Kinesin and myosin: molecular motors with similar engines

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Structure determination of the catalytic domains of two members of the kinesin superfamily reveals that this class of molecular motor exhibits the same architecture as myosin and suggests that these microtubule- and actin-based motors arose from a common ancestor.

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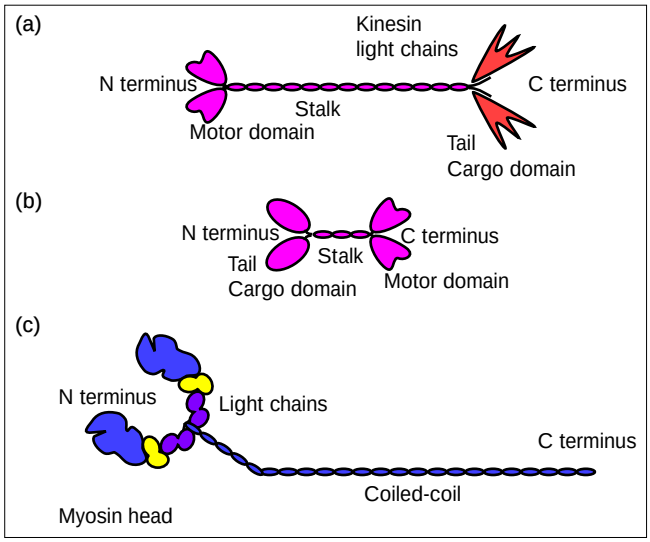
It is obvious that movement, in one form or another, is an essential feature of all life at both the macroscopic and cellular level. Molecular motility participates in many cellular functions including cell division, intracellular transport and movement of the organism itself. Thus, it is not surprising that nature has evolved a series of related molecular motors that fulfill many of these tasks. These fall into three general classes or superfamilies: myosin, dyenin and kinesin. Myosin moves along actin filaments and kinesin and dyenin operate on microtubules. Whereas the existence of myosin and dyenin has been known for a long time from their participation in muscle contraction and movement of cilia and flagella, the kinesin superfamily is a comparatively recent addition to the repertoire of molecular motors [1]. The recent reports of the structures of the motor domains of two microtubule motors, kinesin and NCD (non-claret disjunctional), represent giant steps forward in the study of the molecular basis of motility [2,3]. Kinesin functions in axonal transport and NCD is involved in chromosome segregation in Drosophila meiosis [4]. These structures are the first for this class of molecular motors and are fascinating for many reasons, as described below.

Even though the kinesin family has only been recognized for a comparatively short period of time, enormous progress has been made towards understanding the function of this class of molecular motor. This has arisen because proteins in the kinesin superfamily play important roles in cellular function, including organelle transport, meiosis, and mitosis, and are well suited to manipulation with the tools of molecular biology [4]. In addition, the development of *in vitro* motility assays and instruments that can measure the physical and mechanical properties of single molecules has provided the tools necessary to establish the fundamental properties of these proteins [5]. These studies have shown that kinesins are a diverse family of proteins [4]. Most members appear to be

Figure 1



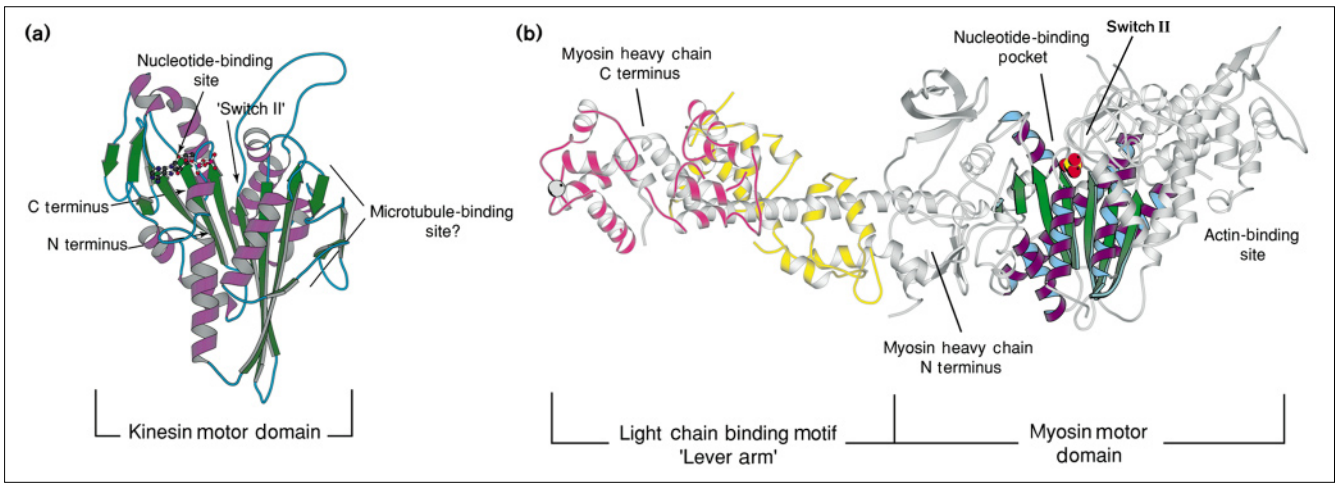
Schematic representation of the architecture of (a) kinesin, (b) NCD (non-claret disjunctional) and (c) skeletal muscle myosin (myosin II). Two light chains are shown attached to the motor domain of myosin and these serve to increase the magnitude of the power stroke.

dimeric molecules that contain a stalk, a tail-like domain and two globular motor domains. The tail-like domain sometimes includes non-covalently attached light chains and is presumably important for transport specificity or organelle recognition (Fig. 1). The globular tail is sometimes referred to as the cargo domain to indicate its role in organelle movement. There are many variations in the size and arrangement of the stalks and tails relative to the motor domains [6].

One of the unusual features of the kinesin superfamily of molecular motors is the existence of two classes of molecule: one, as represented by kinesin, that moves towards the plus end of the microtubules, and a second, as represented by NCD, that moves towards the minus end. This feature is radically different from myosin which only moves in one direction on the actin thin filament and is all the more surprising as this property resides in the motor domains, which share ~40% sequence identity. Also, it appears that kinesin and NCD interact with the same site on tubulin [7,8] and have similar step sizes [5,9,10]. However, the molecular organization of kinesin and NCD do differ as the motor domain resides at the N terminus of kinesin and C terminus of NCD.

The three-dimensional structures of kinesin and NCD in the presence of Mg-ADP (at 1.8 and 2.5 Å resolution,

Figure 2



Ribbon representations of (a) the kinesin motor domain and (b) the chicken skeletal myosin subfragment-1 [13]. They are oriented to look into the nucleotide-binding pocket which is identified by the location of ADP (ball-and-stick representation) in kinesin and a sulfate ion (space-filling model in red and yellow) that binds to the P-loop in myosin subfragment-1. The secondary structural elements in kinesin that are also found in myosin are colored in green (β strands) and dark blue (α helices) and the remainder of the myosin heavy chain is rendered in grey. Kinesin and myosin are drawn to similar scales. (a) was kindly provided by Jon Kull and Robert Fletterick and was modified from [2], with permission.

respectively) now provide the missing link between the biochemical and biophysical properties of members of this superfamily and their cellular function [2,3]. These show (Fig. 2a) that the motor domain is arrowhead-shaped with dimensions of 70×45×45 Å and built around an eight-stranded mostly parallel β sheet with three major α helices on either side. Kinesin and NCD motor domains are very similar with a root mean square (rms) difference of 1.2 Å between 146 structurally equivalent Cαs. The major differences occur in the loops. The nucleotide-binding site is very similar in both proteins and is located in a shallow groove that lies at the base of the arrowhead, above the plane of the β sheet, and exhibits a classical P-loop [11,12] motif. However, the surface loops that surround the entrance and exit to the active sites differ in kinesin and NCD and may contribute to the opposite direction of movement of these two molecules, through alteration of the kinetics of nucleotide release and binding of the motor domains to microtubules [3].

The most remarkable and totally unexpected finding in the structures of kinesin and NCD is that their topologies and tertiary structures are exceedingly similar to the core of the myosin motor domain [13,14] (Fig. 2). Indeed, seven of the eight β strands and six of the α helices overlap with an rms difference of 3.5 Å. This is surprising as there is no significant amino acid sequence similarity, except for residues that coordinate the magnesium-triphosphate component of ATP, between these proteins, and because there is enormous difference in their sizes. Close examination of the nucleotide-binding site shows that the residues that coordinate the α- and β-phosphates and form the putative γ-phosphate binding site are similar in kinesin, NCD and myosin. There are also structural homologs in kinesin for those sections of myosin that change conformation during ATP hydrolysis [13] and that share similarity with the G-proteins [2,11]. At least four regions have been identified in the G-protein α-subunit that exhibit a conformational change between the GTP and GDP bound states [15,16]. In kinesin and myosin, structural elements equivalent to switches I and II are found. These correspond to the metal-binding loop and the DXXG motif, respectively [17,18]. In both myosin and the G-proteins, nucleotide hydrolysis is coupled to a conformational change in switch II that serves to transmit a signal beyond the active site. For myosin, this change appears to prime the molecule for its subsequent force producing step. These observations suggest that the myosin and kinesin superfamilies might have arisen from a common ancestor [2]. This raises the possibility that these proteins convert chemical energy into directed movement by similar molecular mechanisms. However, the comparison between the motor domains of kinesin and myosin (Fig. 2) poses several major questions and points to significant differences between these motors.

It is obvious that the myosin head is considerably larger than the motor domain of kinesin and yet kinesin has a step size of ~80 Å [5] which is similar to that of myosin [9,10] (the step size for myosin is still the subject of considerable discussion). The light chain binding motif in myosin has been shown to amplify the conformational changes that originate in the motor domain [19]. Most kinesins and myosins are dimeric and contain two motor domains that are in close proximity and are usually held together by a section of coiled coil. For kinesin it is clear

that there is an important interaction between the two heads that confers processivity to this motor protein [20] such that a single kinesin molecule is able to remain in contact with and move along a microtubule for a considerable length of time [21]. This suggests that kinesin moves along the microtubules in a 'hand over hand' fashion; the force generating head does not leave the microtubule until its neighbour is attached. This highly processive nature is lost in single-headed kinesin, although a single head can still generate movement at a velocity similar to that of the intact molecule [22]. Thus, although the connection between the heads is important, the ability to produce a significant power stroke must reside in a single head. In contrast, it has been difficult to establish any interaction between the two heads of myosin therefore it appears that these heads operate independently.

There are also significant differences between these motor proteins in the coupling of the chemical cycle to the energy transduction step. In the case of kinesin, it appears that the slow step is the release of ADP; for myosin the rebinding of the metastable myosin–ADP–P_i complex to actin and release of phosphate is clearly rate limiting [23,24]. It is possible that the differences between kinesin and myosin in the loops that surround the nucleotide-binding pocket might account for the alternative rate limiting steps. However, there appears to be a more fundamental difference in the way these molecules work as the kinesins spend a great part of their duty cycle bound to the microtubules whereas myosin remains actin-bound for a very small proportion of the total cycle [23,25]. This may be indicative of different molecular strategies for transmitting the conformational changes associated with ATP hydrolysis into directed movement.

These observations do not answer the question of how kinesin is able to generate a power stroke that is comparable with that of myosin when it is less than one third its length! Unfortunately, not much is known about the detailed interaction between the kinesins and microtubules. The situation is much better for myosin as the structure of actin is known [26] and a model for the interaction between myosin and actin, based on a high resolution image reconstruction and an actin filament model, has been proposed [27]. These studies have identified the sections of the myosin molecule that interact with actin and suggested a communication route between the nucleotide-binding site and the actin-binding interface. This is mediated through the movement of domains whose positions are sensitive to the contents of the active site. These domains are replaced in kinesin and NCD by a series of shorter loops (Fig. 2).

Thus, it is difficult to propose a model for how kinesin functions as a molecular motor on the basis of the structure alone. However, the analogy with myosin and the G proteins provide the basis for speculation about which parts of kinesin and NCD bind to microtubules and are responsible for communicating and amplifying the changes in the active site induced by ATP hydrolysis. The location of conserved residues also provides clues to the location of the microtubule-binding site [2,3]. It is interesting that the C terminus of kinesin lies in a similar location to the reactive cysteine residues in myosin that are well known to undergo a major conformational change during the contractile cycle [28] (although this is still not well understood). Structural studies of the motor domain of *Dictyostelium* myosin complexed with MgADP vanadate, an analog of the transition state for hydrolysis, shows that this section of the molecule moves considerably during this transition from the nucleotide-free state to the metastable myosin–ADP–P_i state [29]. Furthermore, in myosin there is a series of domain–domain contacts that couple the C terminus of the molecule, that contains the reactive cysteine residues, to the actin-binding site. In the current structures of both kinesin and NCD there does not appear to be a direct connection between the putative microtubule-binding site and the C terminus or transducing region of the motor domain. The domain that fulfills this role in myosin is absent in kinesin and NCD. As noted, it is conceivable that the interaction between these sections of the kinesin and NCD might occur, in the ATP-bound state or at the transition state, through a change in the section analogous to switch II of the G proteins [2,3,18]. An alternative possibility is that tubulin plays the role of this missing domain and hence is an active participant in the generation of movement. This is conceivable as there is evidence that the structure of the microtubule changes upon NCD binding [8]. Such a hypothesis would account for the smaller size of the kinesin motor domain relative to that of myosin.

There remain many questions to be answered including the nature of the communication between the two heads of kinesin and the role this plays in energy transduction. There is also the question of how the same protein fold can move in two directions. Answers to these questions will doubtless be forthcoming with the application of structural and molecular biology in conjunction with *in vitro* motility and single molecule studies. It can truly be said that this area of research in structural biology is moving!

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