Neck Rotation and Neck Mimic Docking in the Noncatalytic Kar3-associated Protein Vik1*‡§

Da Duan‡, Zhiming Jia‡, Monika Joshi‡, Jacqueline Brunton‡, Michelle Chan‡, Doran Drew‡, Darlene Davis‡, and John S. Allingham‡1

From the ‡1Department of Biomedical and Molecular Sciences, Queen’s University, Kingston, Ontario K7L 3N6 and the ‡2Department of Cellular and Developmental Biology, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada

* This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada and Canadian Institutes of Health Research (to J. S. A.).

This article contains supplemental Figs. S1–S8, Movies S1–S4, and additional references.

The atomic coordinates and structure factors (codes 4GKP, 4GKQ, and 4GKR) have been deposited in the Protein Data Bank (http://wwpdb.org/).

1 Holds a Canada Research Chair (Tier 2) in Structural Biology. To whom correspondence should be addressed: Dept. of Biomedical and Molecular Sciences, Queen’s University, 18 Stuart St., Rm. 652, Kingston, Ontario K7L 3N6, Canada. Tel.: 613-533-3137; Fax: 613-533-2022; E-mail: allinghj@queensu.ca.

2 The abbreviations used are: MHD, motor homology domain; MD, motor domain; MT, microtubule; N, neck; sN, short neck; CC, coiled coil; AMPNP, adenylyl imidodiphosphate; PDB, Protein Data Bank; ScKar3Vik1, S. cerevisiae Kar3Vik1; CgKar3, C. glabrata Kar3.

Significance:
Kar3 is a kinesin-14 that plays essential roles in mitosis, meiosis, and karyogamy in Saccharomyces cerevisiae and Candida albicans (10–13). These include cross-linking, stabilizing, and sliding spindle pole microtubules, as well as depolymerizing microtubules (10, 14). To accomplish this array of functions, Kar3 associates with two discrete regulatory subunits, Cik1 and Vik1 (14–16), whose motor homology domain (MHD) can bind microtubules but not ATP (17, 18). Amazingly, the affinity of the Cik1 and Vik1 subunits in their respective complexes with Kar3 is reduced when ADP is exchanged in Kar3 for AMP-PNP (a nonhydrolyzable ATP analog), indicating that Kar3 controls disengagement of Cik1 and Vik1 from microtubules (17, 18). This form of intramolecular coordination between a catalytic and noncatalytic subunit-containing complex has not been previously observed in other motor proteins.

The recently determined structure of a truncated version of ScKar3Vik1 by Rank et al. (19) shows that the stalk and neck domain of Kar3 and Vik1, like Drosophila Ncd, forms a continuous coiled coil, nearly to the point of insertion of their neck into the motor (homology) domain (supplemental Fig. S1A). Also shown was that Kar3 and Vik1 interact laterally over two adjacent protofilaments when Kar3 is ADP-bound but that the complex transitions to a single-head bound state involving only Kar3 when ADP is exchanged for AMP-PNP. Uptake of AMP-PNP also caused a minus-end-directed rotation of the stalk-neck element, which resembles the power stroke of Ncd (20, 21). In both theseputative “pre-power stroke” and “post-power

Results:
Vik1 experiences conformational changes in regions analogous to the force-producing elements in catalytic kinesins.

Conclusion: A molecular mechanism by which Kar3 could trigger Vik1’s release from microtubules was revealed.

Background: Kar3Vik1 is a heterodimeric kinesin with one catalytic subunit (Kar3) and one noncatalytic subunit (Vik1).

Significance: These findings will serve as the prototype for understanding the motile mechanism of kinesin-14 motors in general.

It is widely accepted that movement of kinesin motor proteins is accomplished by coupling ATP binding, hydrolysis, and product release to conformational changes in the microtubule-binding and force-generating elements of their motor domain. Therefore, understanding how the Saccharomyces cerevisiae proteins Cik1 and Vik1 are able to function as direct participants in movement of Kar3Cik1 and Kar3Vik1 kinesin complexes presents an interesting challenge given that their motor homology domain (MHD) cannot bind ATP. Our crystal structures of the Vik1 ortholog from Candida glabrata may provide insight into this mechanism by showing that its neck and neck mimic-like element can adopt several different conformations reminiscent of those observed in catalytic kinesins. We found that when the neck is α-helical and interacting with the MHD core, the C terminus of CgVik1 docks onto the central β-sheet similarly to the ATP-bound form of Ncd. Alternatively, when neck-core interactions are broken, the C terminus is disordered. Mutations designed to impair neck rotation, or some of the neck-MHD interactions, decreased microtubule gliding velocity and steady state ATPase rate of CgKar3Vik1 complexes significantly. These results strongly suggest that neck rotation and neck mimic docking in Vik1 and Cik1 may be a structural mechanism for communication with Kar3.

Eukaryotic cells rely on nanometer-sized motors called kinesins to transport cellular components along microtubules (1) or to help build the mitotic spindle and distribute chromosomes between daughter cells (2, 3). Recent studies have shown that dynamic interactions between the neck and a short region of either the N or C terminus of the motor domain form a structure responsible for force generation by the neck (4–7) and that its conformation and interactions with the motor domain core, or regulatory proteins, is linked to the nucleotide–microtubule-binding state of the motor (8, 9). In kinesin-1, this region forms an N-terminal extension of the motor domain, called the “cover strand” (5), and in kinesin-14 motors this region is at the C terminus, after the α6 helix, and has been dubbed the “neck mimic” (8).

Kar3 is a kinesin-14 that plays essential roles in mitosis, meiosis, and karyogamy in Saccharomyces cerevisiae and Candida albicans (10–13). These include cross-linking, stabilizing, and sliding spindle pole microtubules, as well as depolymerizing microtubules (10, 14). To accomplish this array of functions, Kar3 associates with two discrete regulatory subunits, Cik1 and Vik1 (14–16), whose motor homology domain (MHD) can bind microtubules but not ATP (17, 18). Amazingly, the affinity of the Cik1 and Vik1 subunits in their respective complexes with Kar3 is reduced when ADP is exchanged in Kar3 for AMP-PNP (a nonhydrolyzable ATP analog), indicating that Kar3 controls disengagement of Cik1 and Vik1 from microtubules (17, 18). This form of intramolecular coordination between a catalytic and noncatalytic subunit-containing complex has not been previously observed in other motor proteins.

The recently determined structure of a truncated version of ScKar3Vik1 by Rank et al. (19) shows that the stalk and neck domain of Kar3 and Vik1, like Drosophila Ncd, forms a continuous coiled coil, nearly to the point of insertion of their neck into the motor (homology) domain (supplemental Fig. S1A). Also shown was that Kar3 and Vik1 interact laterally over two adjacent protofilaments when Kar3 is ADP-bound but that the complex transitions to a single-head bound state involving only Kar3 when ADP is exchanged for AMP-PNP. Uptake of AMP-PNP also caused a minus-end-directed rotation of the stalk-neck element, which resembles the power stroke of Ncd (20, 21). In both theseputative “pre-power stroke” and “post-power
stroke” configurations, the Vik1 subunit is held in a similar conformation as the previous structure of the ScVik1-MHD monomer (17), and thus conformational changes required to transition Vik1 from a high to low affinity binding state for the microtubule remain uncertain.

Orthologs of Cik1 and Vik1 can be found in numerous fungal relatives of S. cerevisiae (22, 23). By studying the structure and function of these proteins, we hypothesized that they may be possible to identify functionally conserved elements that underlie a general mechanism for communication with Kar3. This study focuses on Kar3 and Vik1 proteins from the human fungal pathogen, Candida glabrata. Its genome encodes a single 692-amino acid Kar3 homolog (CAGL0D049994g) that shares 53% sequence identity with ScKar3, and a 584-amino acid Vik1 ortholog (CAGL0H00638g) that shares 23% identity with ScVik1. We have determined the x-ray structures of the motor domain regions of both CgKar3 and CgVik1, the latter of which crystallized in three unique conformations that were not previously observed in this protein. Two of the conformations exhibit rotation of the neck element in a manner similar to Ncd motors in different nucleotide states, suggesting that they may represent discrete intermediates during Kar3Vik1’s motile cycle. To determine the importance of neck rotation in CgVik1, we mutated the residue that appears to facilitate neck rotation, and as well as one of the neck residues involved in a specific network of interactions with the MHD core. Analysis of these mutants in complex with wild-type CgKar3 showed markedly reduced rates of microtubule gliding and ATP hydrolysis. In these structures, we also observed docking of the C terminus of CgVik1 onto the central β-sheet and its interaction with residues at the neck-core junction in a conformation similar to the C terminus of the ATP-bound form of Ncd (7), the neck mimic of the calcium-regulated kinesin-14 KCBP (8), and the neck linker of kinesin-1 (24). Interestingly, as seen in a separate CgVik1 structure, this docking of the C terminus seems to be completely abolished when the neck loses its α-helical structure and is stabilized in a state where it is undocked from the MHD. Based on this new information, we propose that the neck of Vik1 must change positions during the motile cycle of Kar3Vik1, and interactions between the neck and C terminus of Vik1 may provide the critical link for coupling release of Vik1 and Cik1 from microtubules to microtubule binding and subsequent neck rotation in Kar3 during nucleotide exchange.

**EXPERIMENTAL PROCEDURES**

**Protein Expression and Purification**—Plasmids encoding truncated C. glabrata Kar3 and Vik1 proteins (Fig. 1A and supplemental Figs. S5A and S7A) were constructed by amplifying relevant regions of C. glabrata genomic DNA (ATCC 2001D-5) by PCR and ligating them into either pET24d (Novagen) or a modified version of pET16b (Novagen) in which the Factor Xa cleavage site was replaced with a recombinant tobacco etch virus protease cleavage site. CgVik1 mutagenesis was performed using QuikChange (Stratagene). All plasmids were transformed into Escherichia coli BL21-CodonPlus (DE3)-RIL cells (Stratagene) for protein expression in LB or M9 minimal media. Cells transformed with the pET24d vector encoding CgKar3-N+MHD (MA-Glu324–Asn692) (supplemental Fig. S7A) were grown in LB media supplemented with 50 µg/ml kanamycin and 50 µg/ml chloramphenicol to $A_{600} \sim 0.8$ and were then induced with 1.0 mM isopropyl 1-thio-β-D-galactopyranoside and incubated at 20 °C for a further 16 h. Cells were harvested by centrifugation and lysed by flash freezing and sonication, and the CgKar3-N+MHD protein was purified by ion-exchange chromatography (DEAE SP-Sepharose Fast Flow, GE Healthcare) as described previously (25). Cells expressing CgVik1-sN+MHD (MGH10SSGRENLYFGQM-Leu$^{313}$–Lys$^{584}$) or CgVik1-N+MHD (MGH10SSGRENLYFGQM-Thr$^{302}$–Lys$^{584}$) were grown in M9 minimal media supplemented with 50 µg/ml ampicillin and 50 µg/ml chloramphenicol to $A_{600} \sim 0.8$ and were then induced with 1.0 mM isopropyl 1-thio-β-D-galactopyranoside and incubated at 25 °C for a further 16 h. Both constructs were purified and digested with the recombinant tobacco etch virus protease to remove the polyhistidine tag as described previously for ScVik1MHD (17). Cells co-transformed with plasmids encoding CgVik1-CC+C+N+MHD (MGH10SSGRENLYFGQM-Asp$^{152}$–Lys$^{584}$) and CgKar3-CC+C+N+MHD (Met$^{198}$–Asn$^{692}$) were grown in LB media supplemented with 50 µg/ml kanamycin, 50 µg/ml ampicillin, and 50 µg/ml chloramphenicol to $A_{600} \sim 0.8$ and induced with 1.0 mM isopropyl 1-thio-β-D-galactopyranoside. After continued growth at 20 °C for 16 h, cells were lysed by sonication, and the dimer was purified as described previously (17) followed by gel filtration on a HiLoad Superdex 200 26/60 column (GE Healthcare). All proteins were concentrated and flash-frozen in liquid nitrogen for storage.

**Protein Crystallization, Data Collection, and Structure Determination**—Crystals of CgVik1-sN+MHD grew at 4 °C in 0.1 M HEPES, pH 7.5, 0.075 M NaAc, pH 5.5, 13% PEG6000 and 5% isopropyl alcohol by hanging drop vapor diffusion. Data were collected at the X6a beamline at National Synchrotron Light Source (Brookhaven National Laboratory). CgVik1-N+MHD crystals grew by hanging drop in 0.1 M Tris, pH 8.5, 12% PEG 8000, 0.05 M MgCl$_2$, 0.15 M NaCl, 5% ethylene glycol, and 1 mM tris(2-carboxyethyl)phosphine. Data were collected at the GM/CA-CAT 23-ID-B beamline at Advanced Photon Source (Argonne National Laboratory). CgKar3-N+MHD crystals grew by hanging drop at room temperature in 0.1 M MIB buffer, pH 7.0, 25% PEG 1500, 1 mM tris(2-carboxyethyl)phosphine, and 1 mM ATP. Data were collected at the A1 beamline at Cornell High Energy Synchrotron Source (Cornell University). XDS was used to integrate and scale the reflection data of CgVik1-sN+MHD and CgVik1-N+MHD, and HKL2000 was used for that of CgKar3-N+MHD (26, 27). All three structures were solved by molecular replacement with PHENIX AutoMR (28). For CgVik1-sN+MHD, the motor homology domain region of S. cerevisiae Vik1 (Protein Data Bank (PDB) code 200A) was used as the initial search model (17). For CgVik1-N+MHD, the CgVik1-sN+MHD structure was used as the initial search model. For CgKar3-N+MHD, the motor domain of S. cerevisiae Kar3 (PDB code 3KAR) was used as the initial search model (29). Manual building of the structures were done in COOT and refined in REFMAC to generate the final models 4GKP (CgVik1-sN+MHD), 4GKQ (CgVik1-N+MHD), and 4GRK (CgKar3-N+MHD) (30, 31). Diffraction data collection and structure refinement statistics are presented in Table 1.
Neck Rotation and Neck Mimic Docking in Vik1

Table 1: Data collection and refinement statistics

|                     | CgKar3-N+MD | CgVik1-sN+MHD | CgVik1-N+MHD |
|---------------------|-------------|---------------|--------------|
| **Space group**     | P2₁,2₁,2₁   | P2₁,2₁,2₁     | H3           |
| **Cell dimensions** |             |               |              |
| a, b, c             | 58.7, 84.1, 151.1 Å | 66.7, 77.7, 107.7 Å | 197, 197, 50.2 Å |
| a, β, γ             | 90, 90, 90°  | 90, 90, 120°  | 90, 90, 120° |
| Wavelength          | 0.917       | 0.976         | 1.033        |
| Resolution*         | 40–2.7 Å (2.8–2.7 Å) | 20–2.42 Å (2.5–2.42 Å) | 20–2.99 Å (3.07–2.99 Å) |
| Rmerge*             | 7.6 (64)    | 3.6 (22)      | 7.8 (79)     |
| Completeness*       | 96.2% (94.5%) | 98.5% (93.5%) | 98.8% (100%) |
| Redundancy*         | 11.7 (10.7) | 7.8 (5.4)     | 5.8 (5.8)    |
| No. of observed reflections | 242,709 | 168,643 | 84,512 |
| No. of unique reflections | 20,662 | 14,501 | 7,912 |
| Rwork/Rfree*        | 0.21/0.26   | 0.21/0.26     | 0.22/0.26    |
| No. of atoms        |           |               |              |
| Protein (Chain A)   | 2157       | 1789          | 1752         |
| Protein (Chain B)   | 2214       | 1962          | 1571         |
| Water               | 52         | 80            | 1            |
| **Average B-factors (Å²)** | | | |
| Protein (Chain A)   | 47.9       | 45.2          | 110.2        |
| Protein (Chain B)   | 51.7       | 41.6          | 118.2        |
| Nucleotide (A)      | 32.9       |               |              |
| Nucleotide (B)      | 35.6       |               |              |
| Water               | 34.9       | 40.5          | 71.7         |
| Root mean square deviations | | | |
| Bond lengths        | 0.009 Å    | 0.009 Å       | 0.007 Å      |
| Bond angles         | 1.359°     | 1.253°        | 1.16°        |
| Favored             | 93.4       | 91.2          | 89.2         |
| Allowed             | 6.6        | 8.1           | 10.1         |
| Outliers            | 0.0        | 0.7           | 0.7          |
| PDB code            | 4GKR       | 4GKP          | 4GKQ         |

* Data in parentheses represent highest resolution shell.

Analysis of Kinesin Motility—Motility assays were performed using WT and mutant CgKar3Vik1 complexes at ~0.3 μM. Rhodamine-labeled bovine tubulin (Cytoskeleton Inc.) was mixed with unlabeled tubulin purified from bovine brain at a molar ratio of 1:4, and the mixture was polymerized, centrifuged, and resuspended in BRB80 (80 mM PIPES, pH 6.8 (KOH), 1 mM MgCl₂, 1 mM EGTA). Polarity-marked microtubules were made with rhodamine-labeled microtubules and HiLyte™ 488 microtubules (Cytoskeleton Inc.) by adapting a protocol as described previously by Walter et al. (32). Homemade perfusion chambers were constructed by sandwiching 22 × 60- and 22 × 22-mm glass coverslips using double-sided tape. Microtubules were shredded to generate short filaments before imaging. Anti-histidine antibodies (Fisher) were used to attach the kinesins to the glass surface, which was then blocked with 1 mg/ml BSA. Imaging was performed on spinning disc confocal fluorescence microscope (Leica) in the presence of an oxygen-scavenging system (OSM; BRB80, 1.5 mM MgAc, 1 mg/ml BSA, 200 μg/ml glucose oxidase, 175 μg/ml catalase, 25 mM glucose, 2 mM β-mercaptoethanol, 20 μM taxol, 1 mM AMPPPNP, and 1 mM ATP as described previously (25). To ensure sufficient ATP was present during the imaging process, ATP was regenerated by suplementing the OSM with 0.3 μg/ml phosphocreatine kinase and 2 mM phosphocreatine (25). Quorum WaveFX software (Quorum Technologies Inc.) was used to process the images captured and compile them into movies. Microtubule movement was tracked using Image-Pro Plus (Media Cybernetics Inc.).

Steady State ATPase Activity—ATPase kinetics of the WT and mutant CgKar3Vik1 motors were determined using a pyruvate kinase/lactate dehydrogenase-coupled NADH oxidation reaction (33) as described previously (34).

Steady State Microtubule Binding Affinity—Purified CgKar3-N+MD or CgKar3Vik1 (6 μM final concentration) was incubated with microtubules in ATPase buffer (20 mM HEPES, 5 mM magnesium acetate, 0.1 mM EGTA, 0.1 mM EDTA, 25 mM potassium acetate, 1 mM DTT, 40 μM Taxol, pH 7.2) with either 1 mM MgAMPPNP or MgADP, and bound and unbound motors were separated by centrifugation at 55,000 rpm in a TLA100 ultracentrifuge and analyzed by SDS-PAGE as described previously (34).

RESULTS

Crystal Structures of the CgVik1 Motor Homology Domain—The structures of two different constructs comprising different portions of the neck region (N) and the entire MHD of CgVik1 were determined by x-ray crystallography (Fig. 1A). The structure of the first construct, CgVik1-sN+MHD was solved to 2.42 Å by molecular replacement using the ScVik1MHD crystal structure (PDB code 2O0A) as the search model (17). The solution showed two molecules in the asymmetric unit whose intermolecular interactions are insufficient to indicate complex formation. Similar to ScVik1MHD, the CgVik1MHD structural motif is an α/β fold that lacks a nucleotide binding pocket (Fig. 1B). Unlike ScVik1MHD, the neck in molecule A of CgVik1-sN+MHD is completely disordered N-terminally from Arg325 (supplemental Fig. S2C), and in molecule B it is extended away (undocked) from the MHD core without defined secondary structure (supplemental Fig. S2D). The extended neck confor-
mation is stabilized by symmetry contacts with the β5a/b strands of another molecule in the crystal lattice.

Crystals for the CgVik1-N+MHD construct, which possesses 11 additional residues of the neck (Fig. 1A), also showed two molecules in its asymmetric unit after refinement of its structure to 2.99 Å. In contrast to the CgVik1-sN+MHD molecules, the neck of both molecules of this construct is almost entirely helical, and each differs in their alignment along the MHD core by a ~37° rotation about a conserved glycine residue (Fig. 1, B and C). The neck of molecule A is oriented downward and away from the small β-lobe (β1a, β1b, and β1c) at the edge of the MHD in a manner that is surprisingly similar to several previous Ncd structures (PDB codes 1CZ7, 2NCD, and 1N6M) (supplemental Fig. S3A) (7, 35, 36). In molecule B, the neck is rotated upward relative to molecule A and is in the same position as the ScVik1 neck in the ScVik1MHD and cross-linked SHD-Kar3Vik1 crystal structures (PDB codes 2O0A and 4ETP) (supplemental Figs. S1B and S3B) (17, 19).

**FIGURE 1. Schematic of CgVik1 constructs used for crystallization and overview of their structures.** A, domain architecture of the full-length CgVik1 is shown in a bar diagram, and boundaries of constructs used for crystallization are shown below. The MHD is colored from the N to C terminus with a continuous spectrum of rainbow colors (blue to red), and the coiled-coil regions are in yellow (44). B and C, ribbon representations of the CgVik1-sN+MHD structures (molecules A and B) and CgVik1-N+MHD structures (molecules A and B) are colored the same as the MHD in the bar diagram in A. Secondary structure elements are numbered consecutively from the N terminus of the construct, adopting the system used previously by Allingham et al. (17). Loops with poor electron density were not built into the final models. The “pivot glycine” residue (Gly322) is indicated in each structure and shown as a sphere. This figure was prepared with PyMOL (42).

**Interactions between the Neck and Motor Homology Domain Core of CgVik1—**Core residues in the MHD that interact with the neck of the CgVik1-N+MHD construct are found in the β1 strand, the C-terminal half of helix α1, loop L13, and the N terminus of the β8 strand (Fig. 2). These residues form discrete networks of electrostatic, hydrogen bonding, and van der Waals interactions to stabilize the neck against the core in molecule A and B and cover 317 and 372 Å², respectively. In molecule A, Glu319 of the neck forms salt bridges with Arg325 from strand β1 and Lys552 from β8 (Fig. 2A). Also, Ser316 forms a hydrogen bond with Arg388 from α1, and a single bridging water molecule forms an additional reinforcement between Asn315 and Arg388. In conformation B,
the side chain of Glu319 undergoes a 72° rotation to form salt bridges of different geometries with Lys552 and Arg325 (Fig. 2B). The water-mediated hydrogen bond between Asn315 and Arg388 was also disrupted in favor of a new hydrogen bond between Asn315 and Arg325. These residues, and others immediately surrounding the neck-core junction of CgVik1, exhibit the highest degree of sequence conservation between different Vik1 orthologs and kinesin-14 motors (supplemental Fig. S4) (37, 38). The residue allowing the neck to pivot between these interfaces, Gly322, is completely conserved among all kinesins and sequenced Vik1 and Cik1 orthologs (supplemental Fig. S4C). Additional stabilizing interactions for each neck conformation of CgVik1-N+MHD are formed by contacts with symmetry-related molecules.
Structural Changes in the MHD Core of CgVik1 That Coincide with Neck Isomerization—A major difference in the MHD core that coincides with structural changes in the neck involves the C terminus of CgVik1 (Fig. 2, C–F). In molecules A and B of the CgVik1-N+MHD crystal, and in molecule A of the CgVik1-sN+MHD crystal, five residues beyond the end of helix α6 can be modeled, three of which were not previously observed in ScVik1MHD (Fig. 2, C–E). However, in molecule B of the CgVik1-sN+MHD crystal, the C terminus is completely disordered, and part of the α6 helix melts (Fig. 2F).

When the C-terminal region is visible, we observe it docking onto the central core of the MHD and forming a short two-stranded β-sheet with the neck-core junction. This configuration is structurally analogous to the neck mimic of kinesin-14 family members, which has been observed in recent structures of Ncd (PDB code 3L1C) (7) and the calcium-regulated plant kinesin KCBP (PDB code 1SDM) (8).

In both molecules of the CgVik1-N+MHD construct, stabilizing hydrogen bonds between the neck and C terminus are formed between the backbone amide of Ala324 and the carbonyl of Ile578 and between the backbone carbonyl of Gly532 and the amide of Asn580 (Fig. 2, C and D). In this conformation, the side chain of Ile578 is inserted into a hydrophobic pocket formed by Ala324, Thr530, Phe548, Phe555, and Leu575 within the MHD core, similar to the interaction observed in the ATP-like conformation of KCBP (8, 9). Sequence alignment of the C terminus of other Vik1 and Cik1 proteins shows that Ile578 is moderately conserved (supplemental Fig. S4A), and it also shows some conservation in kinesin-14 motors (supplemental Fig. S4D) (6). Additionally, the side chain of Asn580 is sandwiched directly between Lys552 (β8) and the pivot point of the neck and is only 4.0 Å away from the Glu319–Arg325 and Glu319–Lys552 salt bridges. In this position, it could potentially destabilize either of these interactions and allow the neck to transition from one conformation to the other more readily.

The basis for complete disorder of the C terminus and melting of part of the α6 helix in molecule B of CgVik1-sN+MHD may be related to the repositioning of the side chain of Arg325 in relation to Asn580 when the neck is stabilized in an extended, non-α-helical conformation (Fig. 2F). In this configuration, Arg325 moves toward Ser553 from strand β8 and into the pocket that was occupied by Asn580. Combined with the dramatic change in position of Ala324 and Gly532, which provide hydrogen bond acceptors and donors for aligning the C terminus against the neck-core junction, we speculate that these structural changes may be the basis for undocking the neck mimic from the MHD core. Interestingly, when the C terminus is undocked, Phe577 takes the place of Ile578, perhaps as a means of stabilizing the core.

The fact that the C-terminal segment in molecule A of CgVik1-sN+MHD is nearly identical to that of molecules A and B in the CgVik1-N+MHD crystal is more difficult to reconcile given the nonlocalized position of its neck (Fig. 2E). Similar to molecule B of CgVik1-sN+MHD, Arg325 torsions toward Asn580 and Ser553 (Fig. 2F), but in this case it hydrogen bonds with Asn580. Asn580 also interacts with a nearby water molecule that is bridged to the backbone amide of Ser553 and is within hydrogen bonding distance with the side chain of Glu506 from a symmetry-related molecule. Perhaps these interactions, along with hydrophobic interactions between Ile578 and Pro581 of the C terminus and the MHD core, are sufficient to achieve stabilization of the neck mimic in molecule A of the CgVik1-sN+MHD crystal.

Mutations in the Neck of Vik1 Decrease the Microtubule Gliding Velocity of CgKar3Vik1—We were curious to understand the functional significance of CgVik1 neck isomerization in the motile cycle of CgKar3Vik1. To investigate this, mutations were created in two of the residues that enable the conformational changes observed. Glu319 was chosen for mutation to alanine because we suspected that its involvement in salt bridge formation with the highly conserved MHD core residues Arg325 and Lys552 functions to stabilize each neck conformation and may create a neck position “sensor” for the MHD. The other residue chosen for mutagenesis was Gly322. It was mutated to alanine and proline to probe the importance of rotational freedom in the neck on the motile cycle of CgKar3Vik1. These mutations were generated in CgVik1 constructs that included the MHD, the neck, and an extended length of the native coiled coil to allow dimerization with similar constructs of CgKar3 (supplemental Fig. S5A). Based on the arrangement of subunits in the cross-linked synthetic heterodimer of ScKar3Vik1 (19), none of the mutants should disrupt the inter-helical interactions required for coiled-coil formation between Kar3 and Vik1. Indeed, co-expression of CgKar3-CC+N+MD (Met198–Asn692) and CgVik1-CC+N+MHD (Asp152–Lys552) proteins from E.coli and co-purification of their resulting wild-type (WT) and mutant complexes by nickel-nitrilotriacetic acid affinity chromatography produced stable heterodimers according to analytical size-exclusion chromatography (supplemental Fig. S5, B and C).

In microtubule-gliding assays, the CgKar3Vik1 WT complexes (4.79 ± 0.022 μm/min), which migrated with plus-ends leading (Fig. 3, A–C, and supplemental Fig. S6 and supplemental Movies S1–S3). The larger impact of the G332P substitution on motility is consistent with the additional restrictions proline would place on possible neck conformations relative to alanine. This indicates that conformational freedom of CgVik1’s neck at Gly322 is important for motility of the CgKar3Vik1 motor. With an average microtubule gliding speed of 4.34 ± 0.047 μm/min, the E319A mutant had a more modest effect on motility compared with the G322A and G332P mutants; however, it did show a slightly higher number of microtubule “stalling” events than WT (Fig. 3D and supplemental Movie S4). This suggests that the CgKar3Vik1 motor is sensitive to changes in interactions between the neck and core of CgVik1 and that disruption of the Glu319–Arg325 and Glu319–Lys552 salt bridges may allow the neck to rotate and acquire conformations that are not always conducive to appropriate Kar3-MT interactions or force production events. Notably, in none of the motility studies did we observe obvious dissociation of microtubules from the coverslips, indicating that the CgVik1 subunit was interacting with MTs and that the mutations were not having a negative effect on microtubule binding of the CgKar3Vik1 complex.
To confirm that both CgKar3 and CgVik1 participate in microtubule binding, equilibrium co-sedimentation experiments were performed on our wild-type CgKar3Vik1 dimer construct and a monomeric construct of CgKar3 (CgKar3-N+MD) (supplemental Figs. S7 and S8). Unfortunately, the CgVik1 monomers used for our crystallization studies pelleted in the centrifuge tube regardless of the presence of microtubules, presumably due to aggregation, and thus they could not be used reliably for direct measurement of dissociation constants. Instead, CgVik1’s microtubule binding affinity can be inferred from the difference in $K_d,MT$ of the CgKar3Vik1 WT dimer relative to the monomeric CgKar3-N/H11001 MD construct in the presence of ADP, which confers a weak microtubule-binding state in kinesins. From the binding curves in supplemental Fig. S8, $C$ and $D$, we were able to calculate that the $K_d,MT$ for the ADP complex of the CgKar3Vik1 WT dimer is $4.8 \pm 1 \mu M$, whereas CgKar3-N+MD is nearly twice as weak at $7.6 \pm 2.7 \mu M$. This result indicates that in the ADP state, CgVik1 is contributing to the microtubule binding affinity of the CgKar3Vik1 complex. Moreover, similar $K_d,MT$ values for the AMPPNP complexes of CgKar3-N+MD and CgKar3Vik1 WT dimer (3.05 $\pm$ 0.8 and 3.65 $\pm$ 0.8 $\mu M$, respectively) suggests that, in the presence of ATP, the CgKar3 subunit must form the majority of the interactions between CgKar3Vik1 complexes and microtubules, whereas CgVik1 contributes little to microtubule binding.

Vik1 Neck Mutants Exhibit Reduced Microtubule-activated ATPase Activity—Even though the mutations we engineered were introduced into the noncatalytic subunit of the CgKar3Vik1 complex, we wanted to learn their effect on the biochemical function of Kar3. Compared with the WT dimer (0.95 s$^{-1}$), the steady state ATPase kinetics studies show a 1.8- and 11-fold decrease in $k_{cat}$ for the CgKar3Vik1 G322A (0.54 s$^{-1}$) and G322P (0.088 s$^{-1}$) mutants, respectively (Fig. 4). These data suggest that the slower rate of motility of the mutants is related to their creation of defects in the ability of CgKar3’s ATPase activity to be activated by microtubules. This could be a consequence of the mutants impeding rotational freedom of the CgVik1 neck that is obligatory for appropriate CgKar3-microtubule interactions. An alternative possibility is that ATP binding or hydrolysis by CgKar3 is impeded by the CgVik1 neck rotation defects in a microtubule-independent manner, perhaps as a consequence of disruption of important interactions between the neck and motor domain core of CgKar3.

Similar to but much more dramatic than its effect on the microtubule-gliding assays, the E319A mutant lowered the $k_{cat}$ of CgKar3Vik1 by 3.7-fold (0.26 s$^{-1}$) (Fig. 4), providing further evidence that the interactions between the neck and MHD core of Vik1 form a determinant of the mechanochemical cycle of the Kar3Vik1 motor. As is reflected by their similar $K_{0.5,MT}$ for ATPase activation (Fig. 4C), none of the mutants dramatically lowered the affinity of the CgKar3Vik1 complex for microtubules. However, they did exhibit different trends in their ATP concentration-dependent activities; CgKar3Vik1 G322P and E319A showed lowered $K_{m,ATP}$ values relative to WT, whereas

**FIGURE 3.** Histograms of the velocity distribution of gliding microtubules for CgKar3Vik1 WT dimer, G322A, G322P, and E319A mutants. $A$–$D$, rates for each motor are presented in 0.25 $\mu$m/min bins. Over 250 microtubules were tracked for each motor from movies obtained during 3–6 independent experiments. The supplemental Movies S1–S4 are representative of microtubules gliding by each mutant.
Recent structural studies of the heterodimeric ScKar3Vik1 motor have shown that Kar3 and Vik1 bind side-by-side to adjacent protofilaments when Kar3 is in an ADP-bound state, and there is an ATP-dependent rotation of the neck of Kar3 that is nearly identical to Ncd (19). Unfortunately, high resolution structural information could not be provided to describe in detail the configuration of Vik1 in this state, nor any of the other early states leading up to its release from microtubules when ADP is exchanged for ATP in Kar3. Our crystal structures of CgVik1 provide information about previously unseen states of Vik1 that may be relevant to the initial association of Kar3Vik1 with the microtubule. Through mutational analysis, we also provide evidence of head–head communication in the heterodimeric Kar3Vik1 motor assembly, as well as mechanistic details about the communication route.

Our studies show that the helical neck of CgVik1 can rotate with respect to the MHD, allowing separate clusters of conserved electrostatic, hydrophobic, and hydrogen bond-forming residues in the neck and core to interact. This rotation occurs by a change in the backbone torsion angles at the conserved Gly322, which appears to be critical for the microtubule-activated ATPase activity and motility of CgKar3Vik1. Although motility analysis of the constrained ScKar3Vik1 complex used for structural determination by Rank et al. (19) (cross-linked SHD-Kar3Vik1) suggested that rotation of the Vik1 neck is not critical for the Kar3Vik1 power stroke, a reduction in the rate of motility of this construct relative to the noncross-linked construct (noncross-linked SHD-Kar3Vik1) was observed.

We propose that neck rotation observed in our unconstrained CgVik1-N+MHD construct represents early Vik1-microtubule binding states that facilitate proper Kar3 contacts on the adjacent microtubule protofilament (Fig. 5). Specifically, we suggest that during initial collision of Kar3Vik1 with the microtubule (Fig. 5, Step 1), before Kar3 binds, the neck of Vik1 is in the “up” position (i.e., molecule B of CgVik1-N+MHD crystals). Next, rotation of neck to the “down” position (i.e., molecule A of CgVik1-N+MHD) could help orient the head of Kar3 into closer proximity with the available microtubule binding position on the adjacent protofilament (Fig. 5, Step 2) in accord with the reverse orientation of Kar3 and Vik1 suggested by Rank et al. (19). The resulting two-headed binding state of Kar3Vik1, and the contemporaneous release of ADP, may cause some separation of the heads, placing strain on the neck of Vik1 sufficient to melt its helical structure in a manner analogous to Gly322, critical for the Kar3Vik1 power stroke, a reduction in the rate of motility of this construct relative to the noncross-linked construct (noncross-linked SHD-Kar3Vik1) was observed.

We propose that neck rotation observed in our unconstrained CgVik1-N+MHD construct represents early Vik1-microtubule binding states that facilitate proper Kar3 contacts on the adjacent microtubule protofilament (Fig. 5). Specifically, we suggest that during initial collision of Kar3Vik1 with the microtubule (Fig. 5, Step 1), before Kar3 binds, the neck of Vik1 is in the “up” position (i.e., molecule B of CgVik1-N+MHD crystals). Next, rotation of neck to the “down” position (i.e., molecule A of CgVik1-N+MHD) could help orient the head of Kar3 into closer proximity with the available microtubule binding position on the adjacent protofilament (Fig. 5, Step 2) in accord with the reverse orientation of Kar3 and Vik1 suggested by Rank et al. (19). The resulting two-headed binding state of Kar3Vik1, and the contemporaneous release of ADP, may cause some separation of the heads, placing strain on the neck of Vik1 sufficient to melt its helical structure in a manner analogous to Gly322, critical for the Kar3Vik1 power stroke, a reduction in the rate of motility of this construct relative to the noncross-linked construct (noncross-linked SHD-Kar3Vik1) was observed.

We propose that neck rotation observed in our unconstrained CgVik1-N+MHD construct represents early Vik1-microtubule binding states that facilitate proper Kar3 contacts on the adjacent microtubule protofilament (Fig. 5). Specifically, we suggest that during initial collision of Kar3Vik1 with the microtubule (Fig. 5, Step 1), before Kar3 binds, the neck of Vik1 is in the “up” position (i.e., molecule B of CgVik1-N+MHD crystals). Next, rotation of neck to the “down” position (i.e., molecule A of CgVik1-N+MHD) could help orient the head of Kar3 into closer proximity with the available microtubule binding position on the adjacent protofilament (Fig. 5, Step 2) in accord with the reverse orientation of Kar3 and Vik1 suggested by Rank et al. (19). The resulting two-headed binding state of Kar3Vik1, and the contemporaneous release of ADP, may cause some separation of the heads, placing strain on the neck of Vik1 sufficient to melt its helical structure in a manner analogous to Gly322, critical for the Kar3Vik1 power stroke, a reduction in the rate of motility of this construct relative to the noncross-linked construct (noncross-linked SHD-Kar3Vik1) was observed.

We propose that neck rotation observed in our unconstrained CgVik1-N+MHD construct represents early Vik1-microtubule binding states that facilitate proper Kar3 contacts on the adjacent microtubule protofilament (Fig. 5). Specifically, we suggest that during initial collision of Kar3Vik1 with the microtubule (Fig. 5, Step 1), before Kar3 binds, the neck of Vik1 is in the “up” position (i.e., molecule B of CgVik1-N+MHD crystals). Next, rotation of neck to the “down” position (i.e., molecule A of CgVik1-N+MHD) could help orient the head of Kar3 into closer proximity with the available microtubule binding position on the adjacent protofilament (Fig. 5, Step 2) in accord with the reverse orientation of Kar3 and Vik1 suggested by Rank et al. (19). The resulting two-headed binding state of Kar3Vik1, and the contemporaneous release of ADP, may cause some separation of the heads, placing strain on the neck of Vik1 sufficient to melt its helical structure in a manner analogous to Gly322, critical for the Kar3Vik1 power stroke, a reduction in the rate of motility of this construct relative to the noncross-linked construct (noncross-linked SHD-Kar3Vik1) was observed.

We propose that neck rotation observed in our unconstrained CgVik1-N+MHD construct represents early Vik1-microtubule binding states that facilitate proper Kar3 contacts on the adjacent microtubule protofilament (Fig. 5). Specifically, we suggest that during initial collision of Kar3Vik1 with the microtubule (Fig. 5, Step 1), before Kar3 binds, the neck of Vik1 is in the “up” position (i.e., molecule B of CgVik1-N+MHD crystals). Next, rotation of neck to the “down” position (i.e., molecule A of CgVik1-N+MHD) could help orient the head of Kar3 into closer proximity with the available microtubule binding position on the adjacent protofilament (Fig. 5, Step 2) in accord with the reverse orientation of Kar3 and Vik1 suggested by Rank et al. (19). The resulting two-headed binding state of Kar3Vik1, and the contemporaneous release of ADP, may cause some separation of the heads, placing strain on the neck of Vik1 sufficient to melt its helical structure in a manner analogous to Gly322, critical for the Kar3Vik1 power stroke, a reduction in the rate of motility of this construct relative to the noncross-linked construct (noncross-linked SHD-Kar3Vik1) was observed.

We propose that neck rotation observed in our unconstrained CgVik1-N+MHD construct represents early Vik1-microtubule binding states that facilitate proper Kar3 contacts on the adjacent microtubule protofilament (Fig. 5). Specifically, we suggest that during initial collision of Kar3Vik1 with the microtubule (Fig. 5, Step 1), before Kar3 binds, the neck of Vik1 is in the “up” position (i.e., molecule B of CgVik1-N+MHD crystals). Next, rotation of neck to the “down” position (i.e., molecule A of CgVik1-N+MHD) could help orient the head of Kar3 into closer proximity with the available microtubule binding position on the adjacent protofilament (Fig. 5, Step 2) in accord with the reverse orientation of Kar3 and Vik1 suggested by Rank et al. (19). The resulting two-headed binding state of Kar3Vik1, and the contemporaneous release of ADP, may cause some separation of the heads, placing strain on the neck of Vik1 sufficient to melt its helical structure in a manner analogous to Gly322, critical for the Kar3Vik1 power stroke, a reduction in the rate of motility of this construct relative to the noncross-linked construct (noncross-linked SHD-Kar3Vik1) was observed.
Neck Rotation and Neck Mimic Docking in Vik1

would be free to rotate toward the microtubule minus end as Kar3 binds ATP, completing the power stroke (Fig. 5, Step 4).

We therefore propose that the residues involved in the interactions between the neck, MHD core, and neck mimic in Vik1 constitute part of the communication route between Kar3 and Vik1 (Fig. 5) and that isomerization of the Vik1 neck may be the actuator that “flips” a molecular switch controlling Vik1’s release from the microtubule to allow motility. As noted by Khalil et al. (4), most kinesins, and approximately half of all single-domain proteins in the Protein Data Bank (43), have interacting N- and C-terminal elements that experience dynamic interconversion between different types of secondary structure, or undergo disorder-to-order transitions. It appears that Vik1 may have adapted this behavior for regulation of its microtubule binding interactions by Kar3 in lieu of nucleotide binding and enzymatic function.

REFERENCES

1. Vale, R. D. (2003) The molecular motor toolbox for intracellular transport. Cell 112, 467–480
2. Sharp, D. J., Rogers, G. C., and Scholey, J. M. (2000) Microtubule motors in mitosis. Nature 407, 41–47
3. Wittmann, T., Hyman, A., and Desai, A. (2001) The spindle. A dynamic assembly of microtubules and motors. Nat. Cell Biol. 3, E28–E34
4. Khalil, A. S., Appleyard, D. C., Labno, A. K., Georges, A., Karplus, M., Belcher, A. M., Hwang, W., and Lang, M. J. (2008) kinesin’s cover-neck bundle folds forward to generate force. Proc. Natl. Acad. Sci. U.S.A. 105, 19247–19252
5. Hwang, W., Lang, M. J., and Karplus, M. (2008) Force generation in kinesin hinges on cover-neck bundle formation. Structure 16, 62–71
6. Szczęsna, E., and Kasprzak, A. A. (2012) The C terminus of kinesin-14 Ncd is a crucial component of the force generating mechanism. FEBS Lett. 586, 854–858
7. Heuston, E., Bronner, C. E., Kull, F. J., and Endow, S. A. (2010) A kinesin motor in a force-producing conformation. BMC Struct. Biol. 10, 19
8. Vinogradova, M. V., Reddy, V. S., Reddy, A. S., Sablin, E. P., and Fletterick, R. J. (2004) Crystal structure of kinesin regulated by Ca²⁺-calmodulin. J. Biol. Chem. 279, 23504–23509
9. Vinogradova, M. V., Malanina, G. G., Reddy, A. S., and Fletterick, R. J. (2009) Structure of the complex of a mitotic kinesin with its calcium-binding regulator. Proc. Natl. Acad. Sci. U.S.A. 106, 8175–8179
10. Meluh, P. B., and Rose, M. D. (1990) Kar3, a kinesin-related gene required for yeast nuclear fusion. Cell 60, 1029–1041
11. Saunders, W. S., and Hoyt, M. A. (1992) kinesin-related proteins required for structural integrity of the mitotic spindle. Cell 70, 451–458
12. Saunders, W., Hornack, D., Lengyel, V., and Deng, C. (1997) The Saccharomyces cerevisiae kinesin-related motor Kar3p acts at prophase spindle poles to limit the number and length of cytoplasmic microtubules. J. Cell Biol. 137, 417–431
13. Sherwood, R. K., and Bennett, R. I. (2008) Microtubule motor protein Kar3 is required for normal mitotic division and morphogenesis in Candida albicans. Eukaryot. Cell 7, 1460–1474
14. Page, B. D., Satterwhite, L. L., Rose, M. D., and Snyder, M. (1994) Localization of the Kar3 kinesin heavy chain-related protein requires the Cik1-interacting protein. J. Cell Biol. 124, 507–519
15. Page, B. D., and Snyder, M. (1992) Cik1. A developmentally regulated spindle pole body-associated protein important for microtubule functions
Neck Rotation and Neck Mimic Docking in Vik1

Partial text:

in Saccharomyces cerevisiae. Genes Dev. 6, 1414–1429
16. Barrett, J. G., Manning, B. D., and Snyder, M. (2000) The Kar3p kinesin-related protein forms a novel heterodimeric structure with its associated protein Cik1p. Mol. Biol. Cell 11, 2373–2385
17. Allingham, J. S., Sproul, L. R., Rayment, I. and Gilbert, S. P. (2007) Vik1 modulates microtubule-Kar3 interactions through a motor domain that lacks an active site. Cell 128, 1161–1172
18. Chen, C. J., Rayment, I. and Gilbert, S. P. (2011) kinesin Kar3Cik1 ATPase pathway for microtubule cross-linking. J. Biol. Chem. 286, 29261–29272
19. Rank, K. C., Chen, C. J., Cope, J., Porche, K., Hoenger, A., Gilbert, S. P., and Rayment, I. (2012) Kar3Vik1, a member of the kinesin-14 superfamily, shows a novel kinesin microtubule binding pattern. J. Cell Biol. 197, 957–970
20. Wendt, T. G., Volkmann, N., Skinioitis, G., Goldie, K. N., Müller, J., Mandelkow, E., and Hoenger, A. (2002) Microscopic evidence for a minus-end-directed power stroke in the kinesin motor ncd. EMBO J. 21, 5969–5978
21. Endres, N. F., Yoshioka, C., Milligan, R. A., and Vale, R. D. (2006) A lever-arm rotation drives motility of the minus-end-directed kinesin Ncd. Nature 439, 875–878
22. Gladfelter, A., and Berman, I. (2009) Dancing genomes. Fungal nuclear positioning. Nat. Rev. Microbiol. 7, 875–886
23. Lang, C., Grava, S., van den Hoorn, T., Trimble, R., Philippsen, P., and Jaspersen, S. L. (2010) Mobility, microtubule nucleation, and structure of microtubule-organizing centers in multinucleated hyphae of Ashbya gossypii. Mol. Biol. Cell 21, 18–28
24. Sack, S., Müller, J., Marx, A., Thormählen, M., Mandelkow, E. M., Brady, S. T., and Mandelkow, E. (1997) X-ray structure of motor and neck domains from rat brain kinesin. Biochemistry 36, 16155–16165
25. Sproul, L. R., Anderson, D. J., Mackey, A. T., Saunders, W. S., and Gilbert, S. P. (2005) Cik1 targets the minus-end kinesin depolymerase kar3 to microtubule plus ends. Curr. Biol. 15, 1420–1427
26. Kabsch, W. (2010) Xds. Acta Crystallogr. D Biol. Crystallogr. 66, 125–132
27. Otwinowski, Z., and Minor, W. (1997) Processing of x-ray diffraction data collected in oscillation mode. Methods Enzymol. 276, 307–326
28. Adams, P. D., Afonine, P. V., Bunkóczi, G., Chen, V. B., Davis, I. W., Echols, N., Headd, J. J., Hung, L. W., Kapral, G. J., Grosse-Kunstleve, R. W., McCoy, A. J., Moriarty, N. W., Oeffner, R., Read, R. I., Richardson, D. C., Richardson, J. S., Terwilliger, T. C., and Zwart, P. H. (2010) PHENIX. A comprehensive Python-based system for macromolecular structure solution. Acta Crystallogr. D Biol. Crystallogr. 66, 213–221
29. Gulick, A. M., Song, H., Endow, S. A., and Rayment, I. (1998) X-ray crystal structure of the yeast Kar3 motor domain complexed with MgADP to 2.3 Å resolution. Biochemistry 37, 1769–1776
30. Emsley, P., and Cowtan, K. (2004) Coot. Model-building tools for molecular graphics. Acta Crystallogr. D Biol. Crystallogr. 60, 2126–2132
31. Murshudov, G. N., Vagin, A. A., and Dodson, E. J. (1997) Refinement of macromolecular structures by the maximum-likelihood method. Acta Crystallogr. D Biol. Crystallogr. 53, 240–255
32. Walter, W. J., Koonce, M. P., Brenner, B., and Steffen, W. (2012) Two independent switches regulate cytoplasmic dynein’s processivity and directionality. Proc. Natl. Acad. Sci. U.S.A. 109, 5289–5293
33. Huang, T. G., and Hackney, D. D. (1994) Drosophila kinesin minimal motor domain expressed in Escherichia coli. Purification and kinetic characterization. J. Biol. Chem. 269, 16493–16501
34. Duan, D., Nhatchuck, D. J., Brenner, J., Davis, D., and Allingham, J. S. (2012) Crystal structure of the Kar3-like kinesin motor domain from the filamentous fungus Ashbya gossypii. Proteins 80, 1016–1027
35. Kozielski, F., De Bonis, S., Burmeister, W. P., Cohen-Addad, C., and Wade, R. H. (1999) The crystal structure of the minus-end-directed microtubule motor protein ncd reveals variable dimer conformations. Structure 7, 1407–1416
36. Yun, M., Bronner, C. E., Park, C. G., Cha, S. S., Park, H. W., and Endow, S. A. (2003) Rotation of the stalk/neck and one head in a new crystal structure of the kinesin motor protein, Ncd. EMBO J. 22, 5382–5389
37. Sabin, E. P., Kull, F. I., Cooke, R., Vale, R. D., and Fletterick, R. J. (1996) Crystal structure of the motor domain of the kinesin-related motor ncd. Nature 380, 555–559
38. Sabin, E. P., Case, R. B., Dai, S. C., Hart, C. L., Ruby, A., Vale, R. D., and Fletterick, R. J. (1998) Direction determination in the minus-end-directed kinesin motor ncd. Nature 395, 813–816
39. Endow, S. A., and Waligora, K. W. (1998) Determinants of kinesin motor polarity. Science 281, 1200–1202
40. Deavours, B. E., Reddy, A. S., and Walker, R. A. (1998) Ca2+/calmodulin regulation of the Arabidopsis kinesin-like calmodulin-binding protein. Cell Motil. Cytoskeleton 40, 408–416
41. Reddy, V. S., Day, I. S., Thomas, T., and Reddy, A. S. (2004) KIC, a novel Ca2+ binding protein with one EF-hand motif, interacts with a microtubule motor protein and regulates trichome morphogenesis. Plant Cell 16, 185–200
42. DeLano, W. L. (2002) The PyMOL Molecular Graphics System, DeLano Scientific LLC, San Carlos, CA
43. Krishna, M. M., and Englander, S. W. (2005) The N-terminal to C-terminal motif in protein folding and function. Proc. Natl. Acad. Sci. U.S.A. 102, 1053–1058
44. Berger, B., Wilson, D. B., Wolf, E., Tonchev, T., Milla, M., and Kim, P. S. (1995) Predicting coiled coils by use of pairwise residue correlations. Proc. Natl. Acad. Sci. U.S.A. 92, 8259–8263