The structure of microtubule motor proteins

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7. Summary and Outlook
Microtubules are the intracellular tracks for two classes of motor proteins: kinesins and dyneins. During the past few years, the motor domain structures of several kinesins from different organisms have been determined by X-ray crystallography. Compared with kinesins, dyneins are much larger proteins and attempts to crystallize them failed so far. Structural information about these proteins mainly comes from electron microscopy. In this review we mainly focus on the published crystal structures of kinesin motor domains.

1. Kinesin classes, domain structure and nomenclature

Kinesins constitute a large protein family that realize a wide range of functions within eukaryotic cells, including the transport of different cargoes (vesicles, organelles, protein complexes, chromosomes) and the regulation of microtubule dynamics. The superfamily of kinesins currently includes more than 600 sequences from a variety of species. This large number of proteins led to a confusing variety of names and classifications. To overcome these problems the kinesin research community just proposed a new standardized nomenclature (Lawrence et al., 2004) that subdivide the kinesin superfamily into 14 families. Each family bears the name “kinesin” and is followed by an arabic number (for example the founding member of the protein superfamily, the conventional kinesin or kinesin heavy chain, KHC, is now named as kinesin-1).

Kinesin-1 comprises three major domains: the N-terminal motor domain, that can be subdivided into the core motor domain and the adjacent neck linker and neck region, the central stalk domain and the C-terminal tail or light chain binding domain (Fig. 1a). The core motor domain has a length of about 325 amino acids and contains both the microtubule and the nucleotide binding elements. In different kinesin families, this motor domain can be located at various places within the molecule depending on the function of the specific kinesin family as a plus- (N-terminal location) or minus-end (C-terminal location) directed motor or as microtubule depolymerizing machines (internal location). The different possibilities for the location of the motor domain (correlating with different functions; Fig. 1) led to the classification into N-type, M-type and C-type motors (Vale and Fletterick, 1997). M-type motors were also referred to as Kin-I (for Internal). The motor core domain produces force in concert with the adjacent neck; this region is found on either the N- (in N-type kinesins) or the C-terminus (in C- and M-type kinesins).

Within cells most kinesins are not monomeric, they carry out there functions at least pairwise. For example, kinesin-1 forms dimers through a coiled-coil interaction of the stalk domain. The “kinesin heavy chains” bind to “light chains” thus forming a tetrameric complex. The light chains can dock onto cargo receptors or adaptors, linking kinesin to its different cargoes. Other kinesins fulfill their functions as homotrimers (without light chains, for example Neurospora kinesin-1 or kinesin-7), heterotrimers (kinesin-2, where two different motor molecules are associated with a non-motor subunit), or homotetramers (kinesin-5).

The first structure of a kinesin motor domain was published in 1996. After eight years of kinesin crystallization and structure determination, there are today 26 x-ray structures of motor domains from kinesins and mutants of different sources deposited in the Protein Data Bank (October 2004; http://www.rcsb.org/pdb/, Berman et al., 2000). These structures are from eleven different proteins which belong to six different kinesin families including kinesin-1 (conventional kinesin), kinesin-3 (Unc104/Kif1a-family), kinesin-5 (BimC family), kinesin-7 (CENP-E family), kinesin-13 (MCAK family) and kinesin-14 (C-terminal motors).
The crystallized proteins come from eight different organisms including mammals, *Drosophila*, yeast, *Plasmodium*, *Neurospora* and also a plant (potato). Two of the proteins, a mouse kinesin-3 (Kif1a) and mouse kinesin-13 (Kif2C), have been solved in different nucleotide states. One structure (human Eg5) has been solved together with a class specific inhibitor and another structure (KCBP) contains a short non-motor domain. Referring to the position of the motor domain, members of all three groups (N-type, M-type, and C-type) have been crystallized.

2. Kinesin-1 as prototypical motor

The first structure of a kinesin motor domain - that of human kinesin-1 (formerly named KHC or conventional kinesin) - was determined by Kull and coworkers (Kull et al., 1996). This is still the structure of highest resolution (1.8 Å, PDB-ID: 1BG2) among all structures of conventional kinesins that have been published so far. Yet, it contains several regions of unstructured and therefore invisible amino acids. Shortly after that, the structure of a similar construct from rat kinesin-1 was published (Sack et al., 1997). Though the resolution is lower (2.0 Å, PDB-ID: 2KIN) than that of human kinesin-1, the rat kinesin-1 structure includes some of the structural elements that are invisible in the Kull structure. More recently, another crystal structure of the human kinesin-1 motor domain was found, using crystallization conditions similar to those used for the crystallization of monomeric rat kinesin-1 (Sindelar et al., 2002). This structure (PDB-ID: 1MKJ, 2.7 Å resolution) is very similar to that of rat kinesin-1, demonstrating that the differences between the first human and the rat structure are largely due to the crystallization conditions. Nevertheless, these differences point to “hot spots” of conformational variability that might be significant for the dynamic behavior of the motor domain. The more complete structure of rat kinesin-1 is chosen as a paradigm for the discussion of similarities and differences to other kinesin structures (Fig. 2).

The core domain is an α/β class protein with a three-layer (αβα) sandwich architecture. The central β-sheet consists of eight strands, named β1 to β8 according to their succession in amino acid sequence (spatial sequence: 2-1-8-3-7-6-4-5, Fig. 2b ). These strands are all parallel with the exception of β5 and β6. Strands β6 and β7 form a β-hairpin connected by L10 (a short loop containing a β-turn of type I). β5 consists of two short stretches of three and four amino acids (aa) with a 26 aa insert (loop L8) in between. The β5 strands serve as hinges that anchor loop L8 to the core β-sheet. β4 is the longest β-strand of the motor domain (14 aa), the length of the other strands falling off towards the other edge of the β-sheet (β2 with 3 aa is the shortest strand of the core sheet). As the strands at one end are more or less lined up, the central β-sheet assumes roughly the shape of a right triangle (disregarding the β5-appendix) which is distorted in space.

The central β-sheet is the supporting structure for all the other structural elements and moving parts of the motor domain. Most of the central β-sheet is covered with helices and loops: α1, α2a-L5-α2b, a3-L9-α3a on one face, α4-L12-α5 and α6 on the opposite face (Fig 2c). The overall shape of the core domain is cone-like with the exposed N-terminus of β4 and the loop between β6 and β7 forming the tip of the cone. In addition to the αβα-sandwich, there are two lobes (Fig. 2d): one lobe of about 30 amino acids at the β2-vertex of the central β-sheet, consisting of a short helix (α0) and a small, three-stranded, antiparallel β-sheet (β1a,b,c); the second lobe consisting of loop L8 (already mentioned) is attached to β4 of the central β-sheet via the split strand β5. It is sometimes called "β5-L8 lobe". Besides loop regions of undefined secondary structure, this lobe contains two β-strands (β5a and β5b) in antiparallel conformation.
The monomeric rat kinesin construct (aa 1-354) comprises the head domain (including the core motor domain, aa 2-325, and the neck linker, aa 326-338) and the first half of the neck domain. In the crystal structure, the neck linker consists of two strands, \( \beta_9 \) and \( \beta_{10} \), that form hydrogen-bonds with strands \( \beta_8 \) and \( \beta_7 \) of the core \( \beta \)-sheet (Fig. 2e). The neck linker ends close to loop L10 at the tip of the core domain where the \( \alpha \)-helical neck domain (helix \( \alpha_7 \)) is attached to the core motor domain. Its orientation is roughly in the "plane" of the core \( \beta \)-sheet and perpendicular to the strands.

The nucleotide binding pocket consists of four motifs, N1 to N4, also found in other ATP-binding proteins, like myosins and G-proteins (Sack et al., 1997). N1 is a Walker A motif at the end of \( \beta_3 \) (\(^{86}GQTSSGKT^{93}\), rat kinesin-1 sequence, consensus motif underlined) that forms a "P-loop", a common fold of \( \beta \)-loop-helix type that binds oxygen atoms of the \( \beta \)- and \( \gamma \)-phosphates. N2 (\(^{199}NEHSSR^{204}\), located in \( \alpha_3a \) at the N-terminus of \( \beta_6 \)) and N3 (\(^{232}DLAGSE^{237}\) at the C-terminus of \( \beta_7 \)) are known as switch-1 and switch-2 in analogy to G-proteins and myosins. The switches may function as \( \gamma \)-phosphate sensors that are thought to be first to respond to ATP-hydrolysis. By changing position and conformation of the switches, adjacent elements are forced to move and, thus, the local adjustment in nucleotide coordination will be transduced and amplified. N4 at the C-terminus of \( \beta_1 \) (\(^{14}RFRP^{17}\)) is involved in nucleotide binding by interaction with the adenine moiety. Histidine H94 at the end of the P-loop also interacts with the base.

Structural elements that interact with the microtubule surface have been identified by the effect of point mutations (Woehlke et al., 1997) and by fitting crystal structures of kinesin motor domains to low-resolution electron density maps obtained by cryo-electron microscopy of microtubules saturated with kinesin (Hoenger et al., 1998; Hoenger et al., 2000; Kikkawa et al., 2000; Song et al., 2001). According to these experiments, the structural elements that contribute most to the interaction with microtubules are (1) the \( \beta_5 \)-L8 lobe, (2) the cluster formed by helix \( \alpha_4 \), loop L12, and \( \alpha_5 \), and (3) loop L11, a loop of approximately 15 amino acids length between switch-2 and the \( \alpha_4 \)-L12-\( \alpha_5 \) cluster which is largely disordered in rat kinesin-1 and in most other kinesin structures known so far (see Table I). Exceptions are the structures of Neurospora crassa kinesin-1 (NcKin, PDB-ID: 1GOJ, Song et al., 2001), the monomeric kinesin-3 motor domain complex with ADP and vanadate (MmKIF1a, PDB-ID: 1VFZ, Nitta et al., 2004), the R598A mutant of SeKar3 protein, a member of the kinesin-14 family (PDB-ID: 1F9V, Yun et al., 2001), and the M-type kinesin-13 PfMCAK (PDB-ID: 1RY6, Shipley et al., 2004). Loop L11 is supposed to be disordered in free kinesin and to adopt a rigid structure in the kinesin-microtubule complex, thus establishing a linkage between the \( \gamma \)-phosphate sensor switch-2 and the microtubule-binding cluster \( \alpha_4 \)-L12-\( \alpha_5 \), which is also named "switch-2 cluster". In addition to these elements, neck helix \( \alpha_7 \) may also contribute to microtubule-binding via interaction with the negatively charged C-terminus of \( \beta \)-tubulin.

The kinesin construct shown in Figure 2 ends with K354 (last visible amino acid E351) in the middle of a sequence that is predicted by the program Paircoil (Berger et al., 1995) to form a continuous coiled coil (aa A334 to R371). A longer construct comprising the complete coiled-coil domain (aa 2-379) was crystallized in the form of dimers as shown in Figure 3b (Kozielski et al., 1997). The structures of the two heads are very similar to the 2KIN structure of the monomeric construct. Surprisingly, there is no proper symmetry relation between the heads of the dimer, although the region responsible for dimerization (the neck coiled coil) has almost perfect two-fold symmetry. The heads are related to each other by rotation of approximately 120° about an axis that is inclined to the coiled-coil axis. There is only little
direct contact between the heads via interaction of K160 in the microtubule-binding loop L8 (between β5a and β5b) and the tip of head B (E221 in loop L10 between β6 and β7). It has been suggested on the basis of solution scattering experiments that the asymmetric conformation of the heads in the crystal structure may be representative for the conformation of the isolated dimer (Kozielski et al., 2001). Thus, the special arrangement of the heads in the crystal may not be an artifact caused by crystal packing, but rather the effect of direct and indirect interactions (between residues of the individual heads and residues at the N-termini of the neck coiled coil). In any case, the asymmetric conformation must be of limited stability as both heads can separate during their working cycle and bind simultaneously to adjacent sites on the microtubule (e.g. Asenjo et al., 2003; Hoenger et al., 2000; Skiniotis et al., 2003). This is illustrated in Figure 4 showing schematically how a kinesin dimer could walk along a microtubule protofilament.

A detailed view of the kinesin-microtubule complex has been obtained by combining "high-resolution" structures of the individual components from x-ray crystallography (kinesin) and electron diffraction (tubulin; Lowe et al., 2001; Nogales et al., 1998) with "low-resolution" models of kinesin-decorated microtubules obtained by cryo-electronmicroscopy and image reconstruction (Hirose et al., 1999; Hoenger et al., 2000; Kikkawa et al., 2001; Kozielski et al., 1998; Rice et al., 1999; Skiniotis et al., 2003; Wendt et al., 2002). Figure 5 shows the binding geometry for the motor domain of Neurospora crassa conventional kinesin (NcKin; Song et al., 2001) which is similar to that of human and rat kinesin-1. Helix α4 is almost perpendicular to the protofilament and indents into the cleft between the α- and β-subunits of the tubulin dimer. The major binding regions in kinesin (loop L8/β5a,b and the switch-2 cluster including loop L11) are close to the H12 helices at the outer rim of the microtubule surface, with loop L8 and L12 approaching H12 of β-tubulin, while loop L11 projects to the adjacent α-subunit.

3. Comparison of kinesin structures

3.1 Human and rat kinesin-1

A comparison of the first human kinesin-1 structure (PDB-ID: 1BG2; Kull et al., 1996) and the rat kinesin-1 structure (PDB-ID: 2KIN) has been published previously (Sack et al., 1999). The overall fold of the motor domains is the same, yet the structures differ in significant shifts of surface elements. At that time it was not clear whether the differences that have been spotted are due to variances in the primary structures of human and rat kinesin-1 (86.4% identity, 93.8% with conserved substitutions) or if the observed structures represent two possible conformations of the motor domain that are selectively stabilized by different conditions used for crystallization: the human structure has been determined with crystals grown in polyethyleneglycol (PEG) at pH 4.6, while rat kinesin was crystallized at pH 7.5 with lithium sulfate as precipitant. This uncertainty was resolved by a new structure of the human kinesin-1 motor domain (PDB-ID: 1MKJ; Sindelar et al., 2002). Using the same construct of human kinesin-1 motor domain (HsKHC, aa 2-349) crystals were grown under the conditions used for rat kinesin. The new structure of human kinesin-1 turned out to be very similar to that of rat kinesin-1. The rms distance of the Ca atoms after structural alignment is 0.78 Å (all amino acids included except for the N-terminal alanine; calculated with DeepView Swiss-Pdb Viewer 3.7, Guex and Peitsch, 1997). Thus, the differences
between rat and human kinesin-1 described earlier are not species specific but reveal two conformational states of the motor domain that may be of physiological relevance.

The most obvious difference between the PEG-grown crystal structure of human kinesin-1 and the crystal structures of human and rat kinesin-1 obtained with lithium sulfate is that the neck linker and neck helix are disordered and thus invisible in the “PEG structure”. This correlates with a significant displacement of the switch-2 cluster. In the “PEG structure”, the C-termini of helices $\alpha 4$ and $\alpha 5$ in the switch-2 cluster occlude binding sites for the neck linker at the surface of the core motor domain. This explains why the neck linker is "undocked" (i.e. mostly detached from the motor core) and disordered and, thus, invisible in this crystal structure. In the “lithium sulfate structures”, the switch-2 cluster assumes a different position and orientation, allowing the binding of the neck linker to the motor core. The displacement of the switch-2 cluster can be described to a large extent by a rigid body movement. The rms distance of the C$_\alpha$ atoms in the switch-2 clusters of the human kinesin-1 structures 1MKJ and 1BG2 (K256 to G291, human kinesin-1 numbering) is 3.25 Å, if the superposition is calculated with the core structures. The maximum displacement is 5.8 Å at amino acid Thr273 in loop L12, close to the neck linker binding site. Rigid body superposition of the clusters alone reduces the rms distance to 0.77 Å. The movement consists of a ~2 Å translation towards the nucleotide binding site combined with a tilt away from the neck linker.

How does the "rigid body" movement of the switch-2 cluster comply with its interactions with the rest of the motor domain? Regarding the main chain connectivity, such a movement requires flexible adaptors at both ends of the cluster. At the N-terminus, loop L11 obviously fulfills this function. At the other end, loop L13 (between $\alpha 5$ and strand $\beta 8$ of the central $\beta$-sheet) may provide sufficient flexibility. Loop L13 contains two glycines (G$^{293}$G$^{294}$ in rat kinesin-1; glycine is less restricted in conformational space than any other amino acid) that are conserved throughout the kinesin family with the major exception of the kinesin-13 (formerly MCAK/Kif4) family, which has only one glycine in Loop L13 (according to an alignment of 143 motor domains available on the kinesin home page, http://www.proweb.org/kinesin/KinesinAlign.html). Interestingly, the 2KIN structure of rat kinesin-1 contains a point mutation (G293D) of one of these glycines. This may reduce the flexibility of loop L13 and favour the conformation of the switch-2 cluster that allows docking of the neck linker. This could explain why the rat kinesin-1 mutant produced crystals of rather high quality that could be solved to a resolution of 2.0 Å (compared with 2.7 Å for human kinesin-1 structure 1MKJ which was crystallized in conditions similar to that used to crystallize monomeric rat kinesin). In addition to the flexible main chain connections, the ability to slide over the central $\beta$-sheet may also impose restrictions on the side chain interactions between the switch-2 cluster and its supporting structure. In fact, the cluster seems to be especially suited for gliding because of an hydrophobic patch at its inner surface that faces an extended region of predominantly hydrophobic residues at the central $\beta$-sheet.

### 3.2 Fungal kinesin-1

Compared to animal kinesin-1, conventional kinesins of filamentous fungi are about four times faster and show greater processivity (Steinberg and Schliwa, 1996; Xiang and Plamann, 2003). The motor domain of *Neurospora crassa* kinesin-1 (NcKin355, aa 1-355) differs from that of rat and human kinesin-1 in several distinct features (Song et al., 2001). Most remarkably, loop L11 is ordered (though the B-factors are high) and visible. It comprises a helical part, $\alpha 4a$, that looks like an imperfect extension of the switch-2 helix $\alpha 4$. The conformation of the switch-2 cluster resembles that of rat and human kinesin crystallized with
lithium sulfate, although the NcKin construct has been crystallized using PEGMME 2000 as the precipitant. Thus, it seems that stiffening of loop L11 favours the "permissive" state, i.e. the conformation that allows docking of the neck linker to the core. However, the neck linker (aa 329-342 in NcKin) is only "semi-docked": as expected, the binding pockets that are under control of the switch-2 cluster are occupied by residues Ile330 and Asp332 of the N-terminal half of the neck linker (β9), yet this does not result in binding of the second half, as it does in rat and human kinesin-1. Instead, the C-terminal half of the neck linker as well as the neck region that follows assume a random coil conformation without defined secondary structure and without much direct contact to the core.

Other conformational differences occur in the switch-1 region (α3-L9-α3a): helix α3 of the NcKin structure is extended by five amino acids at its C-terminus compared to rat kinesin-1 (2KIN). This is at the expense of loop L9 and helix α3a, the structural elements that link the end of helix α3 to strand β6 of the central β-sheet (cf. Table I). In rat kinesin-1 these elements comprise 12 – 14 amino acids in total, and α3a is α-helical with two full turns (7 aa, MNEHSSR; here, bold letters highlight the switch-1 motif). In the NcKin structure, the amino acids that correspond to loop L9 in the 2KIN structure are all incorporated into helix α3, and the remaining peptide chain (MNEHSSR in NcKin) is stretched in order to span the distance between α3 and β6. Consequently, L9 of the NcKin structure consists of amino acids MNQE, while the remaining residues (SSR) form a short 310-helix (a helix type that is more extended than the usual α-helix). Thus, it seems that the peptide stretch that connects helix α3 to the distant and roughly antiparallel strand β6 contains a short sequence of amino acids that is prone to form an extension of helix α3, and another short sequence that may form an extension of α3a, but only one of these two possibilities can be realized at any time because of geometric restraints. This could result in a bistable, "switch-like" behavior of the switch-1 region. In the 2KIN conformation of the switch-1 region, the nucleotide binding pocket is partially occluded by L9-α3a residues, while it is easily accessible in the NcKin conformation. Thus it appears that the NcKin conformation facilitates nucleotide exchange and speeds up the ATPase cycle.

3.3 Other N-terminal motors

3.3.1 Monomeric Kinesin-3

Mouse Kif1A is a member of the kinesin-3 (formerly Unc104/KIF1) family of kinesins. Crystal structures of the Kif1A motor domain (Kikkawa et al., 2001) have received much attention for two reasons: Firstly, the Kif1A construct (a chimera of the Kif1A head with an engineered, short neck linker where six residues around β9 have been replaced by corresponding residues of mouse kinesin-1) has been crystallized in several forms mimicking various intermediate states of the ATPase cycle. And secondly, Kif1A is a member of the kinesin-3 family of “monomeric” kinesin-like proteins. This raised the question about a variant mechanism of processive movement. Most models for kinesin-1 movement assume a close coordination of the activities of two heads: at any time, at least one head is tightly bound to the microtubule, preventing rapid detachment and diffusion off the track. In the case of Kif1A, a positively charged insert of eleven amino acids in Loop L12 with a polyllysine motif ("K-loop") may anchor the motor to the microtubule by electrostatic interaction with the negatively charged C-termini of α- and β-tubulin ("E-hook"). This could compensate for the lack of a second motor domain (Okada and Hirokawa, 2000; Tomishige and Vale, 2000). On
the other hand, there is growing evidence for reversible dimerization of Kif1A \textit{in-situ} (Klopfenstein et al., 2002; Tomishige et al., 2002). It appears now that Kif1A uses a mechanism for processive movement that is similar to that used by kinesin-1. A reversible monomer-dimer transition seems to be used as a method to regulate Kif1A's motor activity. This has been confirmed by cryo-electron microscopy showing that the neck of Unc104 protein (the \textit{C. elegans} homolog of Kif1A) consists of two helical segments connected by a flexible hinge region that form an intramolecular coiled coil in the monomer. Under certain conditions the Unc104 neck switches from the self-folded state of the monomer to a true dimeric state by formation of an intermolecular coiled coil (Al-Bassam et al., 2003).

Superposition of the structures with AMPPCP and ADP bound to the active site (Kikkawa et al., 2001) showed little changes in the catalytic core. The conserved serine S215 in switch-1 (corresponding to RnKHC-S203) moves by 1.2 Å around the γ-phosphate, and the conserved glycine G251 of the switch-2 motif (corresponding to RnKHC-G235) moves 0.6 Å towards the nucleotide (AMPPCP form compared to ADP form). In rat kinesin-1 (2KIN) the positions of the corresponding amino acids are nearer to the ADP form than to the AMPPCP form of Kif1A, in accordance with the fact that rat kinesin – as most of the other kinesin structures – was crystallized with ADP.

Although the two crystal forms do not differ very much in the immediate vicinity of the nucleotide, there are considerable changes in more distant regions, especially in the switch-1 and switch-2 regions. In the ADP form, the peptide stretch between helix α3 and the switch-1 motif at the entrance to β6 is roughly similar to the corresponding residues (loop L9 and α3a) of rat kinesin-1; the secondary structure in Kif1A is less well defined but still predominantly helical. In the AMPPCP form this region transforms into a β-hairpin. The switch-2 cluster adopts two conformations that closely resemble the conformations found in rat and human kinesin-1 crystallized with lithium sulfate (permissive for docking of the neck linker; AMPPCP form) and with PEG (obstructive to neck linker docking; ADP form). Accordingly, the engineered neck linker of the Kif1A construct is undocked and disordered in the ADP form, and partially docked in the AMPPCP form. In the ADP form, the switch-2 helix is elongated by two turns at the N-terminal end. This leads to substantial shortening of loop L11 compared to the AMPPNP form and to a considerable shift of the helix towards the neck linker binding site, thus preventing docking of the neck linker. This is remarkable in view of the Nkin structure where stiffening of loop L11 and elongation of the switch-2 helix by α4a goes along with the "permissive" conformation of the switch-2 cluster (cf. Table I).

Recently, three more crystal structures of the same Kif1A motor construct have been determined (Nitta et al., 2004), representing different intermediate states of the ATPase cycle: the motor domain in the complex with AMPPNP (PDB-ID: 1VFV and 1VFW, these two structures of different resolution are virtually identical), with ADP and aluminum fluoride (PDB-ID: 1VFX), and with ADP and vanadate (PDB-ID: 1VFZ). In the light of the new structures, the AMPPCP structure (Kikkawa et al., 2001) is interpreted as an early, "preisomerization" or "collision complex" (Nitta et al., 2004), in accordance with the observation of only minor changes in the nucleotide binding pocket. A better representation of the "prehydrolysis" state is the structure of the AMPPNP complex. Before isomerization (AMPPCP state), the linker between helix α3 and β6 (including the switch-1 motif) assumes a tight β-hairpin conformation and the side chain of the conserved serine S215 points away from the γ-phosphate. In the prehydrolysis state (AMPPNP) the switch-1 region is partially melted (cf. Table I) allowing the conserved serine to rotate and to approach the γ-phosphate. Likewise, the conserved glycine G251 of the switch-2 motif approaches the γ-phosphate by another 0.6 Å, compared with the AMPPCP structure.
The complex with ADP and aluminum fluoride is thought to resemble the early ADP.Pi state immediately after hydrolysis, while the complex with ADP and vanadate may represent a late state in which the phosphate (mimicked by vanadate) has moved quite a long distance (15 Å) from the active centre to the surface of the motor domain. There it is fixed by two hydrogen bonds to the solvent exposed tips of the switch-1 loop region (L9) at one side and the switch-2 loop (L11) at the other side.

In the early posthydrolysis state (with ADP and aluminum fluoride) the switch-1 region folds in a way that resembles that of the preisomerization state (AMPPCP), although the details are a bit different (cf. Table I). Furthermore, there is an overall shift of the switch-1 region of the order of 1 Å away from the nucleotide. Similarly, the switch-2 motif and the switch-2 cluster resemble the corresponding structures in both the AMPPCP and the AMPPNP state. The major change is a relative displacement along the axis of helix α4, with the position of the cluster being nearest to the nucleotide in the prehydrolysis (AMPPNP) state and farthest from it in the posthydrolysis (ADP.AlFx) state. In the preisomerization (AMPPCP) state, the position is intermediate. The displacement between the two extreme states is about 1.5 Å.

The "late ADP phosphate" state as represented by the complex with ADP and vanadate (PDB-ID: 1VFZ) is remarkable as it is the structure with one of the longest α4 helices in all known kinesin structures, and the only structure of Kif1A with loop L11 completely resolved. This is even more notable as the residues which are disordered and invisible in the other Kif1A complexes are surrounded by a structure similar to that observed in the complex with ADP (PDB-ID: 1I5S; Kikkawa et al., 2001). In fact, all the visible part of the ADP structure fits almost perfectly to the ADP.Vi structure (rms deviation for 324 Cα atoms: 0.36 Å). Thus, it seems unlikely that the structure observed with ADP and vanadate be really unique for the "late ADP phosphate complex" and different from that of the structure with only ADP. Rather, the same conformation of loop L11 and helix α4 could also prevail in the ADP state, but with a certain fraction of the molecules in the crystal assuming one or several other conformations, thus reducing the electron density for the dominant conformation below visibility in the most variable regions. The presence of the vanadate ion at the surface of the molecule could decrease this type of disorder and increase the number of the molecules of the predominant conformation, thus raising the electron density above the level of discrimination. Whether trapping of the cleaved γ-phosphate at the surface of the molecule (similar to vanadate) is of physiological relevance is hard to decide solely on the basis of the crystal structures. The main argument for the "late ADP phosphate state" being functionally significant draws on measurements of the (apparent) equilibrium dissociation constants for Kif1A binding to microtubules in the presence of different nucleotides (Nitta et al., 2004). These experiments suggest that the Kif1A cycle includes a state of very low affinity to microtubules. According to the authors, the "late ADP phosphate" state could be a good candidate for this "actively detaching state".

### 3.3.2 Tetrameric kinesin-5

The motor domain of human Eg5 (HsKSP), a member of the kinesin-5 (formerly BimC) family shares more than 40% identity with the kinesin-1 motor domain. The overall structure of a HsKSP construct of the first 368 amino acids (including 10 aa of the class-specific neck linker) complexed with ADP is very similar to the structure of kinesin-1 (PDB-ID: 1II6; Turner et al., 2001). Major differences are (1) an extension of the β-hairpin β1b-L2-β1c in the N-terminal lobe ("L2 finger") due to an insert of eight amino acids, (2) an enlargement of loop
L5 between \( \alpha_{2a} \) and \( \alpha_{2b} \) by another insert of eight amino acids, (3) an elongation of loop L10 between \( \beta_6 \) and \( \beta_7 \) at the tip of the core domain, and most remarkably (4) a novel conformation of the neck linker: although the switch-2 cluster is in the "obstructive state" (very similar to human kinesin-1 crystallized with PEG) and docking of the neck linker is prohibited, the HsKSP neck linker is ordered and well defined in the crystal structure. It is almost straight but without special secondary structure, and it extends roughly perpendicular to helix \( \alpha_6 \). This unusual conformation is stabilized by interactions of conserved residues within the neck linker and within the N-terminal lobe (Turner et al., 2001). Family specific residues were also identified in the region that is involved in regular docking of the neck linker and, therefore, it was anticipated that the neck linker adopts the normal "docked" conformation (roughly in line with \( \alpha_6 \)) when HsKSP switches to the "permissive" state.

Information about the plasticity of the motor domain can be obtained by comparing the two crystallographically nonequivalent molecules of the HsKSP crystal (PDB-ID: 1II6). The most remarkable difference pertains to the length of the switch-2 helix \( \alpha_4 \), which differs by ten amino acids (~3 turns), most of them at the N-terminal end (9 aa). This goes along with a considerable shortening of the disordered (invisible) loop L11. Interestingly, in the case of HsKSP the variability of \( \alpha_4 \) has little effect on the position and orientation of the common part of the switch-2 helix and the entire switch-2 cluster. Other, fairly moderate differences are restricted to the L2 finger and the loop L5 at the surface. The rms difference of the \( C_\alpha \) positions after superposition of 318 amino acids (out of a total of ~340 residues located in the crystal structure, disregarding the variable regions) is 0.65 Å. It should be noted that the high similarity of the two molecules includes the neck linker, which is well defined and structured in both molecules.

While the conformation of the switch-2 cluster (apart from the variable length of \( \alpha_4 \)) of human KSP corresponds to that of human kinesin-1 in the "obstructive state" (PDB-ID: 1BG2), the switch-1 region (\( \alpha_3 \) and the linker between \( \alpha_3 \) and \( \beta_6 \), including the switch-1 motif) is quite different from human and rat but very similar to that of the fungal kinesin-1 NcKin (PDB-ID: 1GOJ). Helix \( \alpha_3 \) is longer by 1-2 turns at the C-terminal end at the cost of the \( \alpha_3-\beta_6 \) linker. The shortened linker is stretched into a rather straight conformation with helix \( \alpha_3a \) restricted to three amino acids and transformed into a short 3\(_{10}\)-helix (SSR of the switch-1 motif). A comparison of the sequences and the secondary structure assignments (Table I) suggests that the conformations of the switch-1 region in human KSP and fungal kinesin-1 on one hand, and human kinesin-1 on the other hand represent two possible and thermodynamically significant (i.e. not singular) states. These conclusions are further substantiated by a recent structure of human Eg5 complexed with the small, class specific antimitotic drug monastrol (Yan et al., 2004). The ligand binds to a pocket formed by loop L5 and the N-terminus of \( \alpha_3 \), close to the nucleotide binding site. Loop L5, which is one of the loops with the highest B-factors in the structure without monastrol, adopts a rigid conformation by binding of monastrol (induced fit). In spite of the vicinity to the P-loop, the drug has little effect on the nucleotide and the core \( \beta \)-structure. Nevertheless, helix \( \alpha_3 \), which is involved in ligand binding, moves about 1 Å in axial direction. This is accompanied by a rearrangement of the linker between \( \alpha_3 \) and \( \beta_6 \) towards a more \( \alpha \)-helical conformation (YSSR) of the switch-1 motif.

The main difference between the structure of the ternary complex of KSP with ADP and monastrol (Yan et al., 2004) and the complex with ADP alone (Turner et al., 2001) is that switch-2 in the complex with monastrol adopts a permissive conformation, similar to rat and human kinesin-1 (PDB-ID: 2KIN, 1MKJ). Consequently, the neck linker binds to the
docking site. As for the switch-1 region, the conformation of the switch-2 cluster is even more similar to that of the fungal kinesin-1 (PDB-ID: 1GOJ). It is remarkable that binding of monastrol to a site antipodal to the switch-2 cluster has such a strong effect on distant structural elements at the periphery, while the central β-sheet and the nucleotide binding site remain virtually unaffected.

Another difference between the ternary and the binary complex is a bent conformation of the L2 finger in the complex with monastrol. Furthermore, the pointed tip of the core structure (β6–L10–β7) has a variable conformation in the structure with monastrol. In the two molecules of the asymmetric unit, the tip bends and moves by about 7 Å. The conformation observed without monastrol is intermediate to the two conformations of the ternary complex. The double conformation of L10 is clearly a crystal packing effect, however, it shows that the tip of the core domain is rather flexible.

Binding of monastrol to loop L5 induces a conformational change of this loop and makes it more rigid. This allows two molecules to form a close-packed dimer of two-fold non-crystallographic symmetry (NCS) with loops L5 of both molecules at the common interface. This NCS dimer would probably not be stable without monastrol because of the intrinsic flexibility of loop L5. As a consequence, crystal packing of the ternary complex is totally different from that of the binary complex. This is most obvious in a curved, four-stranded, intermolecular β-sheet that is formed in the monastrol structure by antiparallel interaction of the L2 fingers of two molecules. Thus, bending of the L2 finger is also a crystal packing effect. It is, however, not clear to what extent changes in the switch regions and the neck linker may also be ascribed to crystal packing effects.

### 3.3.3 Kinesin-7

The centromere-associated protein CENP-E (a member of the kinesin-7 family) is another kinesin-related molecule with an N-terminal motor domain. CENP-E from *Xenopus* has been reported to be essential for the alignment of chromosomes during metaphase and to support slow plus end directed motion *in vitro*, suggesting that its function is to tether chromosomes to the ends of dynamically growing and shrinking spindle microtubules (Wood et al., 1997). The crystal structure of a human CENP-E construct including the motor domain and the neck linker (aa 2-342; PDB-ID: 1T5C, to be released 04-May-2005; Garcia-Saez et al., 2004) shows structural features characteristic for plus-end directed motors. In fact, the CENP-E motor domain is largely superimposable with the human conventional kinesin motor domain. Remarkably, the switch-2 cluster is in the permissive conformation as observed for rat kinesin-1 (PDB-ID: 2KIN) and human kinesin-1 crystallized with lithium sulfate (PDB-ID: 1MKJ), although the CENP-E motor construct has been crystallized with PEG as the precipitant. Accordingly, the neck linker adopts a docked conformation and forms two short β-strands that interact with the central β-sheet in the same way as they do in the case of rat conventional kinesin. Major differences between CENP-E and conventional kinesin are found in the N-terminal lobe and at loop L10 between β6 and β7, at the tip of the motor domain. Human CENP-E has a five residue insert in loop L10 compared to conventional kinesin. Due to the additional residues, the tip of the CENP-E motor domain is more flexible and, thus, invisible in the crystal structure. At the N-terminal lobe, helix α0 of conventional kinesin is replaced by an extended loop without special secondary structure assignments.
3.4. **Kinesin-14 (C-type motors)**

Structures of the motor domains of three different kinesin-14 proteins, kinesins with C-terminal motor domain, have been determined so far: (1) the *Drosophila* non-claret disjunctional gene product (DmNcd), (2) the yeast kinesin-like nuclear fusion protein (ScKar3), and (3) the kinesin-like calmodulin binding protein from potato (PoKCBP).

### 3.4.1. Ncd

C-type kinesins have a class-specific neck at the N-terminal side of their motor domain. In the case of DmNcd, the neck forms a continuous α-helix with the less conserved stalk. Constructs of the motor domain with a sufficiently long part of the neck dimerize by formation of a coiled coil. The first structure of an Ncd motor domain (Sablin et al., 1996) was that of a monomeric construct (aa 335-700) including only part of the neck (aa 328-348). Later, several crystal structures of dimeric constructs have been published. These constructs dimerize by coiled coil interaction of the neck/stalk helices. The PDB database now contains three crystal structures of dimeric DmNcd constructs that differ mainly by the overall conformation (symmetry) of the dimers, while the individual motor domains are very similar in all known structures.

The first structure of a dimeric DmNcd construct (PDB-ID: 2NCD, aa 281-700; Sablin et al., 1998) turned out to be perfectly symmetric (by contrast to dimers of rat kinesin-1): the two molecules of a dimer are related by a crystallographic two-fold axis. The symmetry axis coincides with the axis of the coiled coil (Fig. 3c). A similar construct (PDB-ID: 1CZ7, aa 295-700; Kozielski et al., 1999) crystallized in a different space group with two dimers per asymmetric units. While none of the dimers has a proper two-fold symmetry, their conformation is not far from that. The deviation from the perfect symmetry can be described by 2° and 10° torsions, respectively.

Recently, a novel conformation of dimeric Ncd has been found in crystals of a point mutant (DmNcd-N600K, PDB-ID: 1N6M; aa 293-700; Yun et al., 2003). As crystals of the same type could be produced from the corresponding wild-type construct – although at lower resolution – it is presumed that the new conformation represents a state that plays a significant role in the mechanochemical cycle of Ncd. The new conformation can be obtained from the symmetric conformation by a 75° rotation of one head, leaving the other head and the neck coiled coil untouched (Fig. 3d). The pivot point is G347 at the transition from the neck to the globular motor domain. The axis of rotation is perpendicular to the coiled-coil (i.e. the former symmetry axis).

In spite of the gross conformational differences between the Ncd dimers, there are only minor differences between the individual motor domains. The overall fold of the motor domain is very similar to that of kinesin-1 and other N-type motors. Major differences are: (1) The N-terminal lobe of Ncd is enlarged (+9 aa) compared to rat kinesin-1. Additional residues are located between β1b and β1c (the "L2 finger"). This, however, does not result in a simple elongation of the β-hairpin as in HsKSP and in M-type motors (see below). In fact, the "tip" of the "L2 finger" is rather broadened and forms a short α-helix. (2) Loop L5, the insert in the P-loop helix α2, is quite short (~8 residues compared to 12 in rat kinesin-1), due to three residues that are missing in the primary structure of DmNcd. (3) Switch-1 helix α3 is short and loop L9, the linker between α3 and β6 that includes the switch-1 motif, is rather long, but
without any defined secondary structure. In a superposition of all known Ncd motor domains, loop L9 is the region with the highest variability. (4) Helix α4 and the entire switch-2 cluster is in the "obstructive" conformation (similar to human kinesin-1 crystallized with PEG). The adjacent β5-L8 lobe follows the movement of the switch-2 cluster. (5) According to the obstructive conformation, the C-terminal residues beginning with the end of helix α6 (about 30 aa) are disordered and invisible. The significance of this, however, is unclear so far since the neck linker is N-terminal to β1, and it is ordered and "docked" to the core via multiple interactions with helix α1 and loops L6 and L10 at the tip of the core domain. The neck has also contact to K640 at the start of β8, the strand adjacent to β1. K640 is next to the flexible hinge (loop L13) of the switch-2 cluster and to the principal docking site for the neck linker in N-type motors. It seems that simultaneous binding of Ncd's N-terminal neck and the motor domain's C-terminal extension (which may be considered as a "pseudo-neck"; disordered aa 671-700) to the docking site is excluded by steric hindrance. Thus, it might be hypothesized that the real neck and the C-terminal pseudo-neck compete for binding to the core domain. Then, switch-2 movement could play a critical role in driving the conformation between a state with the neck "docked" (as observed in the Ncd structures) and another state with the C-terminal peptide docked and the real neck displaced. This hypothesis is strongly supported by the structure of PoKCBP that has recently been solved (Vinogradova et al., 2004; see below).

In the N600K mutant, the switch-2 helix α4 of the rotated head moves towards the switch-2 motif by attraction of the mutated residue at the proximal end of α4 (N600K) toward the conserved switch-1 arginine R552, but without substantial changes at the distal end next to the presumed docking site. (Interestingly, attraction towards the switch-1 motif leads to a partial unwinding of helix α4.) Thus, both heads of the asymmetric dimer retain an obstructive conformation and the C-terminal residues beyond helix α6 remain disordered and invisible. Nevertheless, the small change in the switch-2 conformation is accompanied by a large-scale (ca. 75°) rotation of the neck helix relative to the head. Another consequence of the rather limited rearrangements in the switch regions is that the nucleotide (ADP) seems to be less tightly bound to the rotated head compared with the other head, as indicated by a reduced electron density of the adenosine moiety.

3.4.2. Kar3

By contrast to Ncd, native Kar3 is a heterodimer with a single motor domain associated to either Cik1 or Vik1 (Mackey and Gilbert, 2003). Kar3 is involved in spindle assembly and integrity. It is a slow minus end directed motor and seems to have microtubule destabilizing activity. The structure of the ScKar3 motor domain has been solved in two variants that differ in length by eleven amino acids at the N-terminus (PDB-ID: 3KAR, aa 383-729; Gulick et al., 1998 and PDB-ID: 1F9T, aa 372-729; Yun et al., 2001). In addition to that, structures of three point mutants of the shorter construct have been determined (PDB-ID: 1F9U, 1F9V and 1F9W; Yun et al., 2001).

The Kar3 motor domain is very similar to that of other kinesins and especially to Ncd. Like Ncd, Kar3 has an N-terminal lobe considerably larger (+12 aa) than that of kinesin-1. The predominant effect is that the β1a,b,c sheet appears uniformly elongated. Neither the loop between β1b and β1c nor the loop before the β1a,b,c sheet is helical as in Ncd and kinesin-1. The loop L5 insert between α2a and α2b is even shorter than that of Ncd (-5 aa). Another difference on the sequence level consists in a 10-11 amino acids increase in length of the β5-L8 lobe compared to Ncd and kinesin-1. On the conformational level, this is accompanied by a significant relocation of the lobe towards the tip of the motor domain, associated with a 90°
rotation (untwisting) of the β5a-β5b hairpin. This leads to a considerable approach of β5b to the central β-sheet, closing the gap between the β5-L8 lobe and the core domain. Remarkably, in all Kar3 structures (wild-type and mutants), the root of the β5-L8 lobe is partially disordered (residues 532-545, next to the site where the lobe is attached to the central β-sheet).

Other conformational differences between Kar3 and Ncd, that vary to a certain extent between the Kar3 structures presently available, are found in the switch regions. In the wild-type structures, helix α3 moves and tilts away from the central β-sheet and bends towards the nucleotide binding site. The linker between helix α3 and strand β6 containing the switch-1 motif is partially α-helical (α3a), similar to kinesin-1. Compared to kinesin-1 the tilting of helix α3 is even more pronounced, whereas position and orientation of the short linker helix α3a are virtually unchanged. To accommodate the large tilt angle, loop L9 between α3 and α3a moves by 18 Å (Cα distance between ScKar3-Thr587 and RnKHC-Ala194). The switch-2 cluster has a conformation similar to Ncd ("obstructive"), however, the switch-2 helix α4 is longer by nine (3KAR) or six (1F9T) amino acids at the end proximal to the switch motifs. The variation of length of α4 in the wild-type structures differing by eleven amino acids at the N-terminus suggests that residues at the proximal end of the switch-2 helix and the adjacent loop L11 are prone to reversible melting, and the exact conformation may depend on subtle details that cannot be controlled easily.

Among the Kar3 point mutants, the N650K mutant (within helix α4, close to the invariable core of the switch-2 helix; PDB-ID: 1F9U) is almost identical to the wild-type construct. Only small, local differences are observed at the site of the amino acid exchange. Nevertheless, the mutation has marked effects on the kinetics of the construct (Yun et al., 2001): the mutant binds tightly to microtubules, independent of the nucleotide state; it displays no motor activity in the microtubule gliding assay; and its ATPase activity is not stimulated by microtubules. It has been suggested that the N650K exchange disrupts the communication pathway between the microtubule binding site (switch-2 cluster) and the nucleotide binding site (switch motifs, primarily switch-2), and therefore, this mutant has been called "decoupling mutant".

The "salt bridge mutants" R598A (conserved arginine of the switch-1 motif; PDB-ID: 1F9V) and E631A (conserved glutamine of the switch-2 motif; PDB-ID: 1F9W) both have marked functional and structural effects. In some of the kinesin structures a salt bridge between the conserved arginine in switch-1 and the conserved glutamine in switch-2 has been found, and it has been suggested that this salt bridge plays an important role in the coordination of the switch regions. Disruption of the salt bridge by either mutant leads to the loss of microtubule stimulated ATPase activity similar to the decoupling mutant. Interestingly, the switch-2 mutant (E631A) binds tightly to microtubules irrespective of the nucleotide, whereas the switch-1 mutant (R598A) has only weak affinity to microtubules. Surprisingly, the main structural effect of the switch-2 mutant is in loop L9 of the switch-1 region which is largely disordered. The short helix α3a is further reduced to a 3_10-helix of minimal size (three residues, SSR of the switch-1 motif). By contrast, the switch-1 mutant displays strong effects in both switch regions. The switch-1 region is largely disordered: helix α3 is shortened (at the C-terminal end) and partially distorted, its orientation/position is more similar to Ncd and kinesin-1 than to the other Kar3 structures. The linker between α3 and β6 is invisible except for the residues of the switch-1 motif immediately N-terminal to β6. The destabilizing effect on the switch-1 region is accompanied by stabilization of loop L11 between switch-2 motif and switch-2 cluster. The R598A mutant of ScKar3 is one of three structures so far, with loop
L11 ordered and visible. It should be noted that stabilization of loop L11 has virtually no effect on the conformation of the switch-2 cluster. Helix \( \alpha 4 \) is extremely long and its conformation relative to the core is "obstructive" as in the Kar3 wild-type structures. Accordingly, the loop is restricted to nine amino acids (between the end of the switch-2 motif to the first amino acid of helix \( \alpha 4 \)). Three of them in the center of the loop (V635-S636-Q637) form a short \( 3_10 \)-helix antiparallel to helix \( \alpha 4 \). This is remarkably similar to the structure of PfMCAK (Shipley et al., 2004; see below) where loop L11 and \( \alpha 4 \) form a full-fledged helix-loop-helix motif.

The low affinity of the R598A mutant to microtubules has been explained by the rigidity of loop L11 (Yun et al., 2001): strong binding may require a flexible loop that is able to accommodate to the microtubule surface. If this assumption is true the helix-loop-helix conformation of L11-\( \alpha 4 \) seen in the Kar3 mutant and in PfMCAK should be different from the conformation induced by strong binding to microtubules (in the presence of ATP). According to the model proposed by Rice and coworkers (Rice et al., 1999) this unknown conformation should be accompanied by the transition of the entire switch-2 cluster from the obstructive to the permissive state, leading to docking of the neck linker. How does the NeKin structure fit to these assumptions? In NeKin the loop folds in a way faintly similar to the Kar3 mutant. It is much larger (approx. 15 aa), meaning that the switch-2 helix is relatively short, and it displays no well-defined secondary structure, if one neglects \( \alpha 4a \), the short \( 3_10 \)-helix defined in the NeKin structure. Helix \( \alpha 4a \) should not be confused with the short antiparallel helix seen in the Kar3 mutant. It should rather be considered as a part of the switch-2 helix. Thus, the switch-2 helix in NeKin is rather short (compared to Kar3) and kinked, yet it is in the permissive orientation and allows docking of the neck linker. It seems, that loop L11 of the NeKin structure is in an intermediate state that shares features observed in the structure of the Kar3 mutant as well as features anticipated for the tightly bound microtubule complex.

### 3.4.3. KCBP

Recently, the motor domain structure of a kinesin-like calmodulin binding protein from potato (PoKCBP), another member of the kinesin-14 family, has been reported (residues 884-1252, PDB-ID: 1SDM; Vinogradova et al., 2004). The construct has been crystallized with PEG in the presence of 200 mM sodium phosphate. It is the first structure of a minus end directed motor domain that shows most of the C-terminal residues beyond helix \( \alpha 6 \) (aa 1209-1252) in a well-defined conformation. The C-terminal extension of the motor domain contains a calmodulin binding motif (aa 1209-1252) that forms an \( \alpha \)-helix, a short peptide sequence (the "neck mimic" according to Vinogradova et al.) that connects this helix to the end of \( \alpha 6 \), and a negatively charged sequence at the C-terminus, part of which binds to the microtubule binding surface of the core domain while the rest is disordered. Although the construct has ADP bound to the active site, the switch-2 cluster resembles that of Kif1A complexed with AMPPCP (presumed ATP state, "permissive" conformation). Strikingly, the "neck mimic" and the calmodulin-binding helix that follows assume a conformation very similar to the neck linker and neck helix in rat and human conventional kinesin crystallized with lithium sulfate: the neck mimic is docked and runs parallel to the edge of the core structure to the tip, where the calmodulin binding helix is attached at right angle to the neck mimic. The only difference is that the calmodulin binding helix points in the opposite direction compared to the neck helix of rat conventional kinesin. Unfortunately, the present KCBP structure cannot tell anything about the true neck since it was not part of the construct.
3.5. Kinesin-13 (M-type kinesin)

The characteristic feature of the kinesin-13 family is that members of this kinesin family have their "motor" domain surrounded by N- and C-terminal domains. Therefore they are also named internal kinesins (KinI), or M-(middle)-type kinesins. The main function of this class of kinesin-related proteins is to target to the ends of microtubules and to induce depolymerization. While it is not clear, whether these proteins display motor activity in a strict sense, it seems appropriate to include them into this review of motor proteins.

3.5.1. Kif2C

Ogawa and coworkers (Ogawa et al., 2004) have determined the crystal structure of the minimal construct of mouse Kif2C that preserves full microtubule destabilizing activity. The construct (aa S183-S585 of MmKif2C, + 7 His) comprises the catalytic core (aa R254-S585, from β1 to α6, including three additional residues at the C-terminus) as well as 70 residues of the family specific neck that is N-terminal to the core domain. This construct has been crystallized and solved with ADP (PDB-ID: 1V8J) and with AMPPNP (PDB-ID: 1V8K). There is no significant difference between the two crystal forms.

The catalytic domain of MmKif2C is not much different from motor domains of other kinesins. Variations from the structure of kinesin-1 are within the range covered by N- and C-terminal kinesin motors. Loop L9 in the switch-1 region is partially disordered. Switch-2 loop L11 is also disordered as in most other structures, yet helix α4 is quite long and straight. The switch-2 cluster adopts an "obstructive" conformation that would prevent docking of a C-terminal peptide stretch, if present. The most remarkable feature of the catalytic domain is an extension of the L2 finger by 13 additional residues between β1b and β1c, similar to Eg5, but even more pronounced. As the tip of this long β-hairpin contains three KinI conserved residues (K293-V294-D295) that are essential for microtubule depolymerization by Kif2C (Ogawa et al., 2004), this structure has been termed "KVD-finger".

The Kif2C structure also reveals structural features of the N-terminal neck. Though partially disordered (only ~30 of 70 neck residues are visible), the crystal structures show a sequence of 14 amino acids forming an α-helix surrounded by flexible regions that are not visible. Interestingly, this helix is roughly perpendicular to the microtubule-binding surface of the core domain. It has been proposed (Ogawa et al., 2004) that the neck helix serves a double function: it may prevent strong binding to the microtubule side wall, thus facilitating one-dimensional diffusion; once the molecule has reached the end of the microtubule, the neck helix may strengthen the interaction with a terminal tubulin subunit.

3.5.2 MCAK

The catalytic domain of the MCAK homolog of Plasmodium falciparum (named pKinI or PMCAK, PDB-ID: 1RY6; Shipley et al., 2004) has been solved at high resolution (1.6 Å). The crystallographic model corresponds to the core motor domain (residues 68-396). While this construct lacks the N-terminal neck, it still has microtubule depolymerizing activity. The construct was crystallized with high concentrations of sulfate. Interestingly, no nucleotide was
found in the nucleotide binding site. Instead of a nucleotide, a sulfate ion is bound to the P-loop at exactly the same position that is usually occupied by the β-phosphate. Notwithstanding the absence of a nucleotide, the nucleotide binding site shows little changes, if any, compared to kinesin structures with ADP in the active site. There is a 1 Å shift in switch-1 and switch-2 indicating a slight opening of the nucleotide-binding pocket. As a consequence, a hydrogen bond between D236 in the switch-2 motif (DLAGSE) and the conserved P-loop threonine (T99) is broken. However, all conserved amino acids that normally interact with the nucleotide have their side-chains in similar positions as ADP bound motor domains.

By contrast to Kif2C, loop L8 is partially disordered and seems to point in a direction opposite to the normal direction. On the other hand, both switch regions are ordered and completely resolved, including the "loop" between the switch-2 motif and helix α4, which forms a two-turn 3_10-helix in PfMCAK. This short helix is stabilized by hydrogen bonds between residues of the switch-1 motif and residues of the switch-2 loop that are conserved in members of the kinesin-13 family (S210-R242 and R211-D245). These two hydrogen bonds replace the salt bridge between switch-1 and switch-2 that is observed in other kinesin structures and that is considered essential for the communication between the microtubule binding site and the catalytic center (c.f. Kar3 mutants, salt bridge between R598 and E631, corresponding to R211 and E241 in PfMCAK).

4. Conformational switching in kinesin

4.1 Comparison with myosin

Myosins are actin-based motors. The reason for considering myosin in the context of microtubule motors is that the catalytic domains of myosin and kinesin share structural similarities indicating that both families use a similar mechanism for energy conversion. The structural relationship between these families suggests that both descend from a common ancestor, a primordial nucleotide binding protein (Kull et al., 1998).

Like kinesins, myosins form a superfamily of proteins with a growing number of myosin classes. Myosins are present in most, if not all eucaryotic cells and they serve functions in muscle contraction, cytokinesis, cellular locomotion, and actin-based, short-range transport of vesicles and organelles. Myosins are characterized by a heavy chain with a highly homologous, globular, ~80 kDa catalytic domain (motor domain). Most myosin heavy chains consist of an N-terminal motor domain followed by an α-helical light-chain binding domain (LCBD) and a C-terminal tail. For historical reasons, class II myosins, comprising striated muscle myosin-II as well as smooth-muscle and non-muscle myosin-II, are referred to as conventional myosins. Myosin-II consists of two heavy chains, each complemented by two light chains. With its coiled-coil tail domain myosin-II forms bipolar spindles as the active part of a contractile system.

More than 18 classes of myosins have been identified in different organisms, so far. Myosins of class V and class XI (the plant "class V") are most akin to kinesin as they are dimeric, plus end (barbed end) directed, processive motors used for membrane and particle transport along actin filaments. There is also a class of dimeric myosins with reversed motility (class VI). Myosin-I, the first class of "unconventional" myosins that have been identified, comprises monomeric motors with a basic tail that interacts by electrostatic interaction with the cargo.
The structure of the myosin-II motor domain has been determined in various nucleotide states by crystal structure analysis using constructs originating from diverse sources (chicken skeletal and smooth muscle myosin-II, as well as myosin-II from scallop and non-muscle myosin-II of the slime mold *Dictyostelium discoideum*). In addition, structures of unconventional chicken myosin-V (Coureux et al., 2003) and of the monomeric *Dictyostelium discoideum* myosin-IE (Kollmar et al., 2002) have been solved recently. The motor domain of all myosins determined so far are structurally very similar. The motor domain has an elongated shape. The actin binding site is located at one end, and it is split in two subdomains (the "50K upper" and "lower" domains) with a marked cleft in-between. Some of the solved structures include part of the α-helical LCBD originating at the opposite end of the motor domain. The orientation of the LCBD differs by 70° and more in various structures, thus underpinning the hypothesis, that the α-helical extension of the motor domain, stiffened by the light-chains, serves as a lever arm that amplifies conformational changes powered by nucleotide processing in the core of the motor domain (Holmes and Geeves, 2000). The swinging lever arm hypothesis for conventional myosin has strongly been supported by functional analysis of myosin heads with genetically modified LCBD (Uyeda et al., 1996) and constructs with artificial α-helical extension of the motor domain (Anson et al., 1996) showing that the velocity (i.e. the step size) is proportional to the length of the engineered lever arm. Furthermore it is even possible to reverse the direction of motility by redirecting the lever arm by 180°, which is formally equivalent to a motor with a lever arm of negative length (Tsiavaliaris et al., 2004).

As in the case of kinesin, the folding of the motor domain can be described as a central β-sheet that constitutes a supporting structure for the surrounding elements which, however, greatly exceed the peripheral structural elements of kinesin in size and are mostly α-helical subdomains. The nucleotide binding site is buried in the middle of the motor domain. It is structurally and topologically homologous to that of kinesin and comprises four characteristic motifs that are conserved in all myosins and that are similar to the nucleotide binding motifs described for kinesin, the adenine binding motif, the phosphate binding P-loop, and the two switch motifs. Comparison of myosin structures in different nucleotide states showed that the switch-2 conformations fall roughly into two classes, "open" and "closed", with the "open" conformation supposed to occur predominantly in the ADP or apo state (i.e. in the absence of a γ-phosphate), and the closed conformation occurring preferentially in structures with ATP or transition state analogs. Similarly, the lever arm (or the "converter domain" at the base of the lever arm, which is indicative of the lever arm position in structures of truncated motor domains) is either "up" or "down" (or more descriptive: in "pre-power stroke" or "post-power stroke" conformation). Intermediate states may be defined both for the switch-2 conformation (e.g. "half open") and for the orientation of the lever arm (e.g. stepwise rotation accompanied by successive release of Pi and ADP; Houdusse et al., 2000; Veigel et al., 1999). In any case, switch-2 and the lever arm are tightly coupled. The translation of the switch-2 movement into lever arm rotation is ascribed to a rotation of the converter domain that is driven by a long (approx. 40 Å) helix between switch-2 and the converter domain ("relay helix" or "switch-2 helix").

It has long been surmised that switch-2 movement and the concomitant swinging of the lever arm must be controlled by binding to and detachment from the actin filament to avoid futile consumption of ATP. However, direct evidence was lacking as near-atomic resolution crystal structures are necessarily obtained in the absence of the filament. Crystal structures of *Dictyostelium* myosin II (Reubold et al., 2003) and chicken myosin-V (Coureux et al., 2003) recently revealed, that the switch-1 motif can also exist in "open" and "closed" conformations. It has been inferred that switch-1 opening may be coupled to cleft closure and tight binding of
the myosin head to the actin filament. This conclusion is supported by electron microscopy (Holmes et al., 2003) and fluorescence spectroscopy (Conibear et al., 2003) studies of the actomyosin complex which show that the concepts derived from crystal structures of isolated myosin heads are indeed valid for the functional complex.

These observations show that there are striking similarities between myosin and kinesin motors suggesting that both use a similar if not the same mechanism for transforming ATP’s free energy into directed motion. There are, however, also notable differences, both in structure and kinetics which may reflect the diverse functions of the motors.

The myosin motor domain seems to be composed of structurally and functionally well separated building blocks. The actin binding site and the main mechanical actuators (converter domain and lever arm) are located at opposite ends of the head domain, with the catalytic center just in the middle between them. Binding to the actin filament competes with nucleotide binding: the nucleotide-free myosin head binds strongly to F-actin (rigor state) while binding of ATP to the catalytic center leads to detachment from the filament. This competition is due to a mechanical linkage of actin binding site and switch-1. Cleft closure is linked to switch-1 opening and vice versa. On the other hand, switch-2 movement is coupled to rotation of the converter domain and swinging of the lever arm. Communication between actin binding site and lever arm is both mediated and controlled by the catalytic center. It seems that proper coordination of the two poles of the head domain is based on a cooperative behavior of the switches, most probably due to the formation of a salt bridge between switch-1 and switch-2 when both are "open" or "closed", and breakage of the salt bridge in mixed states (Reubold et al., 2003). The important role of this salt bridge and the analogous salt bridge in kinesin (R204-E237 in rat kinesin-1) has been demonstrated by kinetic and structural studies of single and double mutants (Klumpp et al., 2003; Onishi et al., 1998; Yun et al., 2001).

In contrast to myosin, attempts to solve kinesin structures in different nucleotide states had rather limited success so far, and in the few crystal structures with an ATP analogue or without nucleotide, the observed effects on the catalytic center are small. Nevertheless, it is generally assumed by analogy to myosin, that nucleotide processing, microtubule binding, and force generation are coordinated by means of some conformational changes in switch-1 and switch-2. However, there are obvious differences to myosin. (1) By the sheer size of the motor domain, the filament binding site and the mechanical actuator are not well separated from each other. This implicates the possibility of direct interactions, which may not easily and exclusively be controlled by the catalytic core. (2) Main chain connectivity and spatial vicinity of switch-2 and the main microtubule binding site (the "switch-2 cluster") strongly indicate, that filament binding is directly linked to switch-2 movement, not to switch-1 movement as in myosin. (3) The switch-2 cluster with helix α4 serves two distinct functions: one in microtubule binding and another in controlling kinesin`s supposed mechanical actuator (the neck linker). By contrast to myosin, these two functions seem to be coupled in a single chain of actions. (4) The role of switch-1 in kinesin is quite elusive. It seems that in kinesin switch-2 plays a dominant role in controlling both filament binding and force generation. However, there is growing evidence, that switch-1 movement and the conformational variability of the switch-1 region (α3-loop9-α3a) are coupled to the β5-L8-lobe, which is also involved in microtubule binding (Ogawa et al., 2004). It seems plausible, that rigid body movement of a single binding site (the switch-2 cluster) would not change the affinity too much, but only result in repositioning of the motor domain (cf. Kikkawa et al., 2001). To change the affinity, it would be more effective to change the spatial arrangement of two (or more) binding sites. (5) Perhaps the most important difference between kinesin and myosin is
that in kinesin the main microtubule binding site together with the mechanical actuator on one side, and the switch-2 that should control them are largely decoupled according to the crystal structures, as seen in the occurrence of permissive and obstructive conformations without noticeable changes in the catalytic center. This is due to loop L11 which is disordered in most crystal structures, but may assume a rigid conformation when the motor domain attaches to the microtubule surface. This means, that kinesin assumes a fully functional form only while it is bound to the filament.

4.2 Nucleotide binding, switches I and II, and conformational relays

According to the "walking model" for processive motion of kinesin-1, switch-2 induced transition from the "obstructive" to the "permissive" state in one head allows binding of the neck linker to this head in a zipper-like manner, and by this the second (trailing) head is repositioned toward the plus end of the microtubule, thus increasing the probability for the second, now leading head to bind in forward direction. By contrast to the swinging lever arm model of myosin, the movement of kinesin may be considered as a diffusive process that is biased by nucleotide controlled docking of the neck linker (thermal ratchet). At any time, at least one head is attached to the microtubule, which enables single molecules of conventional kinesin to function as "porters" (Leibler and Huse, 1993). Communication between the two heads is probably mediated by mechanical strain that is introduced by simultaneous binding of both heads to adjacent binding sites. Conventional myosin, on the other hand, has a low duty ratio, i.e. it is mostly detached from the actin filament. Productive motion is the result of a high number of myosins (assembled, for instance, in the muscle's thick filament) working in cooperation. The heads of a single myosin molecule seem to act independently, the main advantage of having two heads probably consists in increasing the chances to find a binding site on the actin filament.

For unconventional myosins and kinesins the mode of action may be different. Class V and class VI myosins and other dimeric myosins that function as single molecule transporters of organelles may use a mechanism more similar to that of kinesin-1. Conversely, the minus end directed kinesin Ncd seems to use its α-helical neck coiled coil, stabilized by interaction with one of its heads, as a rigid lever arm that performs a large scale rotation relative to the other head (Yun et al., 2003). Electron microscopy of microtubulus decorated with Ncd indicates that only one head of this dimeric, but unprocessive motor functions as an active, force-generating ATPase, suggesting a hopping type motion that combines a deterministic lever arm movement with a diffusive component (Wendt et al., 2002).

As a general picture resulting from this synopsis it appears that the different members of the myosin and kinesin protein families all combine a common switch-based ATPase mechanism with various transducing elements to convert chemical free energy into mechanical work. A characteristic feature of this type of chemo-mechanical nanomachines is the concerted action of conformational changes on extremely different scales in time and space: local (sub-nanometer scale) rearrangements in the catalytic center induced by virtually instantaneous (probably sub-picosecond) cleavage of ATP into ADP and phosphate drive large scale conformational changes (of the order of tens of nanometers) on a time scale of milliseconds. The combination of small and large-scale movements is intuitively described by the concept of switches. To account for the different time-scales, the existence of elastic storage elements has to be assumed that can match conformational changes occurring at different time scales. These elements should be able to store the energy set free by ATP hydrolysis and to release it slowly during the process of motion like the spring of a clock. The nature of these storage
elements may be as diverse as the types of motor proteins are. Comparison of the molecular structures, however, suggests that reversible melting of helices plays an important role in energy storage and conversion. In myosins, transitions of the relay helix and the SH1-SH2 helix between straight and bent conformations are to mention in this respect (Houdusse et al., 2000). For kinesins, reversible melting of helical regions is most evident in the observed length changes of the switch-2 helix, in the variability of the switch-1 region (helix α3 and the linker between this helix and the switch-1 motif), and possibly in reversible unwinding of the neck coiled coil.

5. Structures of kinesin-related domains

Compared with the structural knowledge about the kinesin core motor domain less is known about its non-motor parts or associated proteins. One exception is the α-helix (neck helix) preceding (as in kinesin-13 or –14) or following (as in kinesin-1) the motor domain. In rat kinesin-1, a short sequence of amino acids (T326-T338) links the neck helix to the motor core. The neck helix (aa A339-W370) is separated from the coiled-coil stalk by a stretch of amino acids with predicted random conformation (hinge, ~aa 371-410; Tripet et al., 1997), allowing the stalk to kink and swivel. The NMR structure of the peptide K357-D386, comprising the second part of the neck helix and the first part of the hinge, supports these predictions. The X-ray and NMR structures agree well in the range between residues K357 and W370. Beyond this tryptophan there is no electron density in the X-ray structure although the crystallized construct comprises nine more residues. The NMR structure show many possible orientations in this region (Seeberger et al., 2000). In C-type kinesins (kinesin-14 family), the conserved neck is entirely helical and connects to the less conserved coiled coil of the stalk without any hinge region (Sablin et al., 1998). The neck helix of the middle type motor MmKif2C (kinesin-13 family) has a conformation very different from other kinesins as it points toward the microtubule surface, i.e. in a direction almost perpendicular to the neck helices found in N- and C-type motors (Ogawa et al., 2004). This may reflect its function as a microtubule depolymerising machine. In contrast, the neck helices of N- and C-type motors are more or less parallel to the microtubule surface when the motor binds to the microtubule lattice.

The structure of the globular tail domain of kinesin-1 is still unknown. It contains determinants necessary for folding into an inactive conformation (Stock et al., 1999). The structure of kinesin light chains (KLC, KAP) also has not been determined crystallographically so far. However, local sequence homologies to other proteins of known structure allow some predictions (Fig. 6; Mandelkow and Mandelkow, 2002). The C-terminal domain of the kinesin light chains (~320 aa) contains six tetratricopeptide repeats (TPR) probably involved in protein-protein interactions. The structure of these repeats can be modelled according to structures of other TPR containing proteins like protein phosphatase PP5 (PDB-ID: 1A17; Das et al., 1998). Each TPR domain comprises about 34 residues presumably folded into two antiparallel helices. The N-terminal part of the light chains (~250 aa) has regions predicted to be engaged in coiled coil interactions linking the light chains to the heavy chains. In the case of heterotrimeric kinesin-2, the two motor molecules are slightly different and they are associated with only one non-motor subunit (KAP). It is a largely α-helical protein containing 11 armadillo repeats, a motif first described in Drosophila armadillo protein, but also found in a lot of other proteins like β-catenin.
6. Dynein structure

Dyneins are microtubule based motors responsible for minus-end directed transport in eukaryotes. Unlike the other motor proteins, kinesins and myosins, dyneins are huge complexes consisting of one to three heavy chains (HC) of > 500 kDa and several intermediate (IC), light intermediate (LIC), and light chains (IC), some of them specific to dynein subclasses (Vallee et al., 2004). Only a limited number of dyneins are found in eukaryotes, most of them are integral parts of axonemes and cause bending of eukaryotic cilia and flagella by sliding of adjacent outer doublet microtubules. Cytoplasmic dyneins are involved in retrograde vesicle transport, mitosis, cell migration, maintenance of the Golgi apparatus, and many other processes. The heavy chains are responsible for ATPase and motor activity, while regulation and specificity for diverse cargoes are mainly due to the combination of HCs with accessory proteins. Notably, single dynein HCs are capable of processive motion along microtubules (Sakakibara et al., 1999; Sale and Fox, 1988; Vale and Toyoshima, 1989).

The dynein heavy chain consists of an N-terminal domain that contains IC interaction and HC oligomerization sites, and a motor domain with a chain of six so called AAA modules of 35-40 kDa each (ATPases Associated with different cellular Activities; Mocz and Gibbons, 2001; Neuwald et al., 1999) and another, supposedly globular domain of 150 to 450 amino acids (depending on the isoform) at the C-terminus (Fig. 7a). Between AAA modules 4 and 5, an insert of variable length appears, that is predicted to form an antiparallel coiled coil with a short microtubule binding motif between the helices. Dynein has four ATP binding sites: four of the six AAA modules contain Walker A and B motifs and bind ATP, modules 5 and 6 are degenerate and have lost their P-loop. ATP binding and hydrolysis by module 1 is absolutely essential for dynein's motor activity, as shown by mutagenesis of the Walker A motif (Eshel, 1995) and domain specific photolysis by UV irradiation in the presence of vanadate (Lee-Eiford et al., 1986). The role of the other nucleotide binding sites is less clear. Binding, but not hydrolysis of ATP to modules 2 to 4 seems also important and has probably regulatory functions.

By electron microscopy imaging, the dynein HC appears as a tripartite molecule, an elongated stem corresponding to the N-terminal part, a ring-like structure or hollow sphere (head) consisting of several globular subdomains, and a stalk of about 10 to 15 nm length, corresponding to the insert between AAA modules 4 and 5 (Vallee et al., 2004). There is no high resolution structure of dynein available so far. Present atomic level structural information is restricted to homology modelling based on the sequence similarity with the AAA class of chaperone-like ATPases (Mocz and Gibbons, 2001). According to this prediction, the AAA modules are α/β type structures with a core β-sheet of 5 strands surrounded by about 8 α-helices (Fig. 7b). Three or four of them form a C-terminal subdomain. The nucleotide binds in a cleft between the N- and the C-terminal domain. Comparison with other ATPases of the AAA family suggests that the modules form a hexameric ring with the N- and C-terminal subdomains of one module contacting the N-terminal subdomain of the adjacent module. The attractive feature of this model is the cooperativity that suggests itself by the geometry of interaction between neighbouring modules. A change of the angle between the subdomains of module 1 due to nucleotide processing should propagate throughout the ring and produce a substantial conformational change of the overall structure, thus explaining the communication.
between the ATPase site at one end of the ring and the change in microtubule affinity of the stalk at the opposite side.

The model derived by homology with other AAA type ATPases certainly needs some elaboration. In electron micrographs, the head often seems to consist of seven or eight globular domains. It has been suggested, that the C-terminal domain following the last AAA module (King, 2000) or part of the stem sequence preceding the first module (Fan and Amos, 2001) account for the additional density. According to a recent electron microscopy study (Burgess et al., 2004) the seventh subdomain is most probably due to the C-terminal amino acids. A large rearrangement of the head following release of hydrolysis products has not been observed. However, the analysis shows further details of the stem structure and motility. The stem consists of four sections: linker, neck, shaft and base. On product release, the linker performs a swinging movement from a position roughly diametrical to the ring to a position rather tangential. This movement can be likened to the docking of the neck linker proposed for kinesin. Docking of dynein's linker could be induced by relatively small conformational changes in the structure of the AAA modules resulting from nucleotide processing in module 1 and propagated throughout the ring. As in the other motor proteins, flexible elements must be present that can match the rearrangements occurring at different length and time scales. In the case of dynein, the neck within the stem, as well as the stalk seem to provide sufficient flexibility.

7. Summary and Outlook

In this review we mainly focussed on the structural comparison of microtubule based motor proteins. Twenty years after the first identification of kinesin as a force generating protein (Brady, 1985; Vale et al., 1985) many details are known about kinesin and various kinesin related proteins due to x-ray structure analyses of their motor domains, whereas the structure of dyneins as well as the structure of the non-motor domains of kinesin are still elusive. A comparison of the known structures, including additional information gained from myosin and G-proteins may help to elucidate the mechanisms that are at work in these motor molecules, which function as highly efficient chemo-mechanical energy converters. These efforts have already led to a certain understanding of the working principles at the level of plausibility, usually expressed in the form of "cartoon models" using switches, pistons, linkers, springs, and lever arms. Of course, our present understanding of motor proteins is not only based on structural data, but also on data obtained by many other methods like single molecule microscopy, biochemical and kinetic experiments, various types of spectroscopy, electron microscopy and so forth, although these methods have not been covered in this review to the extent they would deserve.

Substantial progress in our understanding of motor proteins can be expected from the extension of the presently available data about the structure of the isolated motor domain to the full length protein and its complexes with the filamentous track, the cargo adapter molecules, and any other cofactors; and from a combination of these structural (i.e. mostly static) data with molecular dynamics. X-ray crystallography seems rather restricted with regard to the analysis of large complexes, although this may change with the availability of new technologies, only to mention high throughput methods and the advent of new x-ray sources like the free electron x-ray laser. At present structural analysis of larger complexes is limited to low or medium resolution methods like FRET, NMR, and electron microscopy. Another approach to a better understanding of the mechanisms used by motor proteins like kinesin consists in using computational methods to simulate the dynamics of the motor
molecules. Ideally, molecular dynamics simulation would start from first principles, using quantum mechanics to describe the whole system. However, this is far out of reach because of the sheer size of the problem: the relevant scales cover several orders of magnitude in space and many orders of magnitude in time: from the small scale events involved in hydrolysis of ATP to the large scale conformational changes occurring during the movement along microtubules. The popular "cartoon" models essentially use metaphors (like switch, spring, lever arm) to describe the interrelationship of the processes occurring at the different scales. A possible solution for this problem could be to use ab initio molecular dynamics for the active centre and the nucleotide, and to switch to a coarse-grained model to simulate large scale effects (Lattanzi and Maritan, 2004; Zheng and Doniach, 2003).

The general problem for gaining a thorough understanding of motor proteins seems to be related to other "big" problems of structural molecular biology: the prediction of protein folding from first principles, using only the sequence of amino acids, and the prediction of the interaction of proteins with other proteins or with small ligands like cofactors and inhibitors. As motor proteins are involved in many physiological processes, better models can be expected to be helpful in the search for new drugs (cf. specific inhibition of the mitotic motor Eg5 by monastrol and other small organic compounds that could serve as potential antitumor drugs; Mayer et al., 1999; Sakowicz et al., 2004). Because of their ability to exert molecular control at the nanometer scale, motor proteins and related biomechanical proteins lend themselves as natural models for applications in nanotechnology. Biomolecular motors have been manipulated and used for artificial tasks on nano- and microscales, and they served as models for engineered systems and biomimetic devices (Schmidt and Montemagno, 2004). Progress in bionanotechnology will certainly benefit from future advances in molecular biology of motor proteins.
Table I

Structural alignment of the switch-1 and switch-2 regions of kinesin motor domains with secondary structure assignments and classification of the switch-2 cluster and neck / neck linker conformations.

Legend to Table I

(a) PDB-ID of the coordinates made available by the RSCB Protein Data Bank (http://www.rcsb.org/pdb/; Berman et al., 2000). If the crystal contains several independent molecules per asymmetric unit, the corresponding chain IDs are indicated in parentheses.

(b) Limits for the linker between helix a3 and strand b6 are defined by the last residue of the core of helix α3 (see code assignments below) and the first residue of β6 that participates in a β-ladder. In most cases this is the conserved histidine corresponding to H206 in RnKHC.

(c) Limits for the region marked as "loop 11" are defined by the last residue of strand β7 participating in a β-ladder and the first residue of the core of helix α4 (see code assignments below). In most cases, the residue immediately preceding the "loop 11 region" is the leading aspartate of the switch-2 motif (DLAGSE in RnKHC).

(d) perm = permissive conformation, compatible with docking of the neck linker or neck mimic (Vinogradowa et al., 2004); obstr = obstructive conformation (prohibits docking)

(e) For C-type motors, additional information about the C-terminal residues following helix α6 is given in parentheses.

(f) Although the PDB file contains coordinates for all residues of loop L11, residues V238 to I254 are not part of the model (Kull et al., 1996).

(g) The neck linker is roughly perpendicular to α6 and parallel to the central β-sheet.

(h) Residues preceding strand β1 form a continuous α-helix.

(i) Construct starts with β1.

(j) The PfKinI construct contains an engineering artifact of 30 amino acids C-terminal to helix α6, which is disordered.

(k) The neck helix is roughly perpendicular to the central β-sheet. The construct ends with helix α6.

Code used for secondary structure assignments determined by PROCHECK v.3.5 (Laskowski et al., 1993) using the extended Kabsch/Sander classification (Kabsch and Sander, 1983):

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NNN (bold face)</td>
<td>core region of α-helix</td>
</tr>
<tr>
<td>NNN (bold face, italic)</td>
<td>core region of 3/10 helix</td>
</tr>
<tr>
<td>NNN (normal face, thin underline)</td>
<td>residues in core β- or allowed β-region of the Ramachandran plot.</td>
</tr>
<tr>
<td>NNN (normal face, thick underline)</td>
<td>residues participating in β-ladder or isolated β-bridge</td>
</tr>
</tbody>
</table>
Figure Legends

Figure 1

Domain structures of typical members of the kinesin superfamily. a: Bar diagram of the kinesin heavy chain (KHC) of conventional kinesin (kinesin-1 family) as a typical representative of N-type motors (motor domain at the N-terminus, red); the cartoon model beneath the bar diagram shows the tetrameric complex of two heavy and two light chains. b: M-type kinesin like MCAK of the kinesin-13 family. c: C-type kinesin like Ncd of the kinesin-14 family.

Figure 2

Structure of the motor domain of rat kinesin-1. All panels display ribbon diagrams of the crystal structure of monomeric RnKHC (PDB-ID: 2KIN; Sack et al., 1997) in two standard orientations that differ by rotation of 180° about the vertical axis within the drawing plane. a: overview with all secondary structure elements shown in intensive colors (blue: β-strands, red: helices). b, c, d, e: the same with selected structural elements highlighted and labelled (b: central β-sheet; c: helices surrounding the central β-sheet; d: extra lobes anchored at the left and the right edge of the core structure; e: neck linker and neck helix). The orientation at the right side presents the contact surface with the structural elements that interact with tubulin subunits when the motor is attached to the microtubule (proximal surface), the view shown on the left side is towards the outer (distal) surface. In the orientations selected, the microtubule runs roughly parallel to the drawing plane, plus end down. The figure has been prepared using Deep View Swiss-PDB Viewer (Guex and Peitsch, 1997).

Figure 3

Conformation of the switch-2 cluster and neck linker / neck region in various members of the kinesin superfamily. The upper four panels (a, b, e, f) show crystal structures of N-type kinesins with their motor domain at the N-terminus and the neck at the C-terminus. Panels c, d, g, and h show C- and M-type kinesins with their neck N-terminal to the motor domain, except for PoKCBP (g) where the C-terminal "neck mimick" is shown instead of the N-terminal neck (which is not included in the crystal structure). Each structure is shown in two orientations which differ by a rotation of 90°. Rat conventional kinesin (RnKHC, panel a) has been chosen to define standard orientations with the neck helix α7 parallel / perpendicular to the drawing area. Orientations for the other structures have been determined by least square superposition of their P-loop regions with that of RnKHC (using 11 Cα-atoms of residues F83 to T93 in RnKHC). Panels b, e, and d show the structures of dimeric constructs with the second motor domain in pale colors. The Ncd structure in panel c is 180°-symmetric; the symmetry axis is oblique to the drawing plane and coincides with the axis of the coiled coil which is formed by the two neck helices. In the asymmetric structure of the Ncd N600K mutant (d), the second motor domain (pale) is rotated by about 75° around an axis perpendicular to the coiled coil. The structures shown in panels a, b, f, and g have their switch-2 cluster in "permissive" conformation, whereas the conformation of structures c, d, e,
and h is "obstructive", as can be told by observing the slope of the extended switch-2 helix \( \alpha_4 \).

Color code: red: switch-2 cluster including the extended helices \( \alpha_4 \); magenta: neck region and neck helix, if present; yellow: ADP; orange: AMPPNP; green: monastrol. The figure has been prepared using Deep View Swiss-PDB Viewer (Guex and Peitsch, 1997).

Figure 4

Dimeric kinesin moving along a microtubule protofilament. Four "snapshots" taken from an animated cartoon that illustrates how a kinesin dimer could walk along a microtubule. The pictures shown here are all from one half-cycle: one head (green/blue) is fixed while the other one (yellow/red) orbits around the common neck and stalk (stalk not shown). During the second half-cycle, the motor domains change their roles. (The animated cartoon is available from http://www.mpasmb-hamburg.mpg.de/.)

Figure 5

Binding of the motor domain of \textit{Neurospora} conventional kinesin to the microtubule surface. The microtubule is represented by three tubulin subunits (\( \alpha\beta-\alpha \)) of a single protofilament, plus end at the top. Tubulin subunits according to coordinates determined by electron crystallography (Nogales et al., 1998) and refined to 3.5 \( \AA \) (PDB-ID: 1JFF; Lowe et al., 2001) are colored in green (helices) and magenta (\( \beta \)-strands), except for helices H11 and H12 (olive), the loops formed by residues 35-60 in both \( \alpha \)-tubulins (orange; adopted from PDB-ID: 1TUB; Nogales et al., 1998; this loop is not part of the refined structure 1JFF), and the C-terminal residues not determined by electron diffraction (red; arbitrary random conformation). Small molecule ligands are drawn as space filling models (taxol in red, nucleotides in yellow). The motor domain of NcKin (PDB-ID: 1GOJ; Song et al., 2001) is colored mostly in pink (helices) and cyan (\( \beta \)-strands), except for the major microtubule binding regions \( \beta_5a,b/L8 \) and \( L11-\alpha_4-L12-\alpha_5 \). The \( \beta \)-hairpin \( \beta 6-L10-\beta 7 \) at the tip of the motor domain points to the plus end. Helix \( \alpha 4 \) protrudes into the groove between \( \alpha \)- and \( \beta \)-subunits of a tubulin dimer. Loops L8 and L12 are close to the C-terminal helix H12 of \( \beta \)-tubulin, while loop L11 approaches helix H12 of the adjacent \( \alpha \)-tubulin. The figure has been prepared using Deep View Swiss-PDB Viewer (Guex and Peitsch, 1997) and POVray for Windows (Persistence of Vision Pty. Ltd. 2004, Persistence of Vision Raytracer Version 3.5, retrieved from http://www.povray.org/).

Figure 6

Kinesin domain structure and associated proteins. Conventional kinesin (kinesin-1 family) with two heavy and two light chains attached to a microtubule protofilament (schematically) and a single light chain (KAP3) of the heterotrimeric kinesin KIF3A/B-KAP3 (kinesin-2 family). Head, neck and neck linker are modelled according to the crystal structure of RnKHC (PDB-ID: 3KIN), the mostly \( \alpha \)-helical central part of the heavy chain is depicted schematically as coiled coil (red). The stalk is interrupted by several hinges (not shown), most of the stalk is suppressed, only small pieces at both ends are shown. Regions of the light
chains (yellow) with homology to tetratricopeptide repeats (TPR) are modelled according to the known TPR domains of protein phosphatase 5 (PP5, PDB-ID: 1A17; Das et al., 1998), other parts of unknown structure are represented as spheres. In the case of the heterotrimeric kinesin, the two heavy chains are distinct but similar to each other and to the KHCs of kinesin-1, and there is only one light chain which is predicted to be mostly $\alpha$-helical. (Picture reproduced from Mandelkow and Mandelkow, 2002, with kind permission of the publisher).

Figure 7

Domain structure and homology model of the dynein heavy chain. a: Bar diagram of the sequence of the $\beta$ heavy chain of outer-arm axonemal dynein with six AAA modules highlighted by different colors. The stalk domain between modules 4 and 5 contains a microtubule binding site flanked by two segments that are predicted to form a coiled coil. b: Homology model of the globular part of the dynein heavy chain based on the structures of three AAA proteins (PDB-ID: 1HN5; Mocz and Gibbons, 2001). Module 1 is shown with an ATP in the catalytic cleft. Hydrolysis of this ATP is essential for dynein's motor activity. The figure has been prepared using Deep View Swiss-PDB Viewer (Guex and Peitsch, 1997) and POVray for Windows (Persistence of Vision Pty. Ltd. 2004, Persistence of Vision Raytracer Version 3.5, retrieved from http://www.povray.org/).
References


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Fig. 1

a) kinesin-1 (KHC)

N-type

b) kinesin-13 (MCAK)

M-type

c) kinesin-14 (Ncd)

C-type

- N-terminal domain
- Motor domain
- coiled coil
- C-terminal tail domain
- Light chain

Light chain
Fig. 3

(a) RnKHC (2KIN)
(b) RnKHC (3KIN)
(c) Ncd (2NCD)
(d) Ncd (1N6M)
(e) HsKSP (1II6_A)
(f) HsKSP (1Q0B_A)
(g) PoKCBP (1SDM)
(h) MmKif2C (1V8K)
Fig. 5
Fig. 6
Fig. 7