Myosin and kinesin: mother and child reunited

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The recent solution of the crystal structure of the kinesin motor domain reveals striking similarities to the core region of the myosin motor domain, implying a strong evolutionary relationship between these two motors. However, a complete understanding of the way that motility is generated will require additional structural information, which may explain how the two motors have adapted to their fundamentally different linear substrates, F-actin and microtubules.

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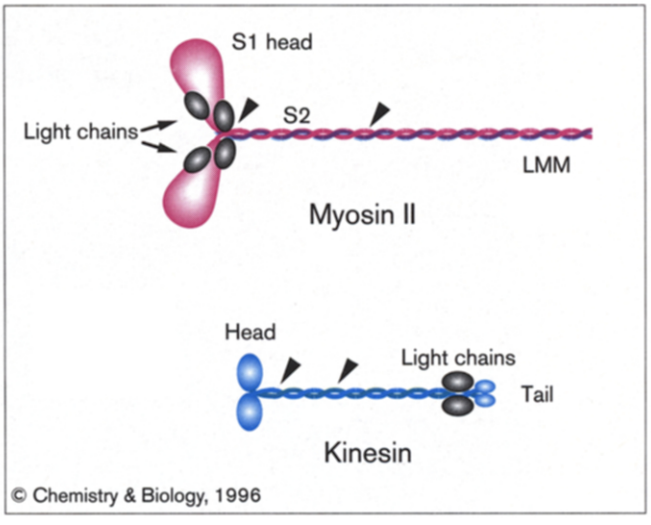
The discovery of kinesin [1] generated considerable excitement, as this molecule produced movement, as does the well-established myosin ATPase, but unlike myosin (which moves along actin filaments and is responsible for the contraction of muscles) this motor moves along microtubules. Kinesin was of great interest to cell biologists, because microtubule-based motors were believed to be involved in many important processes such as vesicular transport and mitosis, but none of these postulated cytoplasmic motors had been previously isolated. It was also of great interest to enzymologists because, on the basis of their very different substrates, kinesin and myosin were expected to be highly divergent evolutionarily. Thus kinesin might have represented a novel solution to the problem of generation of motility, and study of this molecule would provide an opportunity to determine if there were any general rules about how a biological motor could convert the chemical energy of ATP hydrolysis into mechanical energy. The belief that these two systems were very different was reinforced by the cloning of the gene for the heavy chain of kinesin [2], which has no sequence homology with myosin except for the presence of a P loop, which is common to many enzymes that bind nucleotide triphosphates and interacts with the γ-phosphoryl group (see [3]).

Domain organization

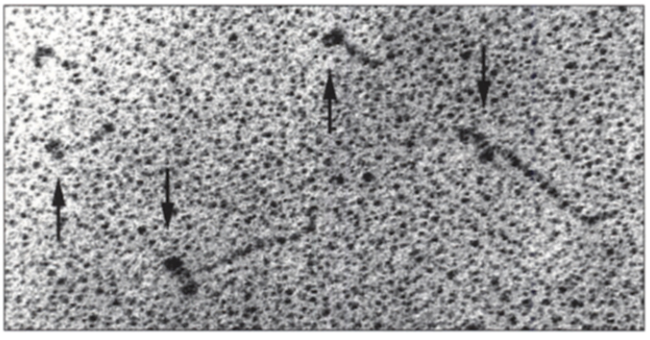
Despite the lack of sequence homology between kinesin and myosin, there is some homology in their domain organization and in the way that this is related to the generation of motility. As shown in Figure 1, both kinesin and conventional myosin II have a three-domain organization; two globular motor domains (heads) contain the active site for ATP hydrolysis and the site for interaction with the filament that provides the road along which movement will occur, and a tail domain attaches the motor to its cargo. A flexible linker region connects the motor to the cargo-binding tail. Both motor proteins are composed of two identical heavy chains and associated light chains. The amino-terminal portion of each heavy chain forms a globular head domain, and the carboxy-terminal region forms an extended α helix. The helical regions from two monomers are wound around each other in a coiled coil that dimerizes the heavy chains. For myosin, the head domain is designated subfragment-1 (S1), and the linker region consists of part of the coiled-coil region, designated S2, extending to the only major discontinuity in the coiled-coil. The remainder of the coiled-coil region, extending from the discontinuity to the carboxyl terminus, is designated light meromyosin (LMM), and this domain is responsible for aggregation of myosin molecules into filaments. In effect, the cargo for myosin is the other myosin molecules in the filament. The combined S2 and LMM coiled-coil regions are referred to as the rod. In kinesin, discontinuities in the predicted coiled-coil are more extensive, with major gaps occurring around amino-acid positions 400 and 600, and there is a small nonhelical domain at the carboxyl terminus. With myosin, two different light chains bind to the back of the head of each heavy chain near the 'neck', whereas kinesin contains only one light chain that binds to the tail region. Other members of the myosin and kinesin superfamilies share the same motor units, but have different arrangements of the other domains [4,5]. Some are monomers, and the ncd subfamily has the

Figure 1



Domain organization of myosin and kinesin. Arrows indicate the approximate locations of major hinge regions. Light chains are indicated by gray ovals. LMM, light meromyosin. See [20] and references therein for reviews and background on the comparative structure and function of myosin and kinesin.

Figure 2



The mother and child reunion. Electron micrograph of myosin (rabbit skeletal) and kinesin (without light chains) obtained by rotary shadowing. The arrows are near the head domains and are pointing up for kinesin and down for myosin. The two head domains of kinesin are not totally resolved at this resolution. The designation of mother and child is in reference to the similar structures, but very different size, as in a parent and a child. It is not meant to imply that kinesin necessarily evolved from myosin. Micrograph courtesy of J. Suhan.

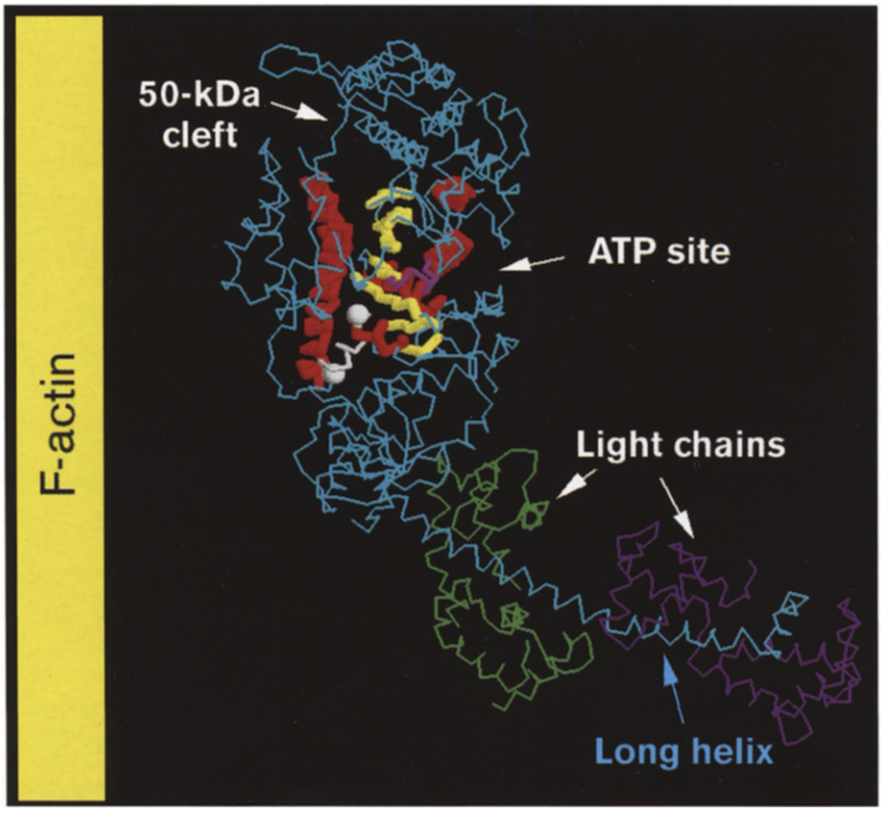
kinesin-related motor domain at the carboxyl-terminus and moves in the opposite direction to kinesin.

One obvious difference between myosin and kinesin is that kinesin is considerably smaller and simpler. In particular, the head domain of kinesin is only 38 kDa and does not contain light chains, unlike myosin S1, which has a mass of ~130 kDa, including the two light chains. The similarity in overall organization but considerable difference in size is illustrated by the electron micrograph (EM) in Figure 2, which shows both myosin and the heavy-chain dimer of kinesin.

Structure of the myosin head

The first high resolution structure of a motor domain was that of the myosin S1 fragment [6] (Fig. 3). The amino-terminal 800 amino acids of the main chain (cyan, with specialized areas highlighted in other colors) folds into a thick globular shape, and contains a long α helix that includes the light-chain-binding region. This is followed by the hinge that attaches S1 to the rod. The two light chains (green and purple) are similar to the calcium-binding protein calmodulin and wrap around the long, heavy-chain α helix, presumably producing a more rigid structure. The S1 heavy chain has a core region composed of a seven-stranded β sheet (yellow) with surrounding helices (red). The view in Figure 3 is oriented with the actin-binding region of S1 to the left. A prominent feature of the actin-binding region is a cleft (called the 50-kDa cleft) that extends towards the face of the central β sheet. This cleft is probably involved in the conformational changes that link actin binding to the ATPase cycle, as the cleft is open in the X-ray crystal structure of isolated S1,

Figure 3



α-Carbon tracing of myosin S1 produced with RasMol from the coordinates of chicken S1 [6]. This view is in a similar orientation as that in Fig. 6a of Rayment and Holden [10], except that the molecule is rotated 180° so that the P loop is in front. Cyan, 800 amino-terminal amino acids of the chicken myosin heavy chain (with specialized areas highlighted in other colors); magenta, P loop; yellow, β sheet of the core region; red, α helices of the core region; white, first helix after the conserved core, containing two cysteine residues (white balls) that can be readily crosslinked when nucleotide is bound to the myosin head; green and purple, myosin light chains.

but appears to be closed in the EM reconstructions of S1 bound to actin [7]. The ATPase active site is in a small cleft on the opposite side of the β sheet from the 50-kDa cleft. The P loop (magenta) connects a strand of the β sheet and a helix at the center of the core region.

Historically, the whole S1 unit has been referred to as the head, with the neck being defined as the junction region with the rod (including, in some cases, the extreme back of S1, which appears more narrow by electron microscopy). From the high-resolution structure it is clear, however, that S1 consists of two fundamentally distinct sections: the thick globular domain composed entirely of the heavy chain, and the long, heavy-chain α helix and its bound light chains. The thick globular region has the ATPase and actin-binding sites and is now referred to as the motor region, while the whole light-chain-binding region is defined as the neck or lever arm. Each of these regions constitutes a stable folding unit, and their structures have been separately determined [8,9].

The currently favored hypothesis for how myosin generates movement is that ATP binding and hydrolysis produces relatively small conformational changes in the motor region, and that these changes are amplified, by movement of the rigid, light-chain-binding domain, into much greater displacements at the end of this region where it attaches to the rod [10]. The rigid light-chain-binding region thus acts as a lever arm, with a pivot point in or near the motor region. Such nucleotide-dependent movements of the lever-arm region have recently been observed with S1 attached to actin [11].

Kinesin structure

The recent solution of the high resolution structures of the head domains of kinesin and its relative ncd by Fletterick, Vale and coworkers [12,13] has shown that they also have a core composed of a β sheet with surrounding helices. When the kinesin and myosin structures are positioned such that the P loops are aligned, the other core elements are also aligned. Thus the core of the kinesin structure, to a first approximation, is equivalent to the core of the myosin structure (depicted in yellow and red in Fig. 3). This common core structure is not present in other proteins that contain P loops, and the striking structural similarity between kinesin and myosin implies a much closer evolutionary relationship between the two motor families than was originally believed.

The differences between kinesin and myosin are mainly in the length of the structural elements, in particular, the lengths of the linking regions that join the core elements together (not highlighted in Fig. 3). These linking regions are much larger in myosin and account for most of the additional mass of the myosin motor unit. For example, helices 4 and 5 of the kinesin structure are joined by a short linker of 9 amino acids, whereas the corresponding linking region in myosin is 140 amino acids long. This region is particularly noteworthy, as it constitutes a part of the actin binding region of myosin and is a candidate for the region where kinesin and microtubules interact, as noted by Kull *et al.* [12]. This loop contains solvent-exposed sidechains that are conserved and may be involved in the interaction with microtubules. The interaction of kinesin with microtubules is also much stronger at low ionic strength; the source of this effect was not clear as microtubules have a large net negative charge, but kinesin heads do not have a significant net positive charge. The structures of both kinesin and ncd are now seen to have conserved patches of positively charged sidechains in the postulated microtubule-binding region that could be responsible for the strong salt dependence of binding, as postulated by Sablin *et al.* [13]. Recent results indicate that mutants in this region interfere with the stimulation of motor ATPase activity by microtubules (G. Woehlke, A. Ruby and R. Vale, personal communication).

Another noteworthy difference in the structures is that the active site of kinesin is more open than that of myosin, despite the fact that the rate of release of the bound ADP is much slower for kinesin than for myosin. The myosin active site also differs from that of kinesin in that the γ-phosphoryl group of ATP is at the bottom of a narrow channel and may depart the active site by a 'back door' mechanism, using a different route than the one used by ATP to enter the channel [14]. Significantly, the region of myosin that is similar to kinesin is at the junction of the major structural regions of the myosin motor unit, and thus kinesin has components contributed from all these regions, although they are smaller in scale.

Conformational changes

The original structure of S1 was obtained in the absence of nucleotide bound to the active site and thus should correspond to the nucleotide-free head of myosin unattached to actin. This structure, in combination with the EM reconstruction of the actin-bound form, constrains the types of conformational changes that may be responsible for production of motility during ATP hydrolysis and has led to a useful working model [7,10]. Direct observation of the conformation in other nucleotide-bound states will, however, be needed to determine definitively the mechanism of coupling. The recent determination of the structure of the motor domain with bound ADP and either beryllium or aluminum fluoride [9,15] provides further insights into these changes. Surprisingly, however, the changes in conformation around the nucleotide site are relatively modest. This may be due in part to crystal packing forces that prevent observation of the full extent of the conformational changes. Also, the nucleotide-free structure has a sulfate group, from the solvent, bound to the site to which the

γ phosphoryl group of ATP binds, and thus may not truly correspond to a totally nucleotide-free state.

Several other results indicate that the conformation observed in the crystal structures may not correspond exactly to that in solution. Myosin contains two highly reactive cysteine groups known as SH1 and SH2 that can be readily cross-linked when nucleotide is bound. In the crystal structure of nucleotide-free S1, these two cysteines are on a short helix directly following the last helix of the core region (highlighted in white in Fig. 3 with the two cysteines as small white balls). These are on opposite sides of this helix and could not be crosslinked without major conformational rearrangement. It was initially anticipated that nucleotide binding would produce conformational changes that would make crosslinking favorable, as is observed in solution, but this helix is preserved in the structures with bound nucleotide that have been recently obtained [9]. An additional observation is that solvent-phase quenching of a fluorescent group attached to the ribose 2' or 3' hydroxyl of ADP indicates that it is not exposed when bound to myosin [16], and yet it should be exposed based on the crystal structure. Comparison of the myosin and kinesin structures with that of the G proteins that are responsible for GTPase-dependent signaling has provided insight into the likely common mechanisms for triggering a conformational change in response to hydrolysis of the triphosphate [13]. Although most of the core elements of myosin and kinesin show no homology with the G proteins, a number of the components of the nucleotide-binding sites are functionally equivalent, with either identical residues or conservative changes in key positions.

Perspectives

Clearly we have come a long way in the last several years with the determination of the structure of the myosin head and now the knowledge that kinesin is built from the same core. Furthermore, key elements of this signaling mechanism may extend to G proteins, which do not share this core structure but may still have common elements around the active site that potentially act as conformational switches [13]. It is now important to determine how such similar structures produce different kinds of motility or signaling. For example, the ncd motor domain is very similar to that of kinesin, yet it moves in the opposite direction. Kinesin lacks the long α helix of myosin in the light chain region, but is still able to take steps of 8 nm and to be highly processive, as determined by both motility data and ATPase kinetics [17,18]. The processivity of kinesin is probably related to the fact that the head domains of the dimer can cooperate in a coordinate head-over-head manner. Solving these questions will require structural information on how the kinesin and ncd motor domains are attached to their coiled-coil regions and how the two motor domains in the dimer interact. An even greater challenge is to determine the structure–function relationships of members of the third major family of motors, the dyneins, which make myosin look small and simple by comparison. The details of those conformational switches will be hard to decipher, however, from just crystal structure determinations. Complementary determinations of structure for motor domains in solution and bound to actin or microtubules will be required. The recent observation of nucleotide-dependent conformational changes of kinesin while bound to microtubules by Hirose *et al.* [19] is an encouraging step in this direction.

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