The Two Motor Domains of KIF3A/B Coordinate for Processive Motility and Move at Different Speeds

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ABSTRACT KIF3A/B, a kinesin involved in intraflagellar transport and Golgi trafficking, is distinctive because it contains two nonidentical motor domains. Our hypothesis is that the two heads have distinct functional properties, which are tuned to maximize the performance of the wild-type heterodimer. To test this, we investigated the motility of wild-type KIF3A/B heterodimer and chimaeric KIF3A/A and KIF3B/B homodimers made by splicing the head of one subunit to the rod and tail of the other. The first result is that KIF3A/B is processive, consistent with its transport function in cells. Secondly, the KIF3B/B homodimer moves at twice the speed of the wild-type motor but has reduced processivity, suggesting a trade-off between speed and processivity. Third, the KIF3A/A homodimer moves fivefold slower than wild-type, demonstrating distinct functional differences between the two heads. The heterodimer speed cannot be accounted for by a sequential head model in which the two heads alternate along the microtubule with identical speeds as in the homodimers. Instead, the data are consistent with a coordinated head model in which detachment of the slow KIF3A head from the microtubule is accelerated roughly threefold by the KIF3B head.

INTRODUCTION

Kinesins comprise a large family of molecular motors that transport intracellular cargo along microtubules using the energy derived from ATP hydrolysis. Of the 14 known classes of kinesins (Miki et al., 2001), kinesin II motors are unique in that they form a heterotrimeric complex consisting of two different heavy chains and a third nonmotor subunit. Members of the kinesin II subfamily are plus end-directed motors that are involved in diverse intracellular functions including intraflagellar trafficking (Cole et al., 1998; Orozco et al., 1999), assembly and maintenance of cilia and flagella (Brown et al., 1999; Cole et al., 1998; Signor et al., 1999), endoplasmic reticulum to Golgi membrane transport (Le Bot et al., 1998), and dispersion of pigment granosomes (Tuma et al., 1998). KIF3A/B, the mouse kinesin II ortholog, functions as a motor for anterograde axonal transport (Kondo et al., 1994; Yamazaki et al., 1995) and plays an essential role in embryonic development. KIF3A and KIF3B knockout mice displayed severe cardiac abnormalities and loss of left-right asymmetry due to immotile nodal cilia (Marszalek et al., 1999; Nonaka et al., 1998; Takeda et al., 1999). Because of their unique heteromeric structure and diverse cellular roles, it is important to better understand the mechanism underlying kinesin II motility.

Conventional kinesin was the first cytoskeletal motor shown to be processive, defined as the ability to take many steps along its filament track before dissociating (Howard et al., 1989). Subsequently other kinesins, myosins, and dynein have also been shown to be processive transport motors (Mallik et al., 2004; Mehta et al., 1999; Okada and Hirokawa, 1999). To prevent detachment and rapid diffusion away from the microtubule, the two heads of a dimeric kinesin must coordinate such that one head is always bound to the microtubule. Because of this coordination, uncovering motor function requires not only defining the ATP hydrolysis cycle and associated conformational changes, but also identifying steps in the cycle in which the activity of one head modulates the kinetics of the second head.

Despite considerable work, there is no consensus mechanism by which conventional kinesin's two heads coordinate their chemomechanical cycles to ensure processivity. Existing models of the kinesin walking cycle incorporate a number of different mechanisms to ensure that the microtubule-bound head does not detach before the tethered head binds to the next binding site. These include 1), strain-dependent detachment of the rear head (Hancock and Howard, 1998; Rice et al., 1999); 2), slowed ATP binding to the forward head when both heads are bound (Rosenfeld et al., 2002, 2003; Klumpp et al., 2004); and 3), very fast attachment and ADP release by the tethered head (Crevel et al., 2004; Hackney, 2002). Because the strain-dependent transitions that ensure processivity are intimately linked to the force-dependent steps, defining this coordination is crucial for understanding chemomechanical coupling in kinesins.

Because kinesin II motors naturally have two different motor domains, they provide an important tool both for testing competing models of motility and for studying intersubunit coordination in dimeric motors in general. To study intersubunit coordination in kinesin II, we used microtubule gliding assays to investigate the motility and processivity of baculovirus-expressed wild-type KIF3A/B

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and chimaeric homodimers created by fusing the head of one subunit to the rod and tail of the other. Our results show that wild-type KIF3A/B is processive and that KIF3B/B homodimer moves 10-fold faster than KIF3A/A homodimer. These results, both of which contrast with previous work on kinesin II motors (Pierce et al., 1999; Yamazaki et al., 1995), suggest that the two heads of kinesin II are biochemically tuned to achieve optimal motor performance.

MATERIALS AND METHODS

Expression constructs

Full-length cDNAs for KIF3A and KIF3B were a gift of L. Wordeman and L. Ginkel (University of Washington, Seattle, WA). Sequences were modified by PCR-based mutagenesis and QuikChange mutagenesis (Stratagene, La Jolla, CA) to introduce proper restriction sites and tags for purification. For KIF3A, a BglII site was added upstream of the coding sequence, the sequence coding for QKLISEEDL was appended to the final E of the coding sequence to generate a Myc tag, and an *Eco*RI site was added downstream of the stop codon. For KIF3B, a sequence coding for a hexahistidine tag was introduced to the 3′ end of the KIF3B coding sequence, and a BamHI site was added following the stop codon. Two transfer vectors, pAcKIF3A and pAcKIF3B, were obtained by ligating the KIF3A and KIF3B genes into pAcUW51 baculovirus transfer vectors (Pharmingen, San Diego, CA). As initially we could only express the KIF3A subunit but not the KIF3B subunit, we compared the upstream sequence of the KIF3B gene to the consensus sequence from 154 native baculovirus genes (Ayres et al., 1994) and to other studies on baculovirus protein expression (Hirokawa and Noda, 2001; Pierce et al., 1999; [illegible] US5194376). We concluded that sequences directly upstream of the [illegible]G start codon must inhibit either transcription of the KIF3B gene or translation of the message. Hence, the sequence AAAT was inserted immediately upstream of the start codon for KIF3B gene by site-directed [illegible]agenesis, which enhanced expression of the KIF3B subunit.

Previous work has shown that KIF3A and K[illegible]B and other kinesin II motors preferentially form heterodimers through the coiled-coil [illegible] (De Marco et al., 2001; Rashid et al., 199[illegible] Yamazaki [illegible]., 1995), so to make homodimeric KIF3 motors containing two identical head domains (KIF3A/A and KIF3B/B), two chimaeric genes were created by switching the heads. By comparing the amino acid sequences of the KIF3A/B heads to conventional kinesin sequences from human, [illegible] and rat, and to the rat kinesin dimer crystal structure (Kozielski et al., 19[illegible] we identified a 10-residue identity region in KIF3A/B spanning the end of the neck-linker and the start of the neck coiled-coil (Fig. 1 *A*). Splicing the heads in this identity region maintained the chimaeric neck-linker and head as an intact domain and the entire predicted coiled-coil as heterodimer.

To make the chimaeric KIF genes, we inserted a NotI site upstream of the KIF3[illegible] gene (this was an existing NotI site upstream of the KIF3B gene) and introduced silent mutations to create an AflII site at LLR in the neck-coil region of both genes (Fig. 1 *A*). For KIF3A, the DNA sequence CTGCTCC[illegible] was changed to CTCTTAAGA and for KIF3B the sequence CTGCTTCG[illegible] was changed to CTCTTAAGA. The resultant pAcKIF3A and pAcKIF3B plasmids were then digested with NotI and AflII restriction enzymes (New England Biolabs, Beverly, MA), gel purified, and the heads spliced to their complementary rod-tail domains (Fig. 1 *B*).

Protein expression and purification

Four different stocks of recombinant viruses were generated by cotransfecting KIF3 plasmids with BaculoGold linearized baculovirus DNA (Pharmingen). Wild-type KIF3A/B motors were expressed by coinfecting *Spodoptera frugiperda* (Sf9) insect cells with wild-type KIF3A virus and KIF3B virus.

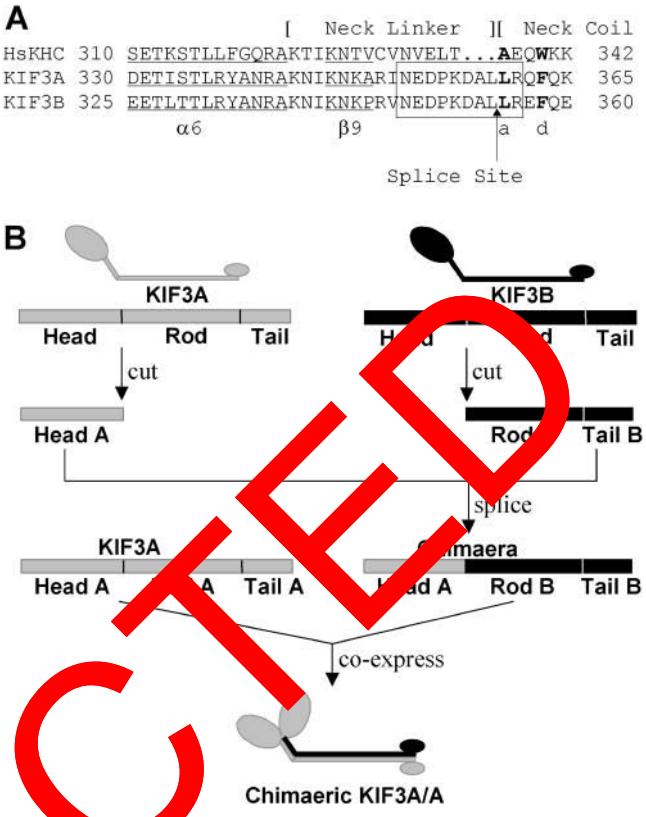

FIGURE [illegible] Amino acid sequence alignment for mouse KIF3A and [illegible]B and human conventional kinesin heavy chain (HsKHC) genes at the neck-[illegible]nction. Secondary structure predictions were taken from the rat KHC crystal structure (Kozielski et al., 1997) and the start of the coiled-coil of KIF3A and KIF3B was inferred by comparison to the conventional kinesin sequence and by predictions from the COILS program. There is an obvious splice site in the neck-linker region of KIF3A and KIF3B; the arrow denotes where the AflII restriction site was introduced. (*B*) Constructing mutant KIF3A/A. KIF3A and KIF3B plasmids were digested and the sequence for the KIF3A head domain was spliced to the sequence for the KIF3B rod and tail domains. This chimaeric gene was then coexpressed with the wild-type KIF3A gene in insect cells, producing a mutant protein that has two KIF3A heads and the normal KIF3A/B rod and tail structure. An analogous approach was used to make KIF3B/B. GenBank accession numbers: KIF3A, NM_008443; KIF3B, NM_008444; HsKHC, X65873.

Mutant KIF3A/A homodimers were expressed by coinfecting cells with chimaeric KIF3A virus and wild-type KIF3A virus; to make KIF3B/B, cells were coinfected with chimaeric KIF3B virus and wild-type KIF3B virus. Maximum yields of functional KIF3A/B, as measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and motility assays, were achieved by growing the cells in Sf-900 II SFM serum-free medium (Gibco-BRL, Gaithersburg, MD) at 27°C, harvesting the cells 60 h after infection and lysing the infected cells in lysis buffer with 1% Triton. For large-scale expression, 25 ml each of two recombinant viral stocks with a titer of $\sim 1 \times 10^8$ plaque-forming units/ml was added into 500 ml of Sf9 suspension cell cultures. After 60 h incubation at 27°C, infected cells were pelleted by centrifuging for 10 min at 1000 × *g*, frozen in liquid nitrogen, and stored at −80°C.

For protein purification, cell pellets were thawed, resuspended in lysis buffer (20 mM TrisHCl, 500 mM NaCl, 10 mM imidazole, 1 mM $MgCl_2$, 1% Triton, 5 mM β-mercaptoethanol (β-ME), 0.5 mM MgATP, protease inhibitor cocktail (Pharmingen), pH 7.5) and lysed on ice for 45 min. The crude cell lysate was then centrifuged for 30 min at 100,000 × *g* to remove

cellular debris and insoluble proteins. His-tagged KIF3 motors were purified by passing through a 2-ml nickel-nitrilotriacetic acid (Ni-NTA) chromatography column (QIAGEN, Valencia, CA). The column was first equilibrated with lysis buffer and then the cleared lysate was loaded onto the column, followed by 10 column volumes of wash buffer (50 mM sodium phosphate, 300 mM NaCl, 60 mM imidazole, 1 mM $MgCl_2$, 10% glycerol, 5 mM β-ME, 0.1 mM MgATP, pH 7.0) to remove contaminating insect host proteins. Motor proteins were eluted from the column by a step elution with elution buffer (50 mM sodium phosphate, 300 mM NaCl, 500 mM imidazole, 1 mM $MgCl_2$, 10% glycerol, 5 mM dithiothreitol, 0.1 mM MgATP, pH 7.0). The protein absorbance at 280 nm was monitored during the purification process. Peak fractions were collected, frozen in liquid nitrogen and stored at −80°C.

Motor concentrations were quantified by running samples on 7% SDS-PAGE gels along with bovine serum albumin standards, and staining with Coomassie blue dye. Gel images were captured by a UVP BioChemi System (UVP, Upland, CA) and the optical density for each band was analyzed with LabWorks 4.0 (UVP).

Hydrodynamic analysis

For sedimentation velocity analysis, 500 μl purified KIF3A/B motors were exchanged into BRB80 buffer (80 mM Pipes, 1 mM EGTA, 1 mM $MgCl_2$, pH 6.9) with 100 μM MgATP, layered on a 5–25% (w/v) sucrose density gradient, and centrifuged at 41,000 rpm for 24 h at 4°C (L8-70M ultracentrifuge, SW 41 Ti rotor, Beckman Coulter, Fullerton, CA). Fractions were collected by gravity from the bottom of the gradient. Standard proteins with known sedimentation values (carbonic anhydrase, 3.2 S; bovine serum albumin, 4.4 S; alcohol dehydrogenase, 7.6 S; β-amylase, 8.9 S) were run in a parallel tube. To determine the peak fractions of the standards, Coomassie blue-stained gels were scanned and the band intensities were fit with Gaussian distributions. Motor peaks were located by motility assays. Sedimentation values of motors were then determined from the standard curves generated by a linear regression of the fraction number versus the sedimentation coefficient.

For gel filtration analysis, 100 μl KIF3A/B motors were loaded onto a Superdex 200 10/300 GL column (Amersham Biosciences, Piscataway, NJ). Due to nonspecific adsorption of motors to the gel filtration beads, the column was run at 4°C in a high ionic strength buffer containing 50 mM sodium phosphate, 300 mM NaCl, 5 mM dithiothreitol, and 10 μM MgATP. The same standards as for the density gradients were run in parallel. Elution volumes and partition coefficients (K_{av}) were obtained by monitoring the absorbance at 280 nm. Motor protein Stokes radius was determined from a linear regression of $(-\log K_{av})^{1/2}$ versus Stokes radius for standard proteins. Motor protein molecular weight was then calculated using the sedimentation coefficient and Stokes radius in the Siegel and Monty (1966) equation. In this equation, partial specific volumes for motor proteins were calculated from those values of the constituent amino acids using a program called SEDNTERP. For example, the partial specific volume for KIF3A/B was calculated to be 0.736 $cm^3\ g^{-1}$. The solvent density and solvent viscosity were chosen to be 0.99823 g/cm^3 and 0.01002 g $cm^{-1}\ s^{-1}$, respectively, which are values for water at 20°C.

In vitro motility assays

Tubulin was extracted from bovine brain by repeated cycles of polymerization and depolymerization using standard recipes (Wagner et al., 1991; Williams and Lee, 1982), labeled with 5-(and 6)-carboxytetramethylrhodamine succinimidyl ester (Molecular Probes, Eugene, OR) (Hyman et al., 1991), and then polymerized into microtubules.

KIF3 motility was tested in microtubule gliding assays following standard procedures (Howard et al., 1993). Flow cells were first preloaded with BRB80 buffer containing 0.5 mg/ml casein to block the glass surface for 5 min, and purified motors diluted in BRB80CA (BRB80, 0.2 mg/ml casein, 1 mM MgATP) were then introduced into the chamber and allowed to adhere to the surface. After 5–10 min, motility solution (BRB80, 10 μM taxol, 1 mM MgATP, 32 nM rhodamine-labeled microtubules, and an oxygen scavenger system consisting of 20 mM D-glucose, 0.02 mg/ml glucose oxidase, 0.008 mg/ml catalase, and 0.5% β-ME) was flowed into the flow cell. To obtain short microtubules with lengths of 1–5 μm, microtubules were sheared by passing the motility solution twice through a 30-gauge needle at a flow rate of 100 μl/s.

To improve the motility in the assay at low motor surface densities, an initial precoating step was added by introducing 10 μg/ml anti-His antibody (Novagen, Madison, WI) into the chamber.

Video microscopy and data analysis

Microtubule gliding was monitored by fluorescence microscopy with an upright Nikon E600 microscope (100×, 1.3 N.A. objective). Fluorescence images were captured by an intensified CCD camera (HamamatsuW, GW-902H, Orangeburg, NY) recorded onto S-VHS videotapes, and analyzed offline using the imaging processing software Scion Image (Scion, Frederick, MD). The distances traveled by microtubules were measured by tracing the microtubule position by hand on a transparent sheet over the video screen or by a custom tracking program. The minimum detectable threshold was 0.3 μm.

To investigate the processivity of KIF3 motors, a landing rate assay was performed at varying motor surface densities by counting the number of microtubules longer than 1 μm that landed and moved for at least 0.3 μm across motor coated surfaces during an appropriate time window in the whole video screen area (equivalent to 3016 μm^2 in the flow cell). The microtubule landing rate data were then fit to a model as previously described (Hancock and Howard, 1998).

RESULTS

Expression and purification of recombinant KIF3A/B protein

It has been reported that KIF3 motors cannot be functionally expressed in bacteria (Kondo et al., 1994; Pierce et al., 1999), most likely due to protein aggregation and improper folding, and our work with KIF3 truncations is consistent with this (Y. C. Lee and W. Hancock, unpublished). Motivated by this, we turned to the baculovirus expression system.

After expression and purification were optimized, purified KIF3A/B appeared as a pair of bands at 85 kD and 95 kD on gels, corresponding to the KIF3A subunit and the KIF3B subunit, respectively (Fig. 2). Sucrose density gradient centrifugation of these motors resulted in a single peak with a sedimentation value of 6.8 ± 0.1, consistent with previous data for sea urchin KRP85/95-GFP dimer (6.3 ± 0.4) (Pierce et al., 1999). When analyzed by gel filtration, there was a motor peak with a calculated Stokes radius of 5.4 nm and predicted molecular mass of 152.5 kD. This agrees well with the predicted 167.7 kD for the KIF3A/B heterodimer, showing that our recombinant KIF3A/B is indeed heterodimeric.

From gel densitometry, some KIF3A/B preparations showed a 1:1 stoichiometry of KIF3A subunits to KIF3B subunits, but in other preparations the stoichiometry of KIF3B to KIF3A ranged from 2:1 to 7:1. Although there was

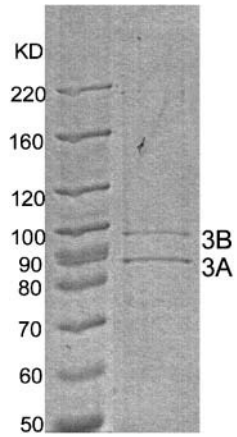

FIGURE 2 SDS-PAGE of purified wild-type KIF3A/B heterodimer. Lane1, molecular weight markers; lane 2, eluate of KIF3A/B.

no observable difference in motility between these preparations and the 1:1 stoichiometry preparations, we wanted to characterize the oligomerization state of this KIF3B and rule out the possibility that any excess KIF3B was affecting our motility assays. From the gel filtration analysis there was no evidence of a KIF3B monomer peak at the predicted 86.3 kD, but there was a large protein peak that eluted after one column volume, which we interpreted as nonspecific adsorption of motors to the column (as seen by o[illegible] (Pierce et al., 1999)), and there was a protein peak that [illegible]n with the void volume (molecular weight >600 kD[illegible] consistent with higher order oligomers of K[illegible]. To tes[illegible] whether KIF3B alone is functional, we i[illegible]ected c[illegible]s with only the KIF3B virus and purified an[illegible]ested the [illegible]esultant protein. In these KIF3B preparati[illegible]s t[illegible]re [illegible]as not an additional ~70 kD band corresp[illegible]ding to the [illegible]ative KIF3A ortholog from the insect cells, i[illegible]ting that KI[illegible]B does not heterodimerize measurably [illegible]ith na[illegible]e Sf9 protei[illegible]s. From gel filtration analysis, [illegible]ere was no [illegible]idence for either KIF3B monomers o[illegible]imers, but again [illegible]re was protein both in the void vo[illegible]me and i[illegible] late fraction, suggesting that this KIF3B formed [illegible]gre[illegible]es and/or was partially denatured and interacted nonspe[illegible]cally wit[illegible]e column. When tested in motilit[illegible] assay, this p[illegible]ified [illegible]F3B showed only minimal microt[illegible]bule bi[illegible]ing (eigh[illegible]d lower than KIF3A/B at comp[illegible]able [illegible] concentrations), and no microtubule movem[illegible]as observed, confirming that they are not functional [illegible]tors. Finally, to test for possible effects on KIF3A/B mo[illegible]ty, we added a sevenfold excess of this purified KIF3B to purified KIF3A/B in motility assays and found no effect on the landing rate and an only minimal effect on the microtubule gliding speed (when 350 nM KIF3B was mixed with 50 nM KIF3A/B, the microtubule gliding speed decreased from 164 ± 36 nm/s to 145 ± 24 nm/s (mean ± SD)). These results led us to conclude that any extra KIF3B in our motor preparations is denatured or partially unfolded protein that has no effect on KIF3A/B motility.

KIF3A/B is a processive motor optimized for long-distance transport

To investigate whether KIF3A/B is processive, the motor activity of KIF3A/B was measured at a series of motor surface densities in the microtubule gliding assay. The surface density of attached KIF3A/B motors was varied by loading different concentrations of motors into the flow cell. Assuming that all molecules loaded are absorbed onto the surface and half of them land on each face of the flow cell, the motor surface density is estimated [illegible] the [illegible]oduct of the protein molar concentration and the [illegible]w cell volu[illegible] divided by the area of both flow cell surface[illegible] Hence, for ou[illegible]tock of purified KIF3A/B with conce[illegible]ration [illegible]0 nM esti[illegible]ated by gel scanning, the maximum [illegible]urface densi[illegible] was c[illegible]ulated to be 3900 molecules/μm^2 [illegible]ased on t[illegible] flow c[illegible]mension of 18 mm × 7 mm × 11[illegible]m.

Velocity of mi[illegible]bule movem[illegible]t is independent of KIF3A/B s[illegible]face d[illegible]sity

Microtubule gliding v[illegible]city was assessed at a variety of KI[illegible]A/B surface densities [illegible]om 19.5 molecules/μm^2 to 3900 m[illegible]ecules/μm^2. As seen in Fig. 3 *A*, the gliding speed was i[illegible]ariant over se[illegible]ral decades of motor density. Even when th[illegible]otor surfac[illegible] density was decreased to single-molecule level[illegible]19.5 [illegible]olecules/μm^2), KIF3A/B was capable of [illegible]ropelling microtubules at the same velocity as at high [illegible] densities. The average velocity was 184 ± 28 nm/s (mean across all densities ± SD, $N = 85$).

This density independence is similar to the behavior of processive conventional kinesin and myosin V (Howard et al., 1989; Rock et al., 2000), and in contrast to the behavior of nonprocessive myosin II, which exhibits a significant drop in velocity as the motor density is decreased (Uyeda et al., 1991).

Pivoting movements of microtubules are observed at low KIF3A/B surface densities

At low KIF3A/B surface densities (3.9–39 molecules/μm^2), microtubules were observed to swivel over single nodal points. The velocity of microtubule pivoting was estimated by measuring how fast the leading end of a microtubule moved away from the nodal point. Pivoting microtubules moved relative to the contact point with the same speed as nonpivoting microtubules at high KIF3A/B densities, indicating a KIF3A/B molecule, not some low-level contaminant, was located at the nodal point.

An example of microtubule pivoting movement is shown in Fig. 3 *B*. The microtubule lands on the surface, presumably tethered to one KIF3A/B molecule, pivots and moves its entire length (3.6 μm) through the single nodal point, then detaches and diffuses away. Assuming 8 nm per step, the single KIF3A/B molecule under this swiveling microtubule took 450 steps until the end of the microtubule was reached.

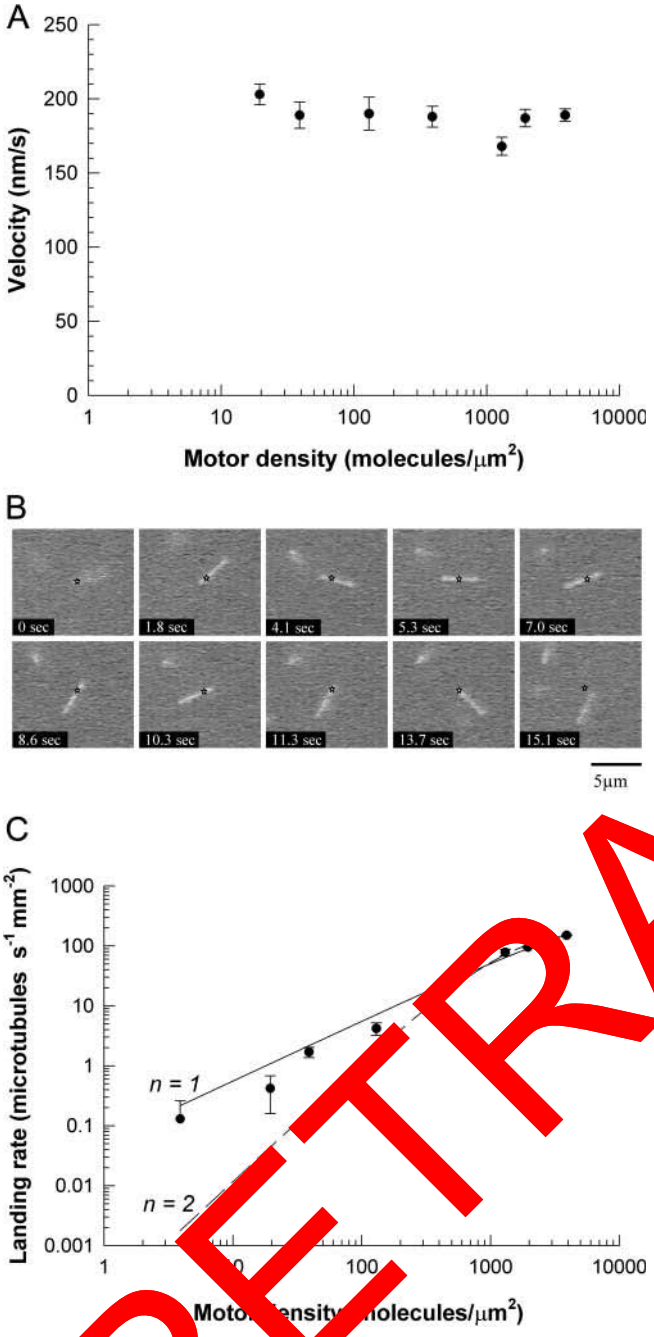

FIGURE 3 (*A*) Microtubule gliding speeds for wild-type KIF3A/B plotted over a range of motor densities. Error bars correspond to standard error of the means of at least seven velocity determinations for each density. (*B*) Microtubule pivoting around a single point on the surface coated with a very low density of KIF3A/B motor. (*C*) Microtubule landing rate of wild-type KIF3A/B plotted as a function of motor density. Error bars correspond to standard error of the means of the landing rate from at least five different windows for each density. The data are best fit with $n = 1$ (*solid line*), indicating that a single KIF3A/B molecule is sufficient to drive the movement of a microtubule. For comparison, the fit for $n = 2$ (*dashed line*) is also shown.

This microtubule pivoting result strongly suggests that KIF3A/B is processive.

One KIF3A/B motor is sufficient to drive microtubule movement

To quantitatively and statistically investigate the processivity of KIF3A/B, landing rate assays were performed to determine the number of motors required to move a microtubule (Fig. 3 *C*). Based on the model described by Hancock and Howard (1998), at low motor densities the landing rate will vary as the nth power of motor density, where n is the number of motors necessary to move a microtubule and appears as the slope of a log-log plot (landing rate versus motor density).

As the surface density of KIF3A/B was decreased, the fall in the microtubule landing rate was proportional to the motor density. The landing-rate data were best fit with $n = 1$, suggesting that one molecule of KIF3A/B is sufficient for motility. This assay provides statistical evidence that a single KIF3A/B molecule, not a chance colocalization of more than one processive motor, is sufficient to move a distance $>$ 100 nm.

Motility of homodimeric KIF3 chimaeras

To understand the coordination between the two different heads of KIF3A/B, we constructed and expressed two types of chimaeric motors, KIF3A/A and KIF3B/B, that retain the wild-type coiled-coil dimerization domain but contain two identical head domains. Both KIF3A/A and KIF3B/B could be functionally produced by the same expression and purification system as wild-type KIF3A/B.

KIF3A/A chimaera moves slowly in the microtubule gliding assay

Homodimeric KIF3A/A was capable of inducing microtubule gliding only when adsorbed at medium surface densities of ~400 molecules/μm^2. At high motor densities, numerous microtubules attached to the surface but no movement was observed. At low densities, no microtubules bound at all. At motor densities where motility could be observed, microtubules that landed on the surface moved at an average speed of 42 $\pm$ 11 nm/s (mean $\pm$ SD, $N = 22$).

To confirm this density-dependent motility, we tested KIF3A/A from four different preparations. The same reliable gliding speed was detected at medium KIF3A/A surface densities for all cases. To ensure that the low velocity is indeed an inherent quality of KIF3A/A rather than a biased result due to improper splicing at the neck-linker region in the chimaera, we coexpressed the 3A chimaeric gene (3A head/3B rod-tail) with the 3B chimaeric gene (3B head/3A rod-tail) to create a heterodimer with one chain having a 3A head and a 3B rod-tail and the other having a 3B head

and a 3A rod-tail. If the splice site is appropriate, we should expect the new heterodimer to have the same velocity as wild-type KIF3A/B.

This chimaeric heterodimer moved microtubules at 169 ± 32 nm/s (mean ± SD, $N = 58$), consistent with the velocity of microtubules driven by wild-type KIF3A/B 184 ± 28 nm/s ($N = 85$). In addition, motility was observed across a range of surface densities and the velocity was independent of density (data not shown). Hence, switching heads between the two subunits at the position of our splice site doesn't affect the motility of both homodimeric chimaeras, and slow motility of KIF3A/A is not an artifact of the splice site.

KIF3B/B chimaera is faster but less processive than wild-type KIF3A/B

KIF3B/B chimaera exhibited very robust motility across a broad range of motor surface densities. The velocity of microtubule movement driven by KIF3B/B remained constant at 446 ± 34 nm/s (mean across all densities ± SD, $N = 135$) through the entire range of motor densities from 15 molecules/μm^2 to 1500 molecules/μm^2 (Fig. 4 *A*).

At low motor densities, most microtubules moved in a straight trajectory suggesting movement by multiple motors, but a few microtubules pivoted with small angles, moved very short distances (<1 μm), and then diff[illegible]d away before the trailing ends passed the contact po[illegible]s. Compared to the wide-angle, long-distance pivoting beha[illegible]ior of single KIF3A/B motors, KIF3B/B chimae[illegible] may hav[illegible] much shorter processive run lengths than K[illegible]F3A/[illegible] hetero-dimer. The lower processivity of KIF3[illegible]B than [illegible]IF3A/B implies that although the 3B head is ca[illegible]ble of [illegible] forward movement faster than th[illegible]3A he[illegible]coordination with the 3A head is requir[illegible] for optim[illegible]processive movement.

Landing-rate assays were perfor[illegible]d to quantitatively determine the process[illegible]ty of KIF3B/B. The best fit of the data was $n = 2$, suggesting the number of KIF3B/B molecules requi[illegible] for mo[illegible]ity is at least two (Fig. 4 *B*). Therefore, KIF3B/B[illegible] processi[illegible] at the detection level of this ass[illegible]0 nm), [illegible]t we ca[illegible]ot rule out the possibility that KI[illegible]B/B is [illegible]rocessiv[illegible] run lengths <300 nm.

The two heads of KIF3B have different motility properties

The two homodimers, KIF3A/A and KIF3B/B, propel microtubules [illegible]10-fold different velocities in the microtubule gliding assay (Fig. 5). KIF3A/A moves at 42 ± 11 nm/s, which is about fivefold slower than wild-type KIF3A/B speed of 188 ± 38 nm/s, whereas KIF3B/B moves at 409 ± 47 nm/s, roughly twice the speed of wild-type KIF3A/B. These results provide the first evidence that the two heads of KIF3 are functionally distinct.

We have constructed three analytical models to interpret these velocity data (Fig. 6). In the Independent Head Model,

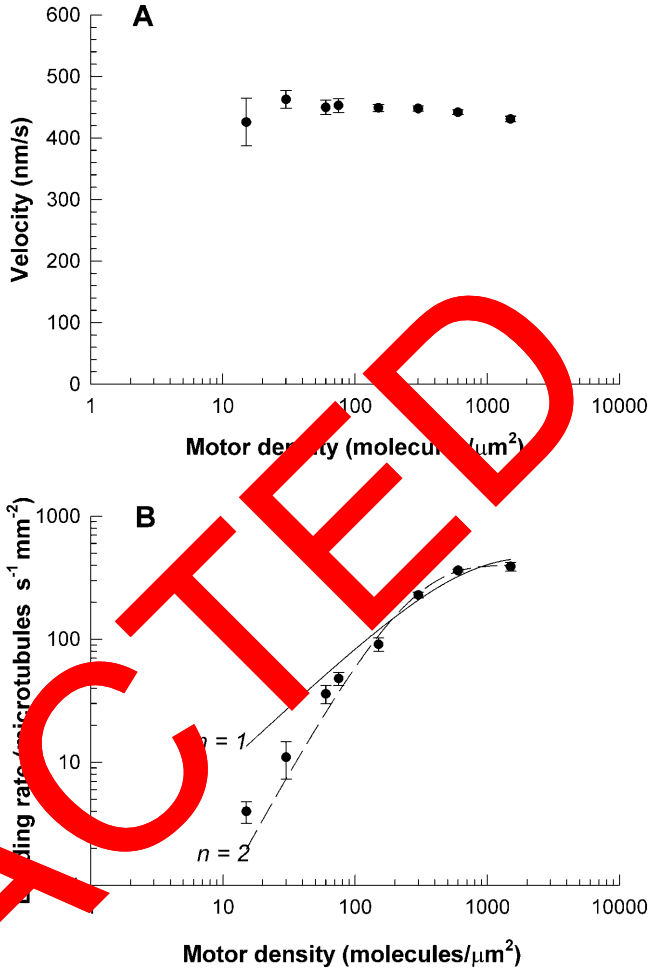

FIGURE 4 Dependence of KIF3B/B motility on motor surface density. (*A*) Microtubule gliding speeds for chimaeric KIF3B/B plotted over a wide range of motor surface densities. Error bars correspond to standard error of the mean of at least 10 velocity determinations for each density. (*B*) Microtubule landing rate of chimaeric KIF3B/B plotted as a function of motor density. Error bars correspond to standard error of the mean of landing rate from at least four different windows for each density.

the cycle rates of each head in the heterodimer are identical to those in the respective homodimers, and there is no correlation between the cycles of the two heads. From this model, which would best describe a nonprocessive motor, the predicted velocity of the heterodimer is an average of the speeds of the two homodimers. Although the data quantitatively agree with the model predictions, we exclude this model based on KIF3A/B's processivity: since the heads remain together as the dimeric motor walks along the microtubule for hundreds of steps, they can't be moving at different speeds.

The Sequential Head Model is a simple hand-over-hand model in which the heads step sequentially along the microtubule and the cycle times for each head match those observed in the homodimers. Hence, the time it takes the heterodimer to take two steps is equal to the time it takes head A to step

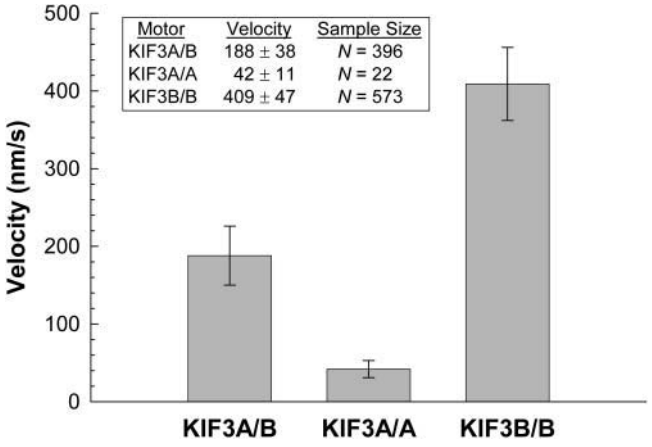

FIGURE 5 Microtubule gliding speeds for wild-type KIF3A/B and chimaeric KIF3 motors. For each motor type, the column bar represents the average of velocities determined at a range of motor densities from at least two protein preparations. Error bars correspond to the standard deviation.

plus the time it takes head B to step, and the predicted velocity of the heterodimer is

$$V_{\mathrm{Dimer}} = \frac{2V_{\mathrm{A}}V_{\mathrm{B}}}{V_{\mathrm{A}} + V_{\mathrm{B}}}.$$

The important result is that the predicted heterodimer speed of 76 nm/s for the Sequential Head Model is significantly less than the measured KIF3A/B speed of 188 nm/s, excluding this model.

In the Coordinated Head Model the kinetic cycle of each head is modulated by the activity of the second head, by simply waiting for the second head to complete its hydrolysis cycle. Hence, by pairing the slow A head with a fast B head in the heterodimer, the stepping rate of the A head must be faster than when it is paired with another A head in the homodimer. This can be interpreted quantitatively as follows. If all three motors take 8-nm steps and we assume that in the homodimers the kinetics of the two heads are identical, then in the homodimers each KIF3A head takes 190 ms to take a step (= 8 nm/step ÷ 42 nm/s) and each KIF3B head takes 19 ms to take a step (= 8 nm/step ÷ 409 nm/s). To account for the 85 ms needed for the KIF3A/B motor to take two successive steps (= 16 nm ÷ 188 nm/s), the cycle of the A head must be sped up from 190 ms in the homodimer to 66 ms in the heterodimer (assuming the kinetics of the B head are unchanged). Hence the Coordinated Head Model fits if the KIF3B head accelerates the stepping rate of the KIF3A head by a factor of 2.9.

DISCUSSION

In eukaryotic cells kinesin II motors carry membranous vesicles and proteins along cytoplasmic microtubules and transport proteinaceous rafts along axonemal microtubules. We are seeking to understand how these kinesins are optimized for their cellular tasks and what role the two different heads play in kinesin II motility. Because intersubunit coordination is central to the mechanism of many homodimeric kinesins and myosins, having two nonidentical heads opens a range of novel coordination mechanisms, and provides a model with which to better understand intersubunit coordination across all molecular motors.

Processivity, the ability to take many steps along the filament track without detaching, is an important property for transport motors, but compared to the body of work on

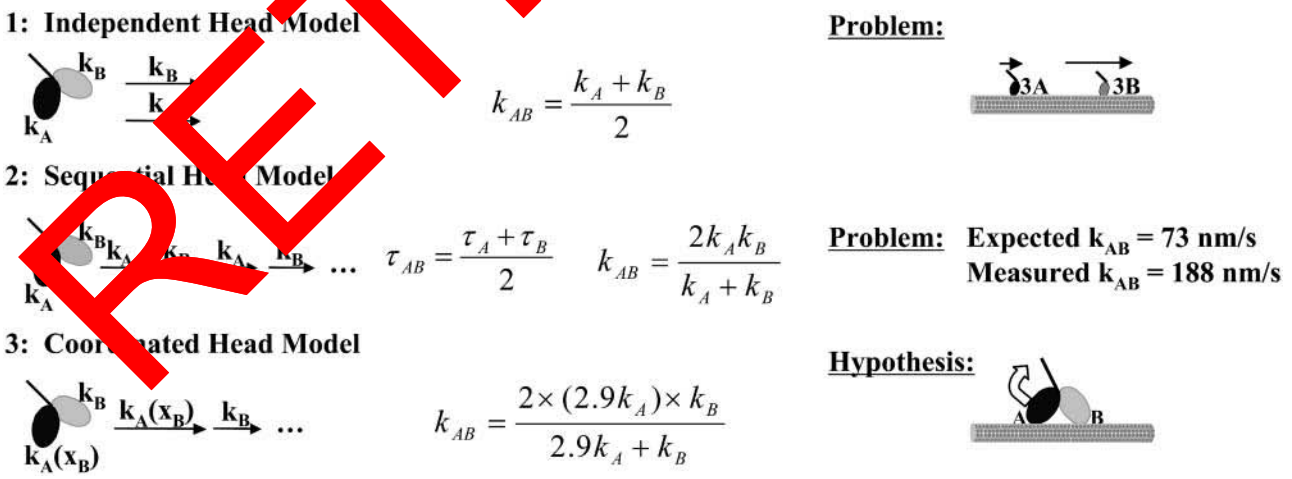

FIGURE 6 Interpreting heterodimer velocity data. Rates are given as stepping rates (k) or stepping times ($\tau = 1/k$). The Independent Head Model assumes no coordination. In the Sequential Head Model the heads alternately step along the microtubule with identical rates as in the homodimers. In the Coordinated Head Model, the heads alternately step along the microtubule, but the rates are different in the context of the heterodimer than in the homodimers. The data can be explained if the fast head B accelerates the slow head A by a factor of 2.9 in the heterodimer. We hypothesize that this is due to accelerated detachment from the microtubule.

conventional kinesin there is relatively little data on the processivity of the kinesin II subfamily. Here, we find for the first time that a member of the kinesin II subfamily is a processive motor, consistent with its role in intracellular transport. This finding for mouse KIF3A/B contrasts with work from Pierce et al. (1999), who failed to measure processive runs of KRP85/95, the sea urchin kinesin II ortholog, using a single-molecule fluorescence-based assay. It is possible that this is simply due to species differences; for example, when assayed under identical conditions chick myosin-Va (M5a) was found to be processive, whereas two yeast class V myosins, Myo2p and Myo4p, were reported to be nonprocessive motors (Reck-Peterson et al., 2001). However, a more plausible explanation for the lack of processivity of Pierce et al. is that the full-length KRP85/95 in solution is inhibited by its tail domain in the absence of cargo binding, similar to conventional kinesin (Coy et al., 1999; Friedman and Vale, 1999; Hackney and Stock, 2000). In our gliding assay experiments the KIF3A/B tail is bound to the glass surface, presumably disinhibiting the motor. Our finding of KIF3A/B processivity supports the notion that intraflagellar transport driven by kinesin II motors is analogous to axonal transport driven by conventional kinesin in neurons.

Why does KIF3A/B have two nonidentical heads? The kinesin II heterotrimeric structure is conserved between humans and *Chlamydomonas*, species that diverged from a common ancestor more than a billion years ago (Hedges, 2002), which suggests that having two nonidentical heads is important for these motors to carry out their intracellular tasks. However, despite a body of both in vivo and in vitro work on kinesin II structure and function, this question remains unanswered. To understand what role the two KIF3A/B heads play in motor function, we have constructed two homodimeric chimaeras with identical head domains dimerized via the wild-type coiled-coil domain. The striking difference in velocity between the KIF3A/A and KIF3B/B chimaeras indicates that the two heads are functionally distinct and raises the intriguing possibility that their chemical kinetics are tuned to complement one another during processive motility.

An important consideration in designing the KIF3A/A and KIF3B/B chimaeras was where to put the splice site. Ideally, the splice site would be located just after the core motor domain and just before the coiled-coil domain that determines heterodimerization, but this is complicated somewhat by the lack of crystal structure for dimeric KIF3A/B. Fortunately, the sequences align reasonably well with conventional kinesin and, based on the crystal structure of dimeric kinesin, there is a stretch of 10 conserved residues in KIF3A and KIF3B that span the end of the neck linker and start of the coiled-coil (Fig. 1 *A*). This is where the splice was made for our chimaeras. The fact that the double chimaera (3A head/3B rod-tail with 3B head/3A rod-tail) has similar motility to wild-type KIF3A/B indicates that the splicing itself does not measurably alter the motor function. Our differential head speeds contrast with an early study on KIF3A/B performed before the crystal structure of the conventional kinesin head was solved. Yamazaki et al. (1995) made two different KIF3B/B chimaeras: when the splice site was positioned in the coiled-coil dimerization region (3B head 1–359/3A tail 365–701), the motors were nonfunctional, and when the splice was positioned in the core motor domain (3B head 1–308/3A tail 314–701), the chimaera moved at the same speed as the reported wild-type speed of ~0.3 μm/s. For the former chimaera, the most reasonable explanation for the lack of motility is that dimerization is disrupted. For the latter chimaera, it is not surprising that it moves because the splice site is in loop 13 between α5 and β8 in the core of the motor, leaving the neck-linker and dimerization domains intact. Taken together, results from the Yamazaki chimaera and our KIF3B/B chimaera suggest that residues responsible for the velocity differences between the two heads are contained in the region 309–346 of KIF3B and 314–351 of KIF3A.

What do the gliding velocities of the homodimeric constructs tell us about coordination between the two heads of wild-type KIF3A/B? If the two heads alternately step along the microtubule with identical rates as in the homodimeric motors (Fig. 6, Sequential Head Model), the predicted heterodimer speed is dominated by the slow head, and is considerably slower than our measured rate. Hence, the data are best explained by a coordinated hand-over-hand model in which the stepping rates in the context of the heterodimer are different than the rates observed in the homodimeric motors. At a minimum, if the two heads alternately step along the microtubule then the KIF3A head must be stepping 2.9-fold faster in the context of the KIF3A/B heterodimer than in the homodimer.

What are potential coordination mechanisms that can account for this acceleration? The best paradigm in which to interpret these KIF3 results is the hydrolysis cycle for conventional kinesin, where interdomain coordination has been shown to be crucial for maintaining kinesin processivity. The problem is there is no consensus as to precisely which transitions in the cycle involve coordination. In one model of the walking cycle, it is proposed that when both heads are bound to the microtubule, forward strain produced by the leading head accelerates detachment of the trailing head (Hancock and Howard, 1998, 1999). Processivity is maintained by ensuring that the rear head will not detach until the leading head binds. However, although this model provides a nice framework for interpreting the KIF3 data, there is debate regarding the degree to which attachment of the leading head does in fact accelerate detachment of the trailing head. Using fluorescent reporters that monitor head detachment, Rosenfeld and colleagues concluded that the acceleration of detachment by the leading head is at most a factor of two- to threefold in a cysteine-modified human conventional kinesin construct (Rosenfeld et al., 2002,

2003). Using ''roadblocks'' on microtubules that prevent the attachment of kinesin's leading head, Crevel et al. (2004) similarly concluded that the leading head accelerates detachment of the trailing head by at most a factor of 2 in rat conventional kinesin. What does this mean for KIF3A/B? As discussed in Results, the KIF3 velocity data can be accounted for by a heterodimer model in which the fast B head speeds up the walking cycle of the slow A head by a factor of 2.9. Hence, if we assume that rear head detachment is the rate-limiting step in the walking cycle, then a model in which the fast KIF3B head accelerates detachment of the slow KIF3A head in the context of the heterodimer is in reasonable agreement with the two- to threefold acceleration of detachment measured in conventional kinesin.

There are other coordination models that also explain the processivity of conventional kinesin. Rosenfeld and colleagues have proposed that when both heads are bound to the microtubule, rearward strain on the leading head slows ATP binding to that head until the rear head detaches and relieves this strain (Rosenfeld et al., 2002, 2003). This mechanism also satisfies the constraint that the rear head detaches before the forward head, ensuring that the motor takes many steps during each encounter with a microtubule. For the KIF3B head to accelerate the stepping rate of the KIF3A head, it must accelerate the rate-limiting step. In the Rosenfeld model the rate-limiting step is most likely detachment of the rear head from the microtubule or a step immediately preceding it (so that the motor waits with both heads bound, the rear head detaches, and then the leading head binds ATP). Hence, the KIF3 data is again best explained by a mechanism in which the KIF3B head accelerates detachment of the KIF3A head in the heterodimer.

There are two recent studies of conventional kinesin that are relevant to understanding the kinetics of the KIF3 walking cycle. Kaseda et al. (2003) generated a heterodimeric conventional kinesin with an ATP binding site mutation in one head and found that the motor took alternate fast and slow steps along microtubule. Interestingly, the step duration in a homodimer consisting of two mutant heads matched the step duration of the slow head in the heterodimer, showing that in this mutant the fast head does not affect the kinetics of the slow head (our Sequential Head Model, Fig. 9). In another study, Asbury and colleagues (2003) found that even in some homodimeric kinesins the stepping rates differ between the two heads, presumably due to structural asymmetries in the coiled-coil region. These and other findings point toward an asymmetric hand-over-hand mechanism for conventional kinesin in which the two heads, due to either structural or kinetic asymmetries, undergo distinct structural or kinetic transitions as they step along the microtubule.

If the two heads of KIF3A/B are biochemically tuned to optimize the performance of the intact heterodimer, then we expect there to be other differences beyond simply the unloaded stepping rate. For instance, if the slow head is responsible for maintaining association with the microtubule, then we would expect the slow homodimer to have a greater microtubule affinity than the fast homodimer. Alternatively, the two heads may be tuned such that the fast head (fast but weak) dominates under the unloaded conditions of our microtubule gliding assay, whereas the slow head (slow but strong) dominates at high loads. These possibilities are currently being tested using single-molecule mechanical techniques to measure the stepping rates and strength of each head.

It is possible that the design of these nonidentical heads plays other roles in motor function. One possibility is that the two heads enable subtle regulation during bidirectional transport either by providing multiple modes of regulation or by enabling different cell signaling pathways to converge on the motor. A second possibility is that the two different heads provide the motor with an enhanced ability to move along axonemal microtubules; neither kinesins outside of the kinesin II subfamily have been shown to transport cargo along axonemal microtubules (Cole, 1999). Though speculative, perhaps these heterodimeric motors walk along the seam of the doublet microtubules or interact optimally with the microtubule-associated proteins found on axonemal microtubules. We now know that the heterodimeric KIF3A/B is processive and that its two heads are functionally distinct. Further studies should uncover both the nature of the intersubunit coordination, and the functional advantage conferred by having two nonidentical motor domains.

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REFERENCES

Asbury, C. L., A. N. Fehr, and S. M. Block. 2003. Kinesin moves by an asymmetric hand-over-hand mechanism. *Science*. 302:2130–2134.

Ayres, M. D., S. C. Howard, J. Kuzio, M. Lopez-Ferber, and R. D. Possee. 1994. The complete DNA sequence of *Autographa californica* nuclear polyhedrosis virus. *Virology*. 202:586–605.

Brown, J. M., C. Marsala, R. Kosoy, and J. Gaertig. 1999. Kinesin-II is preferentially targeted to assembling cilia and is required for ciliogenesis and ormal cytokinesis in tetrahymena. *Mol. Biol. Cell*. 10:3081–3096.

Cole, D. G. 1999. Kinesin-II, the heteromeric kinesin. *Cell. Mol. Life Sci.* 56:217–226.

Cole, D. G., D. R. Diener, A. L. Himelblau, P. L. Beech, J. C. Fuster, and J. L. Rosenbaum. 1998. Chlamydomonas kinesin-II-dependent intraflagellar transport (IFT): IFT particles contain proteins required for ciliary assembly in *Caenorhabditis elegans* sensory neurons. *J. Cell Biol.* 141:993–1008.

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.

Crevel, I. M., M. Nyitrai, M. C. Alonso, S. Weiss, M. A. Geeves, and R. A. Cross. 2004. What kinesin does at roadblocks: the coordination mechanism for molecular walking. *EMBO J.* 23:23–32.

De Marco, V., P. Burkhard, N. Le Bot, I. Vernos, and A. Hoenger. 2001. Analysis of heterodimer formation by Xklp3A/B, a newly cloned kinesin-II from *Xenopus laevis*. *EMBO J.* 20:3370–3379.

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.

Hackney, D. D. 2002. Pathway of ADP-stimulated ADP release and dissociation of tethered kinesin from microtubules. implications for the extent of processivity. *Biochemistry.* 41:4437–4446.

Hackney, D. D., and M. F. Stock. 2000. Kinesin's IAK tail domain inhibits initial microtubule-stimulated ADP release. *Nat. Cell Biol.* 2:257–260.

Hancock, W. O., and J. Howard. 1998. Processivity of the motor protein kinesin requires two heads. *J. Cell Biol.* 140:1395–1405.

Hancock, W. O., and J. Howard. 1999. Kinesin's processivity results from mechanical and chemical coordination between the ATP hydrolysis cycles of the two motor domains. *Proc. Natl. Acad. Sci. USA.* 96:13147–13152.

Hedges, S. B. 2002. The origin and evolution of model organisms. *Nat. Rev. Genet.* 3:838–849.

Hirokawa, N., and Y. Noda. 2001. Preparation of recombinant kinesin superfamily proteins using the baculovirus system. *Methods Mol. Biol.* 164:57–63.

Howard, J., A. J. Hudspeth, and R. D. Vale. 1989. Movement of microtubules by single kinesin molecules. *Nature.* 342:154–158.

Howard, J., A. J. Hunt, and S. Baek. 1993. Assay of microtubule movement driven by single kinesin molecules. *Methods Cell Biol.* 39:137–147.

Hyman, A., D. Drechsel, D. Kellogg, S. Salser, K. Sawin, P. Steffen, L. Wordeman, and T. Mitchison. 1991. Preparation of modified tubulins. *Methods Enzymol.* 196:478–485.

Kaseda, K., H. Higuchi, and K. Hirose. 2003. Alternate fast and slow stepping of a heterodimeric kinesin molecule. *Nat. Cell Biol.* 5:1079–1082.

Klumpp, L. M., A. Hoenger, and S. P. Gilbert. 2004. Kinesin's second step. *Proc. Natl. Acad. Sci. USA.* 101:3444–3449.

Kondo, S., R. Sato-Yoshitake, Y. Noda, H. Aizawa, T. Nakata, Y. Matsuura, and N. Hirokawa. 1994. KIF3A is a new microtubule-based anterograde motor in the nerve axon. *J. Cell Biol.* 125:1095–1107.

Kozielski, F., S. Sack, A. Marx, M. Thormahlen, E. Schönbrunn, V. Biou, A. Thompson, E. M. Mandelkow, and E. Mandelkow. 1997. The crystal structure of dimeric kinesin and implications for microtubule-dependent motility. *Cell.* 91:985–994.

Le Bot, N., C. Antony, J. White, E. Karsenti, and I. V. 1998. Role of xklp3, a subunit of the Xenopus kinesin II heterotrimeric complex, in membrane transport between the endoplasmic reticulum and the Golgi apparatus. *J. Cell Biol.* 143:1559–1573.

Mallik, R., B. C. Carter, S. A. Lex, S. J. King, and S. P. Gross. 2004. Cytoplasmic dynein functions as a gear in response to load. *Nature.* 427:649–652.

Marszalek, J. R., P. Ruiz-Lozano, E. Roberts, K. R. Chien, and L. S. Goldstein. 1999. Situs inversus and embryonic ciliary morphogenesis defects in mouse mutants lacking the KIF3A subunit of kinesin-II. *Proc. Natl. Acad. Sci. USA.* 96:5043–5048.

Mehta, A. D., R. S. Rock, M. Rief, J. A. Spudich, M. S. Mooseker, and R. E. Cheney. 1999. Myosin-V is a processive actin-based motor. *Nature.* 400:590–593.

Miki, H., Y. Setou, K. Kaneshiro, and N. Hirokawa. 2001. All kinesin superfamily protein, KIF, genes in mouse and human. *Proc. Natl. Acad. Sci. USA.* 98:7004–7011.

Nonaka, S., Y. Tanaka, Y. Okada, S. Takeda, A. Harada, Y. Kanai, M. Kido, and N. Hirokawa. 1998. Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. *Cell.* 95:829–837.

Okada, Y., and N. Hirokawa. 1999. A processive single-headed motor: kinesin superfamily protein KIF1A. *Science.* 283:1152–1157.

Orozco, J. T., K. P. Wedaman, D. Signor, H. Brown, L. Rose, and J. M. Scholey. 1999. Movement of motor and cargo along cilia. *Nature.* 398:674.

Pierce, D. W., N. Hom-Booher, A. J. Otsuka, and R. D. Vale. 1999. Single-molecule behavior of monomeric and heteromeric kinesins. *Biochemistry.* 38:5412–5421.

Rashid, D. J., K. P. Wedaman, and J. M. Scholey. 1995. Heterodimerization of the two motor subunits of the heterotrimeric kinesin, KRP85/95. *J. Mol. Biol.* 252:157–162.

Reck-Peterson, S. L., M. J. Tyska, P. J. Novick, and M. S. Mooseker. 2001. The yeast class V myosins, Myo2p and Myo4p, are nonprocessive actin-based motors. *J. Cell Biol.* 153:1121–1126.

Rice, S., A. W. Lin, D. Safer, C. L. Hart, N. Naber, B. O. Carragher, S. M. Cain, E. Pechatnikova, E. M. Wilson-Kubalek, M. M. Whittaker, E. Pate, R. Cooke, E. W. Taylor, R. A. Milligan, and R. D. Vale. 1999. A structural change in the kinesin motor protein that drives motility. *Nature.* 402:778–784.

Rock, R. S., M. Rief, A. D. Mehta, and J. A. Spudich. 2000. In vitro assays of processive myosin motors. *Methods.* 22:373–381.

Rosenfeld, S. S., P. M. Fordyce, G. M. Jefferson, P. H. King, and S. M. Block. 2003. Stepping and stretching. How kinesin uses internal strain to walk processively. *J. Biol. Chem.* 278:18550–18556.

Rosenfeld, S. S., J. Xing, G. M. Jefferson, H. C. Cheung, and P. H. King. 2002. Measuring kinesin's first step. *J. Biol. Chem.* 277:36731–36739.

Siegel, L. M., and K. J. Monty. 1966. Determination of molecular weights and frictional ratios of proteins in impure systems by use of gel filtration and density gradient centrifugation. Application to crude preparations of sulfite and hydroxylamine reductases. *Biochim. Biophys. Acta.* 112:346–362.

Signor, D., K. P. Wedaman, J. T. Orozco, N. D. Dwyer, C. I. Bargmann, L. S. Rose, and J. M. Scholey. 1999. Role of a Class DHC1b dynein in retrograde transport of IFT motors and IFT raft particles along cilia, but not dendrites, in chemosensory neurons of living *Caenorhabditis elegans*. *J. Cell Biol.* 147:519–530.

Takeda, S., Y. Yonekawa, Y. Tanaka, Y. Okada, S. Nonaka, and N. Hirokawa. 1999. Left-right asymmetry and kinesin superfamily protein KIF3A: new insights in determination of laterality and mesoderm induction by kif3A−/− mice analysis. *J. Cell Biol.* 145:825–836.

Tuma, M. C., A. Zill, N. Le Bot, I. Vernos, and V. Gelfand. 1998. Heterotrimeric kinesin II is the microtubule motor protein responsible for pigment dispersion in Xenopus melanophores. *J. Cell Biol.* 143:1547–1558.

Uyeda, T. Q., H. M. Warrick, S. J. Kron, and J. A. Spudich. 1991. Quantized velocities at low myosin densities in an in vitro motility assay. *Nature.* 352:307–311.

Wagner, M. C., K. K. Pfister, S. T. Brady, and G. S. Bloom. 1991. Purification of kinesin from bovine brain and assay of microtubule-stimulated ATPase activity. *Methods Enzymol.* 196:157–175.

Williams, R. C., Jr., and J. C. Lee. 1982. Preparation of tubulin from brain. *Methods Enzymol.* 85:376–385.

Yamazaki, H., T. Nakata, Y. Okada, and N. Hirokawa. 1995. KIF3A/B: a heterodimeric kinesin superfamily protein that works as a microtubule plus end-directed motor for membrane organelle transport. *J. Cell Biol.* 130:1387–1399.