* **113,** 2047–2054
19. Khodjakov, A., Lizunova, E. M., Minin, A. A., Koonce, M. P., and Gyoeva, F. K. (1998) *Mol. Biol. Cell.* **9,** 333–343
20. Kamal, A., Stokin, G. B., Yang, Z., Xia, C. H., and Goldstein, L. S. (2000) *Neuron* **28,** 449–459
21. Deacon, S. W., Serpinskaya, A. S., Vaughan, P. S., Lopez Franarraga, M., Vernos, I., Vaughan, K. T., and Gelfand, V. I. (2003) *J. Cell Biol.* **160,** 291–293
22. Hirokawa, N., Sato-Yoshitake, R., Kobayashi, N., Pfister, K. K., Bloom, G. S., and Brady, S. T. (1991) *J. Cell Biol.* **114,** 295–302
23. Hirokawa, N., Sato-Yoshitake, R., Yoshida, T., and Kawashima, T. (1990) *J. Cell Biol.* **111,** 1027–1037
24. Gindhart, J. G., Jr., Desai, C. J., Beuhausen, S., Zinn, K., and Goldstein, L. S. (1998) *J. Cell Biol.* **141,** 443–454
25. Zhang, J., Li, S., Fischer, R., and Xiang, X. (2003) *Mol. Biol. Cell* **14,** 1479–1488
26. Verhey, K. J., Lizotte, D. L., Abramson, T., Barenboim, L., Schnapp, B. J., and Rapoport, T. A. (1998) *J. Cell Biol.* **143,** 1053–1066