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THE DESIGN PLAN OF KINESIN MOTORS

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ABSTRACT

The kinesin superfamily comprises a large and structurally diverse group of microtubule-based motor proteins that produce a variety of force-generating activities within cells. This review addresses how the structures of kinesin proteins provide clues as to their biological functions and motile properties. We discuss structural features common to all kinesin motors, as well as specialized features that enable subfamilies of related motors to carry out specialized activities. We also discuss how the kinesin motor domain uses chemical energy from ATP hydrolysis to move along microtubules.

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INTRODUCTION

Eukaryotic cells depend on microtubule-based motility for survival. Segregation of chromosomes in mitosis and meiosis, transport of intracellular membrane organelles, localization of cytoplasmic mRNA, and asymmetric localization of morphogens in early embryos are well-known processes that require microtubules. To achieve intracellular transport, microtubules operate in concert with motor proteins that hydrolyze ATP and travel unidirectionally along the microtubule surface. By simultaneously attaching to other macromolecules (e.g. membranes, protein complexes, or other filaments), motor proteins can carry cargo to particular destinations within the cytoplasm or rearrange filament networks.

Two superfamilies of microtubule-based motor proteins have been discovered—kinesins and dyneins. Dynein was first discovered in cilia in the early 1960s and later was shown to be present in the cytoplasm of all eukaryotic cells. Kinesin was discovered in the mid-1980s in squid and mammalian brain, using microscopic in vitro motility assays. Since then, genetic and molecular biological screens have fueled an explosive discovery of new microtubule-based motor proteins (more than 90 kinesin sequences are presently entered into the sequence data base). Dynein and kinesin motors are found in all eukaryotes. The yeast *Saccharomyces cerevisiae* genome encodes one dynein and six kinesin genes, and the numbers of motor genes in higher eukaryotes may be an order of magnitude greater.

Interest in motor proteins derives from their dynamic role in virtually every imaginable cell biological process. Investigators working on biological problems as diverse as yeast cell division, *Caenorhabditis elegans* chemosensory transduction, *Xenopus* early development, and *Drosophila* signal transduction pathways have found their work united by a common theme: the discovery that a kinesin motor protein is involved in their biological phenomenon. The investigators studying microtubule-based motors have driven progress in this field at a remarkable pace, and new biological functions are continually being assigned to kinesin motors. Furthermore, an understanding of the mechanism of kinesin motility is beginning to rival that of the muscle motor myosin, which has been the focus of a century of research.

The focus of this review is on the kinesin motor superfamily, with an emphasis on how the structures of these proteins can provide clues as to their biological functions and motile properties. In particular, our intent is to provide a perspective on following issues: (*a*) What are the structural features of kinesin motors, and how are they related to those of other enzymes and motors? (*b*) How have motors within the kinesin superfamily diverged to achieve distinct activities? (*c*) What conformational changes enable these motors to move

along microtubules? The cell biological roles of kinesin motors are discussed in the context of these issues, but are not reviewed in detail. Roles of kinesin motors are briefly described in Table 1, and references for further reading can be obtained from the table legend. Other recent reviews on kinesin motor proteins include Bloom & Endow 1995, Hackney 1996, Hirokawa 1996, Howard 1996, Scholey 1996, and an excellent world wide web site is available on this group of proteins (Greene et al 1996).

GENERAL STRUCTURAL FEATURES OF KINESIN MOTORS

In 1990, the first hint of the existence of a kinesin superfamily emerged when genes were discovered in *S. cerevisiae* (Meluh & Rose 1990) and *Aspergillus* (Enos & Morris 1990) that contain a ≈350 amino acid region, which is 30–40% identical to the motor domain of the first discovered kinesin (termed conventional kinesin). Beyond the boundary of the motor domain, however, the sequences of these two kinesin-related proteins show no similarity to one another or to conventional kinesin. These findings suggested that a highly conserved motor domain had become combined with different non-motor domains that could target motors to different cargo within the cell and allow them to carry out unique force-generating functions.

In this review, the following terminology is used to describe the different domains of kinesin motors. Motor domain refers to the force-producing element of the protein, which is itself divided into two major parts: One part, the globular catalytic core, is conserved throughout the superfamily and its three-dimensional structure has been solved. The second part termed the neck region is an adjacent ≈40 amino acids found on either the N or C terminus of the catalytic core. The neck, which is conserved only within certain kinesin classes (discussed below), appears to work in concert with the catalytic core to produce movement. Beyond the motor domain, many kinesin proteins contain a long α-helical coiled-coil domain termed the stalk. Finally, there is often an additional globular domain at the end of the stalk. This domain, the tail, is thought to target the motor to a particular cargo within the cell.

MOLECULAR TAXONOMY OF THE KINESIN SUPERFAMILY

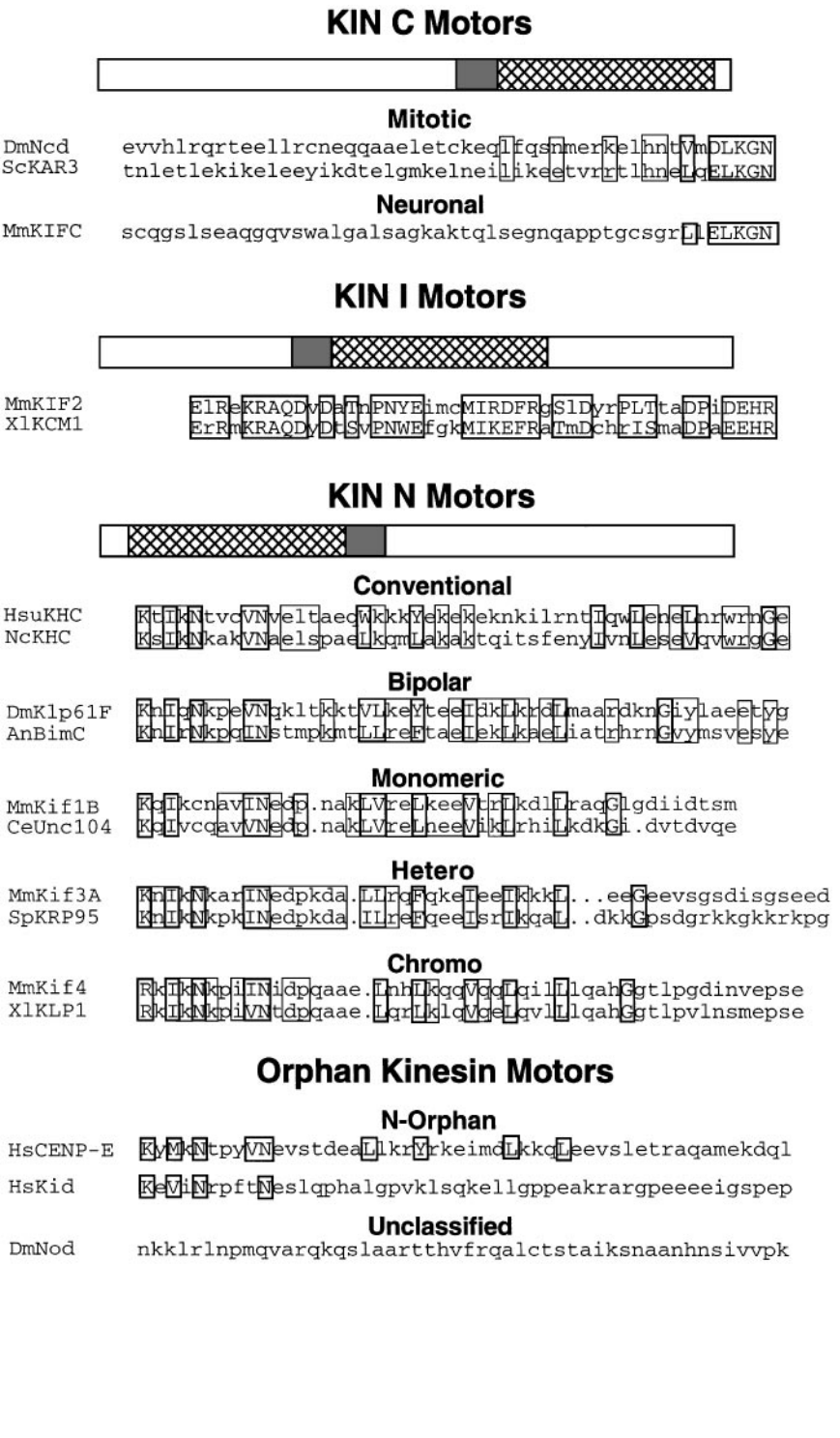
In the last six years, new members of the kinesin superfamily have been discovered in numbers that rival other prominent gene superfamilies such as G proteins and integrins. The escalating discovery of new kinesin motors has brought with it a complex nomenclature that has become impenetrable to all

but the most ardent followers of the kinesin literature. Most kinesin motors are named on the basis of either the genetic mutations that led to their isolation, their chromosomal locations, or their PCR clone number.

Analyses of numerous kinesin motor domain sequences and construction of phylogenetic trees reveal clusters of closely related kinesin motors (Goldstein 1993, Goodson et al 1994, Hirokawa 1996). As has been recently performed with the myosin superfamily (Mooseker & Cheney 1995), phylogenetic sequence analysis can provide a basis for classification of kinesin motors. Two potential benefits arise from such efforts. First, classification of motors can be used to derive a simplified nomenclature that facilitates communication. Second, inspection of sequences from related motor proteins provides clues as to how structure gives rise to specialized motile or regulatory properties.

Sequence comparisons of the motor domains of kinesin superfamily members identify eight major groups of related kinesin proteins (see Greene et al 1996). Approximately 70 to 75 of the 90 motor sequences in the data base align with one of these eight classes; the remainder of the motors are termed orphans (Goldstein 1993) because their sequences do not indicate a close relationship with other motors. The eight kinesin classes fall into three major groups, which correspond to motors that have their catalytic cores positioned either at the N terminus, C terminus, or are internal to the polypeptide chain (Figure 1).

Figure 1 Taxonomy of the kinesin superfamily illustrated here by amino acid sequence conservation and variation in the neck region. The *hatched bar* indicates the approximate position of the catalytic core, and the *solid* bar indicates the position of the conserved neck region corresponding to the sequences shown below. The orphan kinesin motors also have their catalytic cores positioned at the N terminus of the polypeptide chain. A total of ≈80 kinesin-encoding genes were analyzed, and the neck domains of a subset are shown here. Divergent members of each subclass were chosen for display. Phylogenetic analyses of sequences within either the catalytic core or neck region produce the same class division of kinesin motors, as shown here. Neck residues highly conserved and characteristic of the three major kinesin classes (KIN C, I, or N) are *capitalized* and surrounded by a *thick-line box*. Residues highly conserved and characteristic of a subclass are in *lowercase letters* and surrounded by a *thin-line box*. (More members of the subclass were analyzed in making these assignments than shown on this figure). Residues with side chains with similar chemical properties are classified here as conserved. For KIN C and KIN I motors, the class-conserved boxed residues precede the superfamily conserved $\beta 1$ strand of the catalytic core (I392 in NCD and I196 for KIF2). For the KIN N motors, there is a class-conserved motif of K/RxIxNxxxV/IN at the beginning of the neck region, which is C-terminal to helix 6 of the catalytic core (which ends at residue A322 in human kinesin). This neck motif is followed by a hydrophobic repeat pattern of ϕ-xx(x)-ϕ-xxx-ϕ-xx-ϕ (ϕ represents a highly conserved hydrophobic residue), which is then followed by a conserved glycine (G). The KIN N-Conventional motors are unusual in having an insertion of additional residues in the hydrophobic region. Some orphan motors (motors that do not have close relatives) contain similar neck motifs to the KIN N motors and are termed KIN N-Orphan; others do not have neck regions that are similar to other motors (unclassified orphans). An alignment sequences and phylogenetic trees can be found at our web site (Hartman et al 1997).


KIN C Motors
Mitotic
DmNcd evvhlrqrteellrcneqqaaeletckeqlfqsnmerkelhntVmDLKGN
ScKAR3 tnletlekikeleeyikdtelgmkelneillikeetvrrrtlhneLqELKGN
Neuronal
MmKIFC scqgslseaqggqvswalgalsagkaktqlsegnqapptgcsgrLLELKGN
KIN I Motors
MmKIF2 ElReKRAQDvDaTmPNYEimcMIRDFRgSlDyrPLTtaDPiDEHR
XlKCM1 ErRmKRAQDvDtSvPNWEfgkMIKEFRaTmDchrISmaDPaEEHR
KIN N Motors
Conventional
HsuKHC KtIkNtvcVNveltaecWkkkYekekeknkilrntIqwLeneLnrwrmGe
NcKHC KsIkNkakVNaelspaeLkqmLakaktqitsfenyIvnLeseVqvwrqGe
Bipolar
DmKlp61F KnIgNkpeVNgkltkktVLkeYteeIdkLkrdLmaardknGiylaeetyg
AnBimC KnLrNkpqLNstmpkmtLLreFtaeIekLkaeLiatrhrnGvymsvesye
Monomeric
MmKif1B KqIkcnavINedp.nakLVreLkeeVtrIkdlLraqGlgdiidtsm
CeUnc104 KqIvcqavVNedp.nakLVreLneeVikLrhiLkdkGi.dvtdvqe
Hetero
MmKif3A KnIkNkarINedpkda.LLrqFqkeIeeIkkkL...eeGeevsgsdisgseed
SpKRP95 KnIkNkpkINedpkda.ILreFqeeIsrIkkqaL..dkkGpsdgrkkgkkrkpg
Chromo
MmKif4 RkIkNkpiINidpqaae.InhIkqqVqqLqilLlqahGgtlpgdinvepse
XlKLP1 RkIkNkpiVNtdpqaae.LqrLklqVgeLqvlLlqahGgtlpvlnsmepse
Orphan Kinesin Motors
N-Orphan
HsCENP-E KyVkNtpyVNevstdeaLlkrYrkeimqLkkqLeevsletraqamekdql
HsKid KeViNrpftNeslqphalgpvklsqkellgppeakrargpeeeeigspep
Unclassified
DmNod nkklrlnpmqvarqkqslaartthvfrqalctstaiksnaanhnsivvpk

In this review, we refer to these three major branches as KIN N, KIN C, and KIN I motors, respectively. The KIN N motors can be subdivided into five classes. For the purpose of refering to them in this review, we have named them based on a notable structural feature or currently popularized name: KIN N-Conventional refers to the class containing the originally discovered kinesin motor. KIN N-Bipolar corresponds to a group of mitotic kinesin proteins that form homotetrameric, bipolar filaments. KIN N-Monomeric are monomer organelle transport motors. KIN N-Hetero contain two different motor subunits and appear to function in organelle and ciliary protein transport. KIN N-Chromo refers to a kinesin class in which some members have been shown to contain DNA-binding motifs and are commonly called chromokinesins. Members of this class also serve as organelle transport motors. KIN C motors can be divided into two classes. We refer to them here as KIN C-Mitotic and KIN C-Neuronal, which alludes to their distinct activities in mitotic/meiotic spindle formation and neuronal organelle transport, respectively.

The above nomenclature may be convenient for the non-specialist because it divides the superfamily into three branches based on the position and structure of the motor/neck domain and then into classes that are named in reference to a property of these motors. However, this nomenclature also has pitfalls because the property that is featured in the name may not be universally true of all motors in a given class. For example, it remains unclear whether proteins in either the N-Bipolar, N-Monomeric, or N-Hetero classes will all share the same oligomeric features because quaternary structure has been determined in only a few cases. Furthermore, this is not a foregone conclusion because oligomerizaton is not determined by the motor domains, whose sequences are used in generating class assignments (see below). Classification by biological function also is imperfect, as illustrated by the fact that motors in N-Chromo class can perform functions unrelated to chromosomes. In the future, property-based class names may become inappropriate, as exceptions to the rule grow. A numerical nomenclature similar to that used for myosin may be most suitable, but such a system will have to be derived from a consensus of investigators in the field.

Surprisingly, the most class-specific region of the kinesin motor domain is not located in the catalytic core, but is the $\approx$40 amino acid neck region that emerges from either the N or C terminus of the catalytic core (Figure 1). In the KIN C and KIN I motors, the conserved neck is N-terminal to the catalytic core, and the sequence C-terminal to the catalytic core is poorly conserved. Lack of conservation suggests that the chain immediately following the C terminus of the catalytic core in both KIN C and KIN I motors is not critical for motility, although this remains to be tested. Conversely, for the KIN N motors, the conserved neck is C-terminal to the catalytic core, and residues N-terminal to

the catalytic core are poorly conserved in sequence and length, which suggests that they also are not essential for motor activity.

Sequence analysis of the ≈40 residues of the neck generates a phylogenetic tree that reveals a clear clustering of related motors similar to trees based upon the 330 residues in the catalytic domain (R Case & R Vale, manuscript in preparation). This finding suggests that the neck and catalytic core function as a unit and have evolved together. The relatedness of motor proteins within classes can be easily appreciated from the neck sequence shown in Figure 1. KIN C motors have a characteristic motif of LxE/DLKGN preceding the first β strand of the catalytic core defined by the three-dimensional structure. In contrast, the KIN N motors contain a consensus sequence of K/RxIxNxxxV/IN at the beginning of the neck region. Analysis of peptides containing this motif suggest that it may be part of a β-strand structure (Morii et al 1997, Tripet et al 1997). Beyond this motif, KIN N motors share a similar pattern consisting of ϕ-xx(x)-ϕ-xxx-ϕ-xx-ϕ, where ϕ represents a highly conserved, hydrophobic residue. This is a common motif in α-helical coiled-coils, and conventional kinesin proteins have been shown to contain such a structure in this region (see below). Four to 10 residues beyond the repeat of hydrophobic residues is a conserved glycine in KIN N motors, which may mark the beginning of a flexible hinge.

Several of the currently classified orphan motors also contain the KxIxNxxxxN motif (e.g. HsKid), and some even have the subsequent hydrophobic repeat (e.g. HsCENP-E) (Figure 1). Thus some orphans share structural similarity to the KIN N motors in the neck, and hence we refer to them in this review as KIN N-Orphan motors. Other orphan motors (e.g. DmNod), however, have no sequence similarity to other motors in the neck and may have novel structures in this region. This diversity of structure is intriguing given the importance of the neck in kinesin motility (discussed below). However, it is also possible that divergent members of the kinesin superfamily may not be capable of microtubule-based movement and may instead perform other functions (reversible microtubule binding, for example).

Can the above classification of kinesin motors be used to predict biological properties? There is no a priori expectation that this will be the case because the amino sequence alignments used to classify motors are based upon the catalytic core and/or neck, regions that are involved in motor output and/or regulation of motor activity. In many cases, however, biological function appears to correlate well with motor domain sequence, presumably because attributes of motor function (e.g. velocity) are tuned for a particular cellular function. This is illustrated by KIN N-Bi polar motors (found in yeast, *Drosophila*, and mammals), which all appear to be involved in spindle pole separation and spindle morphogenesis (Table 1). In contrast, the biological activities of the KIN

Table 1 Properties of kinesin motors

Class	Representatives[a]	Heavy chain oligomerization	Other chains (kDa)	Motility (μm/sec)	Biological activity
KIN C	DmNcd[b]	Homodimer	N.D.	−0.2	Meiotic/mitotic spindle
Mitotic	ScKar3[c]	N.D.	77 (CiK1)	−0.03	Karyogamy/mitotic spindle
Neuronal	MmKIFC2[d]	N.D.	N.D.	N.D.	Organelle movement
KIN I	MmKIF2[e]	Homodimer	None	+0.5	Axonal transport
	XlKCM1[f]	N.D.	N.D.	N. D.[*]	MT dynamics/Chromosome segregation
KIN N	MmKIF1B[g]	Monomer	N.D.	+0.7	Mitochondria transport
Monomeric	CeUnc104[h]	Monomer	N.D.	+1.0	Synaptic vesicle transport
Bipolar	DmKLP61F[i]	Tetramer	None	+0.04	Centrosome separation/Spindle formation
	AnBimC[j]	N.D.	N.D.	N.D.	Centrosome separation/Spindle formation
Hetero	SpKRP85/95[k]	Heterodimer	115	+0.4	Organelle movement/Ciliary Transport
	MmKIF3A/3B[l]	Heterodimer	100 (KAP3)	+0.3	Organelle movement
Chromo	MmKIF4[m]	Homodimer	N.D.	+0.2	Organelle movement
	XlKLP1[n]	N.D.	N.D.	N.D.	Chromosome movement/Germ plasm aggregation
Conventional	HsuKHC[o]	Homodimer	70 light chain	+0.6	Organelle movement
	NcKHC[p]	Homodimer	None	+2.5	N.D.
Orphans	HsCENP-E[q]	N.D.	N.D.	N.D.	Kinetochore function
KIN N-Orphan	HsKid[r]	N.D.	N.D.	N.D.	Chromosome movement
Unclassified	DmNod[s]	N.D.	N.D.	N.D.	Chromosome movement

[a]The first two letters of the motor representative's name refer to the species name: Dm, *Drosophila melanogaster*; Sc, *Saccharomyces cerevisiae*; Mm, *Mus musculus*; XI, *Xenopus laevis*; Ce, *Caenorhabditis elegans*; An, *Aspergilus nidulans*; Sp, *Strongyloscentrotus purpuratus*; Cr, *Chlamydomonas rheinhardtii*; Hs, *Homo sapiens*; Nc, *Neurospora crassa*; Dd, *Dictyostelium discoideum*. References for properties described in Table: [b]DmNCD: Chandra et al 1993, Matthies et al 1996, McDonald et al 1990; Walker et al 1990; [c]ScKar3: Meluh & Rose 1990, Saunders & Hoyt 1992, Endow et al 1994, Page et al 1994; [d]MnKIFC2: Hanlon & Goldstein 1997, Saito et al 1997; [e]MmKIF2: Noda et al 1995; [f]XlKCM1: Walczak et al 1996; [g]MmKIF1b: Nangaku et al 1994; [h]CeUnc104: Hall & Hedgecock 1991; D Pierce, A Ohtsuka & R Vale, unpublished data; [i]DmKLP61F: Heck et al 1993, Cole et al 1994, Kashina et al 1996a,b; [j]AnBimC: Enos & Morris 1990; [k]SpKRP85/95, Cole et al 1993, Scholey 1996, Wedaman et al 1996, Morris & Scholey 1997; [l]MmKIF3A/B: Aizawa et al 1992, Kondo et al 1994, Yamazaki et al 1995, 1996; [m]MmKIF4, Sekine et al 1994; [n]XlKLP1, Vernos et al 1995, Robb et al 1996; [o]HsuKHC, Bloom & Endow 1995; [p]NcKHC, Steinberg & Schliwa 1995; [q]HsCENP-E, Yen et al 1992; [r]HsKid, Tokai et al 1996; [s]DmNod, Afshar et al 1995, Murphy & Karpen 1995. N.D. means not determined. For motility, + and − refer to movement directed toward the plus end and minus end of the microtubule, respectively, [*]XlKCM1 has been shown to be capable of destablizing microtubules.

N-Chromo motors are broader and include chromosome movements, organelle transport, and oocyte germ plasm aggregation. However, tail domains, which probably dictate cargo binding and hence biological activity, are not considered in this classification scheme. Different groupings of motors based upon tail domains could emerge in the future when there is a better understanding of this region of kinesin molecules.

In contrast to biological function, class-specific variation within the motor domain must be directly related to aspects of motility, either motor output or regulation. In particular, the high degree of class conservation of the neck region suggests that it may be an essential yet modular element of the motor domain. Thus analyzing neck sequence alignments may not only provide information on evolutionary linkages but should also provide a valuable resource for deciphering how kinesin motors work. However, the information contained in these sequences is still cryptic and invites further study. Some motile properties, such as speed and direction of movement along a microtubule, appear to be shared by members of a class. For example, 4 KIN C motors have been shown to move to the minus-end of the microtubule. In contrast, 12 KIN N motors have been shown to be plus-end motors. Recent work suggests that the neck and not the catalytic core may dictate the direction of movement (Case et al 1997).

THE ARCHITECTURE OF KINESIN MOTORS

Kinesin motors are used for many force-generating tasks. In primitive single-cell organisms, kinesin motors probably evolved primarily for the purpose of chromosome segregation. As organisms became more complex and developed additional requirements for intracellular sorting (including developmental and tissue-specific processes), new kinesin motors evolved for membrane and protein transport. Concomitant with the evolution of new functions, the kinesin motors have acquired considerable structural diversity. Such structural diversification is a legacy of the adaptation of motors to perform particular functions and provides clues as to their biological roles and mechanisms. Likewise, common structural themes within the superfamily suggest features that are indispensible for motor activity. Below we examine the structural elements of kinesin motor proteins and discuss how they are involved in function.

The Motor Domain

THE CATALYTIC CORE The structures of the *Drosophila* NCD (KIN C-Mitotic; a minus-end directed motor) (Sablin et al 1996) and human kinesin (KIN N-Conventional; a plus-end directed motor) (Kull et al 1996) catalytic cores were determined to 2.5 and 1.9 Å resolution using multiple isomorphous replacement. In each case, the core consists of a single domain with an open

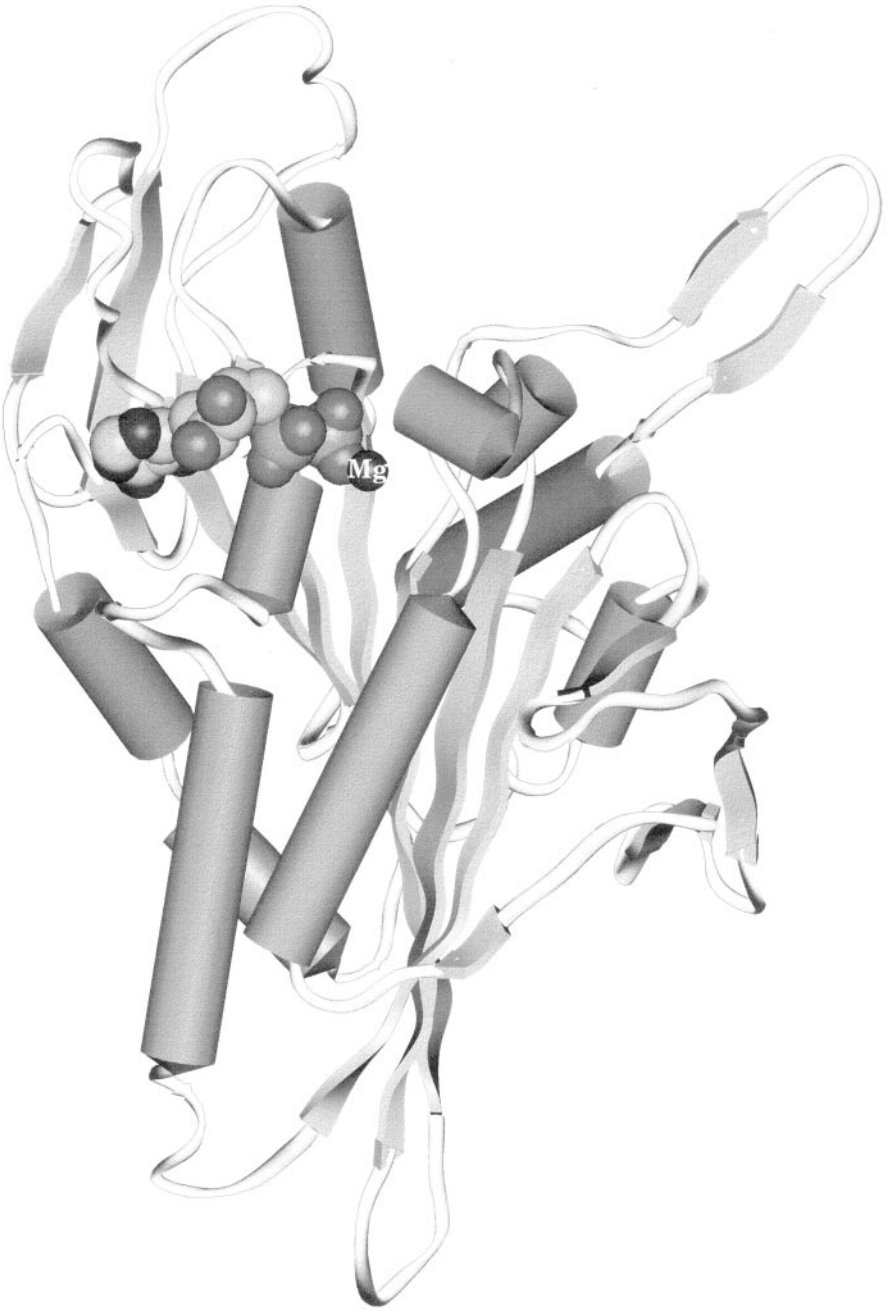
nucleotide pocket containing a bound ADP. Many nucleotide-binding enzymes are comprised of multiple domains, as seen in adenylate kinase and myosin and pyruvate kinase, but the kinesin and NCD catalytic cores are not articulated into obvious flexible elements. However, the neck segments are disordered and are not visible in the published crystal structures.

The three-dimensional structure of the motor is shown in Figure 2. It is described as an α/β class protein with three helices covering either side of an eight-stranded, mostly parallel, β sheet. The nucleotide ADP, with its attending Mg ion, resides in a relatively exposed pocket and nestles among four loops that converge at the C-terminal ends of four β strands of the sheet.

Other features are noteworthy, although their functional significance is unknown. One obvious appendage to the single domain structure is the towering loop, L11, whose 15 amino acids splay away from the nucleotide-binding site. This β-ribbon extension very likely alters its position during the enzymatic cycle because the base of the loop contacts the nucleotide. Preliminary data also suggest that this loop contacts the microtubule. A subdomain composed of three β strands, whose function is unknown, is perched above the core β strands. Two of the central β strands are unusually long, 8–10, rather than 6–7 amino acids for most α/β domain proteins. Electron microscopic studies suggest that these extended β strands may contact a second catalytic core in a microtubule-bound NCD dimer (Sosa et al 1997). Also of structural interest is an α helix on the side of the sheet opposite the nucleotide, which is canted by $\approx 45^\circ$ rather than parallel with the strands, as is true for most helices in α/β domain proteins. As discussed below, this helix may change its conformation during the enzymatic cycle.

Despite the fact that NCD and kinesin move in opposite directions along microtubules, the lengths and relative positions of the secondary structural elements in these two catalytic cores are nearly identical. This is not necessarily unexpected because the catalytic cores are $\approx$40% identical in amino acid sequence. This degree of identity among homologues is compatible with significant additions and/or deletions of surface loops, subtle changes in arrangements of secondary structural elements, and possibly larger changes in the chemical characteristics of the molecular surfaces. These variations are all found when the kinesin and NCD structures are compared. It does not appear that these modest alterations to the catalytic core give rise to different directions

Figure 2 A schematic cartoon of the catalytic core of kinesin motors (the NCD motor domain is shown here). The β strands are shown as *ribbons*, and the helices are indicated as *cylinders*. The nucleotide ADP with a bound magnesium (Mg) is shown. The Mg ion is near the position expected for the γ-phosphate when ATP is bound.

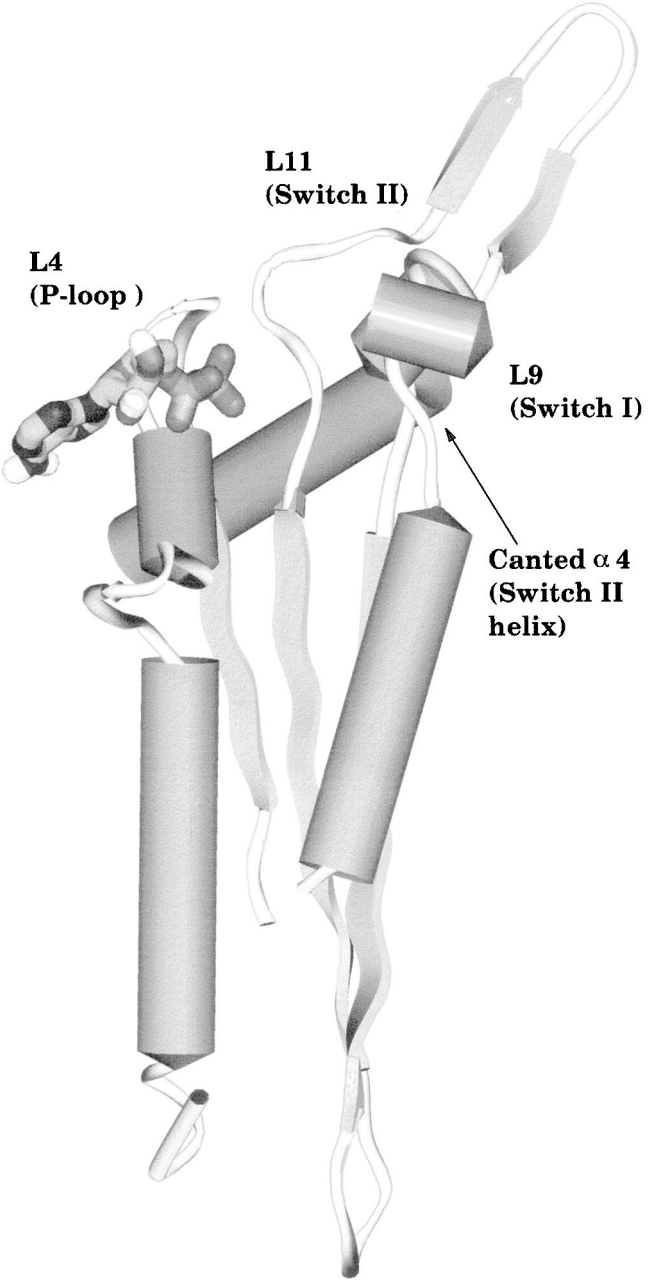

Mg

of movement. Indeed, when the catalytic core of NCD is exchanged for the kinesin catalytic core in a dimeric kinesin construct (K560), the chimeric motor moves in the kinesin direction (Case et al 1997).

THE NECK REGIONS The placement of the N and C termini of the catalytic core is an important issue, because the KIN N and KIN C motors link through these elements to their neck regions to form dimers and to convert ATP hydrolysis energy into movement. Interestingly, the N and C termini of the catalytic cores are only 7 Å away from one another and are close to the 45° canted helix that may change conformation during the enzymatic cycle. The structures of the neck regions in kinesin and NCD were not resolved in the initial crystallographic studies because they were disordered. However, studies of the conventional kinesin neck using synthetic peptides indicate that the first 10 amino acids of the neck may form a β-strand structure and the next 30 amino acids may form an α-helical coiled-coil (Morii et al 1997, Tripet et al 1997). The position of these residues suggests that it might act as a mechanical transducer that responds to cues from the catalytic core (discussed below). Information on the structure of the neck regions of KIN I and KIN C motors is not available.

COMPARISON WITH MYOSIN AND G PROTEINS: TWO EVOLUTIONARY COUSINS Unexpectedly, the structure of kinesin and NCD revealed a similarity to two other families of proteins, the myosins and the G proteins (a diverse group of molecular switches) (reviewed in Vale 1996). The similarity is particularly apparent in the nucleotide active site, which consists of three loops, called the P-loop, switch I, and switch II, that emerges from adjacent β strands in the center of the sheet (Figure 3). The switch regions in the G protein family were so named because they can detect whether NTP or NDP is bound to the active site and then respond by undergoing conformational changes between these two nucleotide states. This nucleotide-dependent switch in conformation is then transmitted to adjacent regions that interact with other target proteins, which results in changes in binding affinity. Beyond the switch regions, structural elements

Figure 3 The nucleotide-binding environment of NCD. This assembly, three parallel and adjacent β strands, three interconnecting loops, and a nucleotide and Mg ion, is observed in kinesin, myosin, and G proteins. Strand 1 is always N-terminal in sequence to the others. The third (edge) strand is always antiparallel to the other two, and strand 3 proceeds directly into strand 2. The three strands project functional loops. The loop (P-loop) emanating from strand 1 is a widely recognized sequence motif, appearing as the signature loop in many nucleotide-binding proteins. The loops emerging from strands 2 and 3 are termed switch II and I, respectively, a nomenclature derived from the G protein field. (The switch I loop contains two very short helices.) These loops contain residues thought to form hydrogen bonds to the γ-phosphate of the nucleotide and change conformation between ATP and ADP states.


L11
(Switch II)
L4
(P-loop)
L9
(Switch I)
Canted α 4
(Switch II
helix)

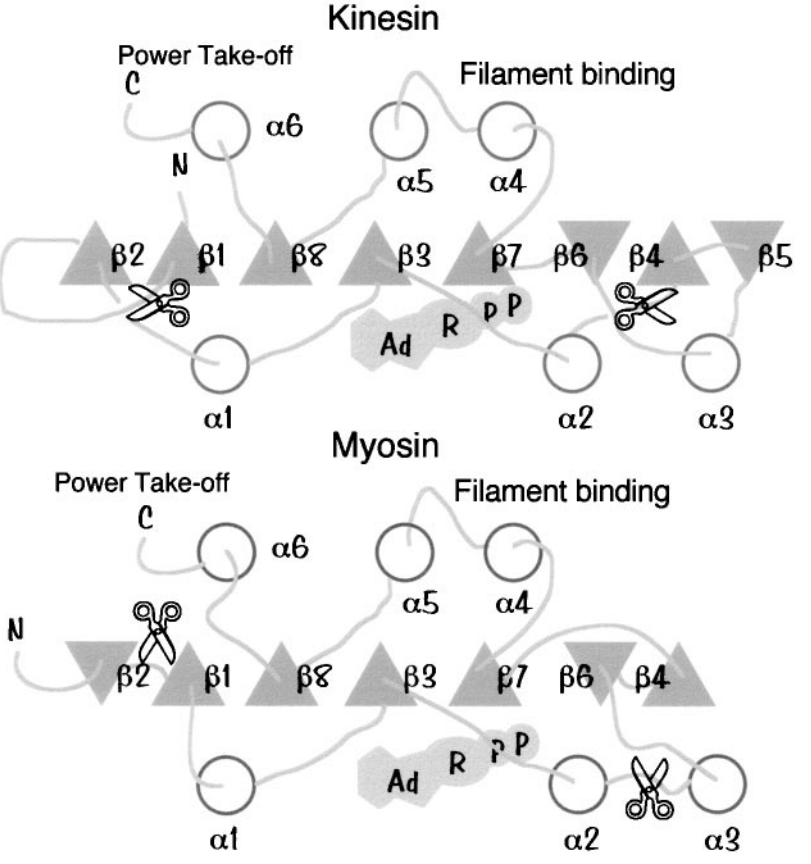
of motor proteins and G proteins superimpose poorly. However, remarkably, seven of the eight β strands and all six helices of kinesin superimpose well with corresponding structural elements in the actin-based motor myosin. Despite this structural similarity, there is little amino acid identity between kinesin and myosin, other than a group of key nucleotide-sensing residues in the switch regions and two prolines residues that define turns.

Are kinesin, myosin, and G proteins evolutionary relatives or homologues that arose by convergent evolution? It is generally agreed that two proteins share a common ancestor when their sequence identity is extensive. When the sequence identity drops below ≈20% (as is true for kinesin, myosin, and G proteins), the question must be asked more carefully. Generally accepted criteria for common ancestory include conserved function, tertiary structure, and topology (the order of linkages between secondary structural elements). Myosin and kinesin share common attributes in their core regions surrounding the nucleotide, although the order of linkage is somewhat different (Figure 4). However, the similarities in topologies and the fact that similar groups of amino acids are positioned nearly identically in three-dimensional space in the phosphate pocket argue that myosins, kinesins, and perhaps G proteins evolved from a common ancestor. Myosin, kinesin, and G proteins are also clearly

Figure 4 Comparison of the topological connectivity of kinesin and myosin in the core region surrounding the nucleotide. The *triangles* are β strands (the point indicates the direction of the chain), helices are represented as *circles*, and ADP is shown on the front face of the central β sheet. The numbering of strands and helices follows kinesin in the order that the secondary structures appear in the linear sequence. Note that $\beta 5$ is missing in myosin (myosin has a seven-stranded sheet and kinesin has an eight-stranded sheet). Two large, inserted domains in myosin, a ≈180 amino acid domain between $\beta 4$ and $\beta 7$, and a ≈150 amino acid domain that falls between $\alpha 4$ and $\alpha 5$ and contains actin-binding elements, are not shown. Myosin and kinesin have different positional and directional linkages between the α helices and β strands at both ends of the β sheet, and the *scissors* mark positions where the chain topology diverges in comparing myosin with kinesin. This can be explained by distinct genetic insertions that occurred as these two motor proteins diverged from a common ancestor. At the left edge of the sheet in kinesin, a secondary structure insertion occurs between $\beta 1$ and $\alpha 1$ that adds $\beta 2$ to edge of the sheet. In myosin, a large N-terminal domain, not shown, and $\beta 2$ (amino acid) is added at sheet edge. The left hand side of the nucleotide-sensing core, is $\beta 1$-$\alpha 1$-$\beta 3$-$\alpha 2$- for kinesin and $\beta 1$-$\alpha 1$-$\beta 3$-$\alpha 2$- for myosin. At the opposite edge of sheet, the sensing core is $\beta 6$-$\beta 7$-$\alpha 4$-$\alpha 5$-$\beta 8$-$\alpha 6$ for kinesin and $\beta 6 \ldots$-$\beta 7$-$\alpha 4$-$\alpha 5$-$\beta 8$-$\alpha 6$ for myosin. The elements $\beta 4$-$\beta 5$-$\alpha 3$ are inserted at sheet edge in kinesin, and $\alpha 3$-$\beta 6$-$\beta 4$ is added at the sheet edge in mysoin. Note that the positions of the power take-offs (regions that connect to the myosin lever arm helix or the kinesin neck), the nucleotides, and filament-binding sites are similarly configured in the two proteins. These comparisons suggest that these two proteins could have been derived from a common progenitor and then diverged by different genetic insertions. Similar arguments can be made to link G proteins and molecular motors to a common ancestor, although more significant alterations in connectivity must be postulated.

more closely related to one another than they are to any other known P-loop containing, nucleotide-binding protein.

COMPLEXES OF MOTOR DOMAINS WITH MICROTUBULES Several groups have decorated microtubules with bacterially expressed kinesin or NCD motors and reconstructed three-dimensional images of the motor-microtubule complex at 20–30 Å resolution (Hirose et al 1995, Hoenger et al 1995, Kikkawa et al 1995). Truncated monomeric motor domains form tadpole-shaped structures on the microtubule and appear to interact with both α- and β-tubulin subunits. Although there was initial disagreement about the orientation of the motor with respect to the polarity of the microtubule, this issue is now settled, and both



kinesin and NCD motor domains clearly are oriented in the same direction (reviewed in Amos & Hirose 1997). Hence, the opposite directions of movement of kinesin and NCD cannot be explained by binding of these two motors in opposite orientations on the microtubule. In contrast, cryoelectron microscopy of motor dimers has revealed differences between kinesin and NCD that may be relevant to directionality. In the dimer structures of kinesin and NCD in the presence of AMPPNP (an ATP analogue that promotes tight microtubule binding), one motor domain is bound to the microtubule, whereas the partner head is dissociated (Arnal et al 1996, Hirose et al 1996). The dissociated head is clearly visible in these EM reconstructions, indicating that it is relatively immobile and well-ordered, perhaps by interactions with the bound motor head (Sosa et al 1997). However, the position of the dissociated motor domain is different for kinesin and NCD. In the case of kinesin, the dissociated head is directed closer to the plus-end of the microtubule; in the case of NCD, this head points closer toward the microtubule minus-end.

The microtubule-binding interface of kinesin motors has recently been explored by alanine-scanning mutagenesis of human kinesin (Woehlke et al 1997) and by docking of the atomic structure of NCD onto EM reconstructed images of NCD dimers bound to microtubules (Sosa et al 1997). Both studies indicate that L12 (a short, well-conserved loop following the switch II helix; Figure 4) constitutes the major microtubule interacting site on kinesin, although other regions contribute as well. Interestingly, this loop corresponds topologically to a larger domain of ≈150 amimo acids in myosin that binds to actin filaments.

Oligomerization Domains

A DIVERSITY OF QUATERNARY STRUCTURES Structural elements beyond the catalytic core control the oligomerization of kinesin polypeptides. These elements are best characterized in conventional kinesin, which is composed of two identical motor-containing polypeptide chains (termed α or heavy chains) and two non-motor polypeptides (termed β or light chains) (Bloom et al 1988, Kuznetsov et al 1988). By electron microscopy, conventional kinesin has a shape reminiscent of myosin; its two globular motor domains are connected to an elongated stalk (Hirokawa et al 1989). Unlike myosin, however, the light chains are not part of the motor domain but are located at the opposite end of the stalk. A structural mechanism for homodimerization of the heavy chains was suggested from the amino acid sequence, which revealed a heptad repeat characteristic of a coiled-coil just C-terminal to the motor domain (Yang et al 1989). Dimerization via a coiled-coil interaction was proven directly by CD spectroscopic analysis of the bacterially expressed stalk fragments (de Cuevas et al 1992) and synthetic neck peptides (Morii et al 1997, Tripet et al 1997). Neurons contain at least two closely related conventional kinesin heavy chains

encoded by separate genes; these two polypeptides preferentially form homo- rather than heterodimers (Niclas et al 1994).

The sequences of many new members of the kinesin superfamily show an extended region of α-helical coiled-coil heptad repeats, which suggests that dimerization is a common feature of kinesin motors. However, biochemical analyses have shown that these coiled-coils can give rise to a surprising diversity of quaternary structures. For example, the *Drosophila* KLP61F kinesin motor (a KIN N-Bipolar motor identical to the biochemically identified KRP130; Kashina et al 1996b) has a central coiled-coil heptad sequence, but biochemical studies indicate that it forms a homotetramer (Cole et al 1994). Electron microscopy shows that the homotetramer is arranged as two antiparallel dimers (Kashina et al 1996a). The forces giving rise to this bipolar filament formation are unknown, although electrostatic interactions between the two coiled-coils (analogous to interactions in myosin thick filaments) or interactions between the head and tail domains may be important. The quaternary structures of the KIN N-Bipolar motors, which are found in yeast as well as in humans, have not been established, but given the similar biological functions of these motors in mitotic spindle formation (Walczak & Mitchison 1996), it is likely that they will prove to be bipolar kinesin motors as well.

Even more surprising was the discovery of a heterotrimeric kinesin motor in sea urchin (KRP 85/95 or kinesin II) (Cole et al 1993, Scholey 1996) and mouse (KIF3A/3B) (Kondo et al 1994). These KIN N-Hetero motors consist of two distinct motor domain-containing polypeptides encoded by different genes and a third non-motor polypeptide (discussed below). Recently, a third close relative in mouse has been identified. Immunoprecipitation experiments indicate that KIF3A can form a heterodimer with KIF3B or KIF3C but that KIF3B and KIF3C do not form heterodimers (Yang et al 1996). Heterodimerization is preferred to homodimerization for all these motors (Rashid et al 1995).

Hydrodynamic and electron microscopy studies have shown that at least some of the baculovirus or bacterially expressed KIN C (NCD; Chandra et al 1993), KIN I (KIF2; Aizawa et al 1992, Noda et al 1995), and KIN N-Chromo motors (KIF4; Sekine et al 1994) also form homodimers. Sequence analysis of KIN C and KIN N-Chromo motors predicts an extended coiled-coil like that of conventional kinesin. However, only $\approx$50 residues of KIN I motors (C-terminal to the catalytic core) are strongly predicted to form a coiled-coil. Hence, whether the dimerization of the KIN I motors involves interactions other than a coiled-coil remains an open question.

Sequence analysis predicts that KIN N-Monomeric motors, similar to KIN I motors, contain two short regions ($\approx$50 residues) that may form coiled-coils. However, hydrodynamic studies of mouse KIF1A (Okada et al 1995) and KIF1B (Nangaku et al 1994) indicate that these motors are monomeric. Electron

microscopy confirms this result: KIN N-Monomeric polypeptides expressed in baculovirus appear globular, have a small tail, and clearly lack an extended stalk. Sequence analysis of a subset of the orphan kinesin motors, such as Nod from *Drosophila* (Zhang et al 1990), also fail to predict an extended α-helical coiled-coil, and hence they may be monomeric as well. However, the quaternary structure of these motors needs to be established by direct biochemical methods.

WHY OLIGOMERS? The KIN N-Monomeric motors are monomeric, yet they produce some of the fastest velocities observed for kinesin motors (Table 1). Thus dimerization cannot be an absolute requirement for generating motility. Moreover, monomeric fragments of conventional kinesin also generate motility in vitro (Yang et al 1990, Berliner et al 1995, Vale et al 1996). What then is the purpose of forming dimers or tetramers?

Conventional kinesin may utilize its dimeric structure to coordinate the activities of the two motor domains during movement. Accumulating evidence suggests that the two motor domains operate in a hand-over-hand manner, so that at least one motor domain is in contact with the microtubule at all times (discussed below). This enables the motor to move processively over many subunits on the microtubule without dissociating. Processive movement serves a distinct biological advantage, because one or at least relatively few motors can transport organelles or protein complexes efficiently by moving continuously for prolonged time periods. Whether all dimeric kinesin motors operate in a hand-over-hand manner, however, remains an open question.

Providing a suitable geometry for force-generation in microtubule bundles may be the reason for forming tetrameric, bipolar structures. In muscle, the bipolar nature of the myosin thick filaments enables them to pull antiparallel actin thin filaments closer together. The bipolar architecture of KIN N-Bipolar motors may serve a similar function, although owing to their polarity of movement, they would be expected to push overlapping microtubule arrays apart rather than pull them together (Kashina et al 1996a). This activity is consistent with their biological roles in separating spindle pole bodies, which are connected by an interdigitating antiparallel array of microtubules. Other types of microtubule-microtubule sliding may also be important in spindle morphogenesis (Walczak & Mitchison 1996).

More mysterious is the advantage gained from having two distinct motor polypeptides in KIN N-Hetero motors. Heterodimerization does not appear to be essential for motor function because the individuals subunits can generate movement at approximately the same speed as the heterodimer (Kondo et al 1994, Yamazaki et al 1995). An alternative possibility is that heterodimers are designed for the purpose of cargo binding rather than for motor function.

Although the motor domains are very similar, the C-terminal tail domains of the KIN N-Hetero polypeptides are distinct. Having two distinct tails (or generating different tail combinations) may be important for specifying different cargo binding.

SMART COILED-COILS The globular motor and tail domains of kinesin motors have been the object of considerable attention and interest. However, it is clear that the coiled-coil domains of kinesin motor proteins are also carefully designed for function. This is evident in the coiled-coil of conventional kinesin, which is divided into three regions separated by flexible hinges. The first coiled-coil is a ≈40 amino acid region within the kinesin neck domain. Kinesin polypeptides truncated at the C-terminal boundary of the neck domain are capable of assembling into dimers (Huang et al 1994), and synthetic peptides encompassing the neck region form stable coiled-coils in solution (Morii et al 1997, Tripet et al 1997). Thermodynamic analyses show that the first two heptads form strong coiled-coil interactions, the next two form weak interactions, and the last two again form strong interactions (Morii et al 1997, Tripet et al 1997). The weak interaction of the middle two heptads is primarily the result of three non-ideal residues (Tyr344, Glu347, and Asn351 (human kinesin residue numbers) in the hydrophobic core of the coiled-coil. Tyr and Glu are strongly conserved in many of the KIN N motors (Figure 1). Moreover, many of the electrostatic interactions across the hydrophobic core of the predicted KIN N-Conventional neck coiled-coil (e and g positions of the heptad repeat) are destabilizing. Collectively, these results indicate that the kinesin neck coiled-coil is divided into discrete subdomains and that a region of low stability in the middle of the coiled-coil has been evolutionarily conserved. This raises the possibility that the neck coiled-coil is a dynamic structure and perhaps partially unwinds during the ATPase cycle.

Distal to the neck region, the stalk of conventional kinesin contains two coiled-coil domains (the N- and C-terminal portions termed coil 1 and coil 2; de Cuevas et al 1992) that are separated by a ≈40 amino acid hinge (Figure 5). The bacterially expressed coil 1 is less stable than coil 2 and melts at a 20°C lower temperature. This differential stability is conserved in *Drosophila*, squid, and human kinesin, which suggests an important but unknown function of coil 1 and coil 2 (Goldstein 1995). The Tm1/2 of coil 1 is close to physiological temperature, and it was speculated that unmelting of coil 1 (although not essential for movement) may augment the force-generation process (Goldstein 1995). An important role for coil 2 is suggested by the presence of a highly conserved sequence among KIN N-Conventional motors, which could be involved in membrane attachment or in interactions with light chains (Hirokawa et al 1989).

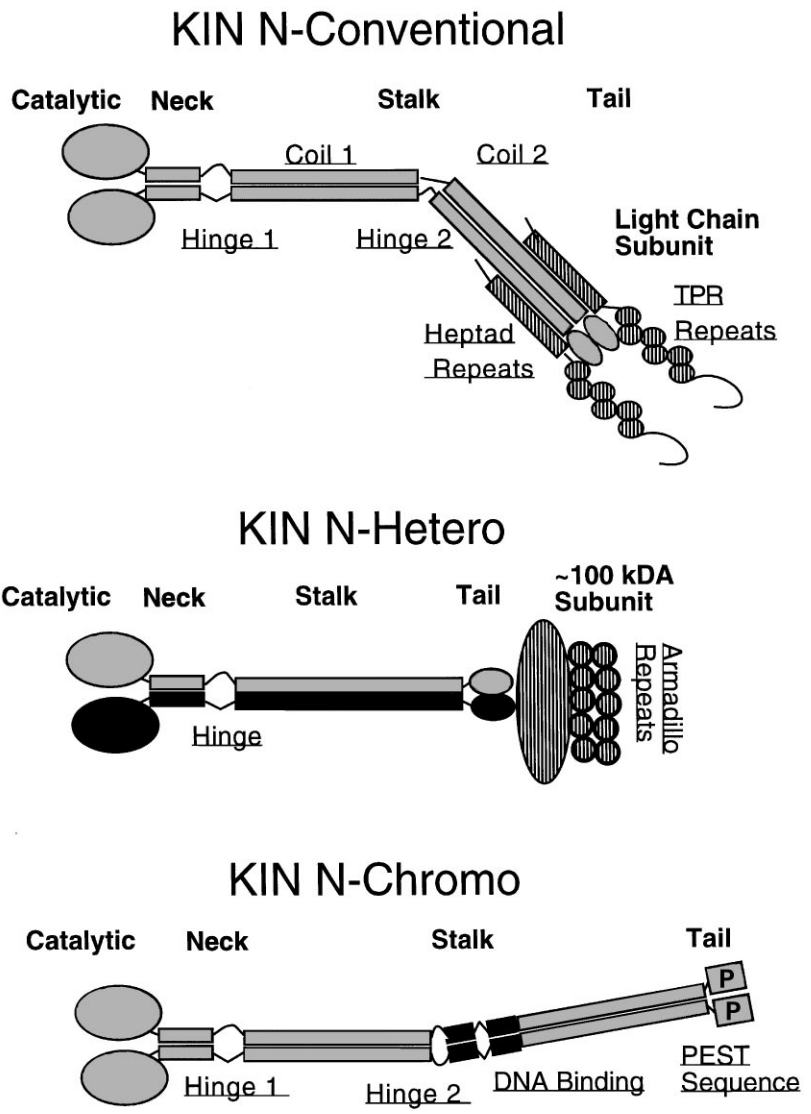


Figure 5 Architecture of kinesin motors. The overall structural features of three well-studied kinesin motors are shown here. Details can be found in the text. The precise organization of the tail domains and how subunits interact with the tail domains are speculative at this time. Motor-containing polypeptide chains are indicated (by *gray* or *black area*), and associated subunits are indicated by *hatched area.* The KIN N-Conventional and KIN N-Chromo motors are homodimers of two identical motor-containing polypeptides, whereas KIN N-Hetero motors are heterodimers of two non-identical subunits (indicated by the *black* and *gray* coloring).

Unique oligomerization properties of other kinesin motors must also be specified by their coiled-coils. From examining the sequence of the neck coiled-coil domain, Rashid et al (1995) noted a series of glutamic acid residues in sea urchin KRP85 (and the mouse homologue KIF3a) and a corresponding set of lysine residues in KRP95 and KIF3B (see Figure 1). These complementary electrostatic interactions may be important for specifying heterodimerization between KIN N-Hetero motor polypeptides. Other KIN N-Hetero motors (e.g. *C. elegans* Osm3) do not have such highly charged residues in their neck domains, which raises the question of whether these motor proteins form heterodimers using different interactions. Alternatively, they may not form heterodimers at all.

Hinges

As described above, kinesin motors contain helix-disrupting residues within their coiled-coil domains that may signify regions of flexibility. The positions of these flexible regions (termed hinges) appear to be conserved within a motor class, suggesting that they are important for motor function. Conventional kinesin, for example, contains two hinges. The first hinge is located between the neck coiled-coil and coil 1 of the stalk (human kinesin residue 370 to $\approx$415), and a second hinge is found between coil 1 and coil 2 ($\approx$amino acid 570) (Hirokawa et al 1989, Yang et al 1989, Navone et al 1992). These hinge regions contain several proline and glycine residues, which suggests flexibility.

Mechanical and geometrical considerations suggest that the first hinge serves as a swivel point for the motor domains, allowing them to rotate and position themselves independently of the geometry of the stalk/tail domains. This is illustrated by motility experiments showing that microtubules rotate about a perpendicular axis while being transported by a single kinesin molecule attached to the glass surface (Howard et al 1989). Detailed analysis of the phenomenon by Hunt & Howard (1993) showed that Brownian motion of the microtubule can twist the bound kinesin molecule by $>360^\circ$. Furthermore, the transport speed is independent of the orientation of the microtubule relative to the kinesin fixed onto the glass surface. Because of this remarkable torsional flexibility, the motor domains of kinesin can rapidly rotate and achieve the correct stereospecific binding to a microtubule, irrespective of the orientation of the tail domain on the organelle and the orientation of the organelle relative to the microtubule. Perhaps related to this observation, optical trap experiments have shown there is a slack region in the kinesin molecule that becomes taut when the molecule is pulled (Svoboda et al 1993). The structural element(s) that allows the motor domains to rotate freely and the linkage to extend have not been identified experimentally. However, it is likely that the hinge between the neck and coil 1 contributes to these phenomena.

The flexibility of the hinge between coil 1 and coil 2 was confirmed directly by electron microscopy, which shows that conventional kinesin can bend in the middle of the stalk (Hirokawa et al 1989). In fact, the flexibility of the hinge probably allows the C-terminal tail domain to interact directly with and regulate the ATPase activity of the motor domains of conventional kinesin (Hackney 1996). The coil 1-coil 2 hinge may also be important for cargo attachment; electron microscopic images of motor-organelle complexes suggest that coil 2 wraps around the surface of the organelle membrane, while coil 1 projects the motor domains away from the organelle (Hirokawa et al 1989).

Are hinges a general feature of kinesin motors that have coiled-coils? The neck sequences of the KIN N motors contain a highly conserved glycine (and sometimes multiple glycines) at the end of the neck (Figure 1). These glycines probably mark the beginning of a flexible region analogous to the first hinge in kinesin. Thus it is likely that a swivel point close to the motor domain is a common feature within the kinesin superfamily. The presence of a second hinge (analogous to the hinge between coil 1 and coil 2 in conventional kinesin) appears to be more variable. Electron microscopy of the KIF4 (KIN N-Chromo) shows a clear bend in the middle of the molecule (Sekine et al 1994) (Figure 5). On the other hand, electron microscopy of NCD (KIN C-Mitotic) (Chandra et al 1993) shows no noticeable bend in the stalk domain. Hence, stalk domain hinges may reflect specialized, class-specific functions of kinesin motors.

Interaction Domains with Other Macromolecules

TIGHTLY ASSOCIATED SUBUNITS Conventional kinesin from metazoans contains two tightly associated polypeptides of ≈60–70 kDa (termed light or β chains). There are at least two light chain genes in mammals (L Goldstein, personal communication) and several mRNAs are produced by alternative splicing (Cyr et al 1991). In *Drosophila*, the light chain is essential to kinesin function because a disruption of the gene produces a phenotypic defect that is similar to a heavy chain gene disruption (Gindhart et al 1996). Although it has been speculated that light chains are involved in cargo binding, there are no direct experiments to substantiate this claim. Fungal KIN N-Conventional motors (which contain sequence identity to metazoan kinesin motor in the tail domain), however, do not contain associated polypeptides (Steinberg & Schliwa 1995). This suggests that KIN N-Conventional motors evolved light chains later in evolution.

The binding interface between the light and heavy conventional kinesin chains was examined by Gauger & Goldstein (1993) who showed that the N-terminal third of the *Drosophila* kinesin light chain is sufficient for heavy chain binding. This region is composed primarily of an extended coiled-coil heptad repeat, and it was speculated that this light chain coiled-coil forms a

heterodimer with the heavy chain coiled-coil, possibly in a four-helix assembly (Gauger & Goldstein 1993). Consistent with this idea, the C-terminal residues of the heavy chain coiled-coil are highly conserved among conventional kinesin motors, suggesting that this region is involved in a protein-protein interface.

Tightly associated subunits have been described for several other members of the kinesin superfamily besides conventional kinesin (Table 1). Two KIN N-Hetero motors (KRP 85/95 in sea urchin and KIF3A /KIF3B in mouse) contain a ≈100-kDa polypeptide that forms a 1:1:1 complex with the two motor subunits (Wedaman et al 1996, Yamazaki et al 1996). Like the kinesin light chains, the ≈100-kDa subunit associates with the C terminus of the motor polypeptides (Figure 5).

KIN C motors may also have tightly bound subunits. The *S. cerevisiae* Kar3p motor interacts by genetic and physical criteria with a 77 kDa protein, Cik1 (Page et al 1994). Deletion of the *cik1* gene produces a phenotypic defect that is identical to a Kar3 deletion. Like the kinesin light chain, Cik1p contains a predicted coiled-coil domain. A KIN C-Mitotic motor from *Xenopus* (XCTK2) also coimmunoprecipitates with one or two other polypeptides, although they remain to be characterized (Walczak et al 1997). Calmodulin represents another potential light chain for a KIN C motor in *Arabadopsis.* Both the expressed motor polypeptide and a 23 amino acid peptide found just C-terminal to the motor domain bind calmodulin with high affinity (Reddy et al 1997), and calcium-calmodulin inhibits motor activity (Song et al 1997). However, it is not known whether the native protein from plants is normally complexed with calmodulin. Calmodulin is also a regulatory light chain of several classes of unconventional myosin motors (Mooseker & Cheney 1995).

CARGO INTERACTION DOMAINS Kinesin motor-receptor interactions are poorly understood. Best understood at a structural level are kinesin motors that are uniformly distributed over the surface of chromosomes: KIN N-Chromo motors (Vernos et al 1995, Wang & Adler 1995), HsKid (KIN N-Orphan; Tokai et al 1996), and DmNod (an unclassified orphan; Afshar et al 1995). These motors are thought to transport chromosomes away from the spindle poles so that they can migrate to metaphase plate. Interestingly, the DNA-binding motifs used by three chromosomal kinesin motors appear to be distinct from one another. Chromokinesin contains a 59 residue motif similar to the basic-leucine zipper family of transcriptional factors (Wang & Adler 1995). Constructs containing this domain bind to DNA, and deletion of the first leucine zipper region largely abolishes this activity. DmNod, on the other hand, contains a putative DNA-binding region with similarity to one used by the non-histone chromosomal proteins HMG14/HMG17 (Afshar et al 1995). In the case of HsKid, mapping

experiments have identified a 52 amino acid region important for DNA binding (Tokai et al 1996), but this region is distinct from other known DNA-binding motifs. Because the DNA-binding motifs of these motors differ from one another, it is possible that chromosomal association of kinesin motors has evolved convergently on multiple occasions.

Several protein-protein interaction motifs have also been identified in kinesin motors. The KIN N-Monomeric motors contain two sequences that bear similarity to both pleckstrin and AF-6/cno domains (Ponting 1995). The pleckstrin domain is a common motif that binds inositol phosphates as well as proteins, and *Drosophila* canoe (cno) plays a role in the Notch signal transduction cascade, possibly by mediating protein interactions. Sequence analyses of kinesin-associated subunits also reveal possible protein-protein interaction motifs. The conventional kinesin light chain has six putative tetratrico peptide repeats (TPR), likely forming an amphipathic helix that interacts with a target molecule (Gindhart & Goldstein 1996b) (Figure 5). Kinectin, a candidate kinesin membrane receptor, has an extended coiled-coil (Kumar et al 1995, Yu et al 1995), and it was proposed that the TPR repeats in the kinesin light chain interact with the kinectin coiled-coil (Gindhart & Goldstein 1996b). The $\approx$100 kDa non-motor subunit associated with the KIN N-Hetero motors was also discovered to have a series of armadillo repeats (Gindhart & Goldstein 1996a) (Figure 5), an important protein-protein interaction motif first discovered in the *Drosophila* armadillo gene product.

THE STRUCTURAL BASIS OF KINESIN MOTILITY

The study of biological motility has focused on muscle and muscle myosin for several decades. However, in recent years, an intensive effort has been directed toward understanding the structural and biophysical basis of kinesin-driven motility. Investigators have been attracted to kinesin because of the relative simplicity of its motor domain and because of the ease of preparing active protein by bacterial expression. Most studies have been performed on conventional kinesin, but biophysical studies on other kinesin motors are underway. As a prelude to discussing possible structural changes that might occur during the ATPase cycle, we first briefly discuss the motile properties of kinesin motors. More detailed descriptions of this subject and kinetic analysis of the enzymatic cycle can be found in other recent reviews (Hackney 1996, Howard 1996).

Kinesin Motility: Processivity, Step Sizes, and Forces

The conventional kinesin motor is endowed with the remarkable ability to take multiple steps along a microtubule ($>$100 tubulin subunits) before dissociating. The observation that single kinesin molecules can transport microtubules

continuously was first shown by diluting the motors to low density on a glass slide (Howard et al 1989) or bead surface (Block et al 1990) and demonstrating by statistical arguments that transport was driven by single motor proteins. More recently, single kinesin molecules have been observed moving along microtubules by a microscopic technique that can detect a single fluorescent dye molecule attached to kinesin (Vale et al 1996). Processivity has also been detected by ATPase measurements, which show that a kinesin molecule undergoes multiple rounds of ATP hydrolysis per diffusional encounter with the microtubule (Gilbert et al 1995, Hackney 1995, Ma & Taylor 1997). Processivity requires that the motor maintains almost continuous contact with microtubule, since dissociation for even 0.1% of its ATPase cycle would result in the motor diffusing away from the microtubule.

The most widely accepted model for kinesin processivity is that the enzyme coordinates the activities of its two motor domains and moves in a hand-over-hand manner. Two pieces of experimental data are consistent with this idea. First, truncated, monomeric kinesin motors are incapable of processive movement, even though they are still functional and can produce movement if many motors are interacting simultaneously with a microtubule (Berliner et al 1995, Vale et al 1996). Second, Hackney (1994) reported the intriguing observation that dimeric kinesin releases ADP rapidly from one of its heads and very slowly from the second head. This finding clearly indicates that the two heads are not equivalent and suggests the possibility that the chemical cycles of the two heads work in an alternating manner. This idea has gained further support from experiments by Ma & Taylor (1997), comparing kinetic properties of monomeric and dimeric kinesin.

Are other motors in the kinesin superfamily also processive? This question is not resolved. However, single molecule fluorescence motility assays with the *C. elegans* Unc104 motor (KIN N-Monomeric) (D Pierce & R Vale, manuscript in preparation) and *Drosophila* NCD (KIN C-Mitotic) (Case et al 1997) have failed to detect processive movement. These results demonstrate that processivity or the degree of processivity may vary considerably among different kinesin motor classes.

During processive movement, kinesin follows the path of a microtubule protofilament (Ray et al 1993). This could occur by two general mechanisms (Block & Svoboda 1995). First, the two kinesin heads could straddle two adjacent protofilaments and the motor would move by waddling between the two. Alternatively, motion could be confined to a single protofilament, and the two heads could undergo alternating hand-over-hand movement. Although the latter notion seems more likely, it is interesting that kinesin is unable to move along zinc-induced macrotubes (Ray et al 1995), an alternative microtubule form in which adjacent protofilaments are antiparallel instead of parallel. This

result suggests that kinesin either moves along two adjacent protofilaments or interacts with the groove between two protofilaments.

A number of microscopy techniques have been developed to assay discrete displacements taken by single kinesin motor proteins. The processivity of kinesin has facilitated such analyses because kinesin, in contrast to myosin, executes multiple mechanical steps per encounter with the microtubule. Pioneering studies by Svoboda et al (1993) using an optical trap microscope with a high-resolution motion detector showed that dimeric kinesin moves in 8-nm incremental steps along a microtubule. The dimension of these steps, which also have been observed by other investigators (Coppin et al 1996, Higuchi et al 1997), corresponds to the distance between sequential α/β tubulin dimers along a microtubule protofilament. These findings indicate that the kinesin motor undergoes a step-wise motion from one binding site to the next along a microtubule, with each step corresponding to the hydrolysis of a single ATP molecule (Hua et al 1997, Schnitzer & Block 1997). The 8-nm step size does not necessarily reflect the magnitude of the protein conformational change taken by kinesin, which may be considerably smaller (discussed below). The timing of the power stroke (the force-generating conformational change) in the ATPase cycle also has not been fully resolved, although it is generally agreed that it occurs after the ATP hydrolytic step and is probably associated with product release (either Pi or ADP) (Gilbert et al 1995, Higuchi et al 1997).

Several groups have also determined that a single kinesin molecule can exert maximal forces of 5–7 pN (Hunt et al 1994, Svoboda & Block 1994, Meyhofer & Howard 1995, Coppin et al 1997). Combined with an 8-nm step, this would result in work production of ≈40 pN-nm per ATPase cycle, which would correspond to an efficiency of ≈50% (maximal energy from ATP hydrolysis is ≈80 pN-nm). A biophysical characterization of other motors in the kinesin superfamily has not been reported, although several groups have begun such investigations.

Remarkably, members of the kinesin superfamily can move either to the plus-end or minus-end of microtubules, although a given motor only moves in one direction. All KIN C motors tested thus far move to the minus-end, whereas KIN N and KIN I motors thus far move to the plus-end. The orphan motors are less well characterized. Truncation experiments have shown that the direction of movement is an intrinsic property of the motor domain (i.e. the catalytic core plus neck region) and does not involve the stalk or tail (Stewart et al 1993). Chimera experiments between KIN C and KIN N motors (discussed previously) suggest that the neck region may dictate the polarity of movement.

Possible Structural Changes in the Motor Domain

Ultimately, one would like to describe biological movement in terms of a sequence of structural changes that occur in the motor and polymer during the

enzymatic cycle. A considerable effort has been devoted to these issues for the myosin motor. The most widely accepted model for myosin proposes that the catalytic core binds to the actin filament in a fixed orientation and that a long helix C-terminal to the catalytic core undergoes a large lever-arm like motion that powers the movement of the motor relative to the polymer. This swinging cross-bridge theory has received recent experimental support, although uncertainties remain (reviewed in Block 1996). Given that kinesin and myosin share a similar fold, it is natural to wonder whether these two motors share a similar conformational change strategy for transmitting information from the nucleotide-binding site and whether kinesin, like myosin, uses a lever arm to power movement. The definitive answers to these questions must await the crystallization of kinesin in different nucleotide states, as well as in a kinesin-tubulin complex. However, the existing crystal structures, in conjunction with functional studies of kinesin, provide some clues into possible similarities and differences between kinesin and myosin.

As discussed above, the functional kinesin motor domain has two components: the catalytic core and the neck. The catalytic core is the information transducer that undergoes conformational changes in response to transitions in the ATPase cycle (e.g. binding of ATP, ATP hydrolysis, and release of products). The crystal structure of myosin's catalytic core has been obtained in different nucleotide states (e.g. Smith & Rayment 1996), and the structural element that undergoes the largest nucleotide-dependent change has a counterpart in kinesin (α4: the 45° canted helix on the side opposite to the nucleotide). Because the α4 helix in kinesin is connected at its C terminus to the switch II loop, which contacts the nucleotide, and at its N terminus to a loop that interacts with microtubules, it is possible that microtubule affinity could be altered by events occurring in the active site via the α4 helix. Thus it is likely, based on many common structural features in their catalytic cores, that the mechanisms for sensing whether ATP or ADP is in the active site and is for transmitting this information to the rest of the molecule will have similarities in kinesin and myosin motors.

The kinesin catalytic core is a compact, single domain unlikely to undergo large conformational changes ($>$10 Å) that may be necessary to elicit motion. However, the catalytic core may work in concert with a mechanical transducer that is responsible for force and motion. In the case of kinesin motors, we propose that the mechanical transducer is the class-conserved neck region discussed above. The location where the necks join the catalytic cores in KIN N motors (the C terminus of α6) and KIN C and I motors (the N terminus of β1) is close in space to the beginning of the lever arm helix of myosin. Does this indicate that kinesin will also operate by a lever arm mechanism?

Although information on this question is sparse, the current data suggest that kinesin may not operate by a lever arm. Most significantly, deletion of

the helical region in the kinesin neck (which forms a coiled-coil) produces a monomeric motor that is still capable of movement, albeit at a fivefold reduced velocity and without the capability of moving processively as a single motor (Yang et al 1990, Berliner et al 1995, Vale et al 1996). This does not rule out the possibility that the coiled-coil helix in the kinesin neck undergoes a lever-arm swing during the ATPase cycle that augments motility, but it strongly suggests that such an action is not essential. The fact that myosin and kinesin appear to differ in structure beyond catalytic core also argues that they may have evolved different mechanical transducers.

How does kinesin's mechanical transducer work? Truncation experiments on conventional kinesin suggest two different roles for the neck domain in motility. First, the catalytic core can work in conjunction with a short segment of the neck (10–15 amino acids, the majority of which may form a β-strand structure) thus producing a functional, although non-processive conventional kinesin motor. This first segment of the neck (also termed the neck linker region to distinguish it from the coiled-coil segment of the neck) could function as a spring-like element, contracting or extending to produce force. Hence, it is essential to determine the structure of this short stretch of amino acids and resolve how it changes conformation during the ATPase cycle. The second role of the neck domain in motility involves the helical coiled-coil portion of the neck, which appears to be essential for processivity of single kinesin motors. Nucleotide-dependent conformational changes in this region may be necessary for communication between the two kinesin heads. One model proposes that a segment of coiled-coil unwinds to allow the partner head in the dimer to bind to a microtubule (Tripet et al 1997), but this idea awaits experimental verification.

Our image of how different elements in motor proteins contribute to motility, although blurry, is intriguing and surprising. The information transducer (the catalytic core) is very well conserved and, perhaps, can be traced back to a common ancestor of both G proteins and molecular motors. However, the mechanical transducer that is connected to the catalytic core (the neck region for kinesin and the lever arm helix for myosin) can differ dramatically in sequence and presumably in structure as well. This is dramatically illustrated by the different positions of the neck relative to the N and C termini of the catalytic core in KIN C and KIN N motors. To a molecular engineer, the variation in the putative mechanical transducers is surprising given the degree of cooperation that has to occur with the catalytic core and between the two motor units within the dimer. On the other hand, what present data may be revealing is that the most accessible means for evolving new motors with very distinct properties may be by constructing a new mechanical transducer, although changes

in the catalytic core must occur as well. If this is true, then biophysical and structural studies of different classes of kinesin motors would be an important step toward understanding motor mechanism. As has emerged from comparisons of kinesin and myosin, common and distinct structural and mechanical strategies for generating movement within the kinesin superfamily may become apparent.

CHALLENGES FOR THE FUTURE

Ten years ago, our *Annual Review of Cell Biology* article on kinesin contained hardly any structural information (Vale 1987). In the intervening decade, a staggering amount of information has been gathered on this protein and its relatives. While much progress has been made, large questions remain unanswered or unexplored. First, although the structure of the motor domain has been solved and high resolution measurements of kinesin motility have been made, this information clearly has not answered the question of how movement is generated. Because motor proteins undergo a choreographed cycle of conformation states as ATP hydrolysis is converted to movement, much will be learned from high resolution structures of various stable intermediate states in the cycle and of a motor-tubulin complex. In addition, although this review has argued for the importance of the kinesin necks as key regions that determine motor function, very little is known about their structures and activities. Furthermore, while the motor domain has taken center stage in the first decade of kinesin research, the tail domains will undoubtedly become a subject for increasing research efforts. Indeed, receptors for tail domains are expected to be identified, and NMR and crystal structures of tail domains and tail-receptor complexes will provide clues into the specificity and regulation of these important protein-protein interactions that govern the biological actions of kinesin motors.

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