

Fifty Ways to Love Your Lever: Myosin Motors

Review

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A Century of Effort

The ability to produce directed motion is a distinguishing characteristic of practically all living organisms. Over a century has passed since Kühne first extracted the proteins actin and myosin from muscle (Squire, 1981), yet the molecular origin of the force produced between these two components remains one of the outstanding puzzles in biology. Progress towards an understanding of how muscles develop force was made in the middle of this century with the development of the sliding filament model, immortalized today in undergraduate cell biology textbooks (for historical perspectives, see A.F. Huxley, 1980; H.E. Huxley, 1996). It is now well-established that molecules of the ATPase myosin bind to, and slide along, filaments of actin. In fact, direct visualization of this motion at the macromolecular level is possible today, using *in vitro* motility assays consisting of purified components (Scholey, 1993). The force produced by actomyosin is not only harnessed on a grand scale in muscle, but also underlies a host of microscopic motions, including cell motility, cytokinesis, vesicle transport, and cellular shape changes. Along with myosin, other linear motor families have since been identified, including dynein and kinesin, which move along microtubules. There is reason to believe that the molecular mechanism of these other motor proteins may, at the end of the day, resemble that of myosin. But what physical and chemical changes hold the key to the action of motor proteins? And how is the hydrolysis of ATP thereby coupled to motion? These fundamental questions have proved very hard to answer.

Over the decades, the motility problem has spawned endless debates and countless numbers of competing models. A vast literature of biochemical and biophysical data has been amassed, particularly for myosin, which is arguably the best-characterized of proteins (Squire, 1981; Bagshaw, 1993). Despite the wealth of information, the fundamental questions remain. Testifying to the ongoing controversy, there is not even consensus about whether movement is powered by changes taking place primarily in the myosin head — the conventional dogma — or by shape changes within the actin filament itself (e.g., Schutt and Lindberg, 1992), and both alternatives remain formally possible. Despite this, many investigators hold to the view that it is the myosin head that undergoes some kind of conformational change, or “power stroke”, causing it to step forward cyclically along the actin, ratcheting in the direction of motion. This notion emerged from seminal work on muscle fibers (H.E. Huxley, 1969; Huxley and Simmons, 1971) and led to the suggestion that myosin might produce a power stroke of around 12 nm: an enormous distance for a protein, even one as big as myosin. One way to leverage up the power stroke would be for the entire myosin head

to rock as an entity about its point(s) of contact with the actin filament: the swinging crossbridge model. Throughout the 1970's and 80's, sophisticated biophysical techniques were used to hunt for signs of cross-bridge rotation, including EPR and fluorescence spectroscopy, X-ray and neutron scattering, electric birefringence, etc. Much of the evidence was equivocal, at best. For example, spin probes attached to the primary reactive thiol of the myosin head (known as SH-1) reported little, if any, net angular movement during active muscle contraction, as compared to at rest (reviewed by Highsmith and Cooke, 1983; Cooke, 1986). These sorts of negative result led to the salvage proposal that the bulk of the head might not rotate after all, but that its more distal tail portion (i.e., the remainder of the S1 proteolytic head fragment) wagged nevertheless (Cooke, 1986).

Biophysical Breakthroughs

During the first half of this decade, two significant breakthroughs occurred in diverse areas of biophysics. These advances, in conjunction with established methods in molecular biology, have renewed hope that a resolution of the myosin problem might be close at hand, perhaps by the dawn of the coming millennium. First, the atomic structures for both the actin monomer (Kabsch et al., 1990; Schutt et al., 1993; McLaughlin et al., 1993) and the S1 head fragment of myosin (Rayment, et al., 1993a) were solved, in a tour de force of X-ray diffraction. Both proteins had defied crystallographers for decades. Armed with high-resolution structural data for the monomers, it became possible to combine this information with lower-resolution data, obtained by X-ray diffraction of actin fibers or electron microscope-based reconstructions of actomyosin complexes, and thereby formulate atomic-level models for the actin filament (Holmes et al., 1990) and the actin filament decorated by myosin heads bound in rigor (a reference to the Latin rigor mortis, the ATP-depleted state that frequently follows death) (Rayment et al., 1993b; Schröder et al., 1993). Here, at last, was a plausible picture of actomyosin during at least part of its mechanochemical cycle.

The second breakthrough occurred when *in vitro* motility assays were successfully married with ultrasensitive optical instrumentation, capable of recording both force and displacements down to the molecular level, all in the light microscope. This made it possible for the first time to measure directly the steps taken by individual motor molecules, such as kinesin or myosin. For this purpose, laser-based optical traps (“optical tweezers,” Svoboda et al., 1993; Finer et al., 1994; Molloy et al., 1995) and fine glass microneedles have been used (Ishijima et al., 1994). Single myosin interactions have been scored whose mean displacements range from 5–25 nm (with forces developed of 1–5 pN). However, a key point of controversy remains as to whether these individual mechanical events correspond to a single ATP hydrolysis, or whether one ATP might somehow lead to multiple steps (Yanagida et al., 1993; Finer et al., 1994).

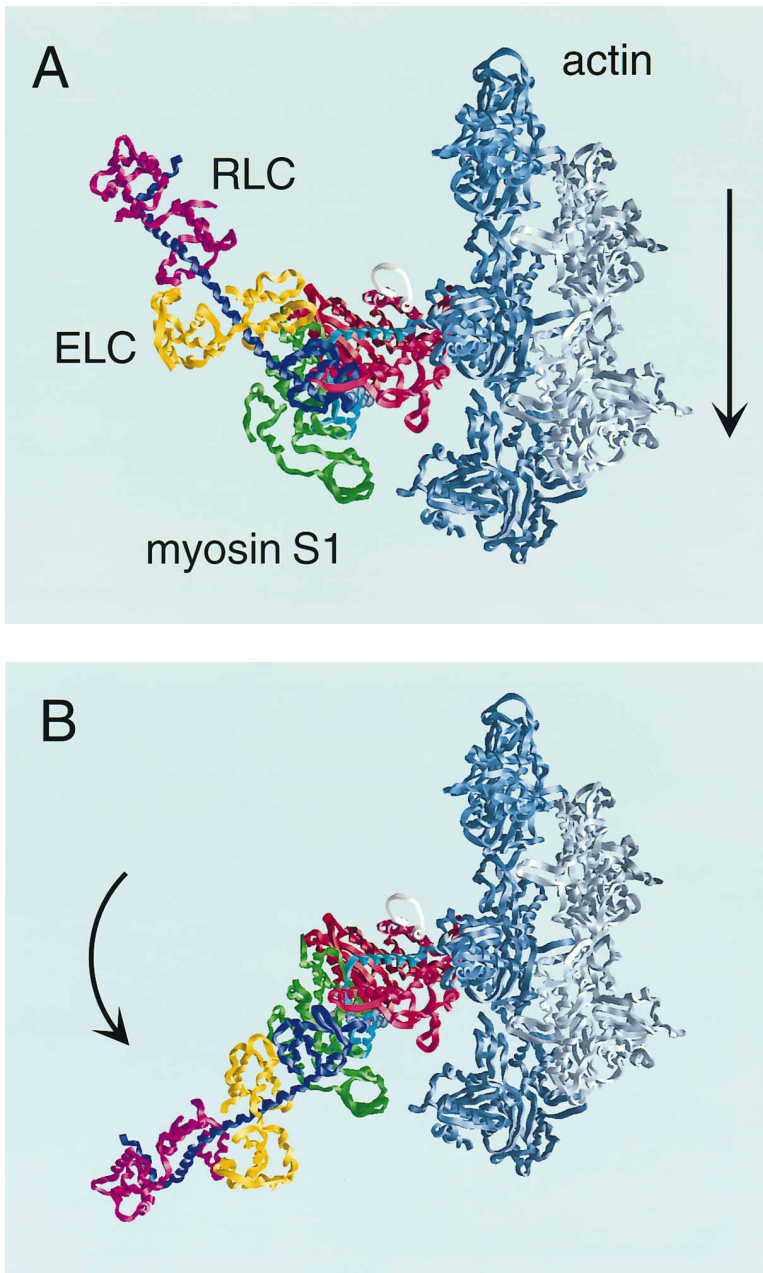


Figure 1. Hypothetical Model of the Swinging Lever Arm

(A) A computer-based visualization of the prestroke complex. Right, running vertically: a ribbon representation of a portion of an actin filament, positioned according to the model of (Lorenz et al., 1993), showing five identical monomers colored either slate blue or light grey. The barbed end of the filament is towards the bottom; myosin moves along the direction shown (arrow). Left: A ribbon representation of a single chicken myosin S1 head bound to actin, based on Rayment et al., 1993b. The color convention follows (Rayment et al., 1993a): magenta, regulatory light chain (RLC); yellow, essential light chain (ELC); green, heavy chain 25 kDa domain (amino acids 4-217); red, heavy chain 50 kDa domain (amino acids 218-625); dark blue, heavy chain 20 kDa domain (amino acids) 648-843). The α helices of the 20 kDa subunit bearing reactive thiols SH-1 and SH-2 have been colored cyan. A short loop (amino acids 626-647) at the junction of the 50 kDa and 20 kDa domains, unresolved in the chicken crystal structure, has been colored white: this loop has been implicated in control of the ATPase cycling rate (Spudich, 1994). A part of the bound nucleotide (grey) can be seen in the cleft between the 25 and 50 kDa domains, just to the right of the 20 kDa domain. To model the conformational change, the myosin structure has been deliberately altered, rotating the long helical portion of the 20 kDa domain and associated light chains about a pivot near the base of the lever arm, to depict what this complex might resemble prior to the power stroke. The 50 kDa and 25 kDa domains have not been altered, and molecular collisions occur in the model: rearrangements are anticipated for these regions as well, particularly near the SH1-SH2 helices (see text).

(B) The post-stroke state. This panel depicts the actomyosin complex in a rigor-like configuration, as in (Rayment et al., 1993b). It is assumed here, as in previous work, that the myosin S1 crystal structure has the same shape as in rigor (no bound nucleotide), and also that the rigor configuration displays a similar orientation with respect to actin as that following the power stroke, prior to ADP release. A rotation of the "lever arm" through $\sim 90^\circ$ would produce a step of ~ 12 nm, thereby pulling the remainder of the myosin

molecule (not shown) downwards. Smaller steps would correspond to less severe rotation. Composition and color color scheme are identical to (A). Models in (A) and (B) were created by K. C. Holmes using GRASP software.

This question lies at the heart of issues about mechanochemical coupling (Burton, 1992).

Structural Implications

The S1 crystal structure and the corresponding model for myosin bound to actin led Rayment and colleagues to propose a conformational change-based model that bears unmistakable similarities to the tail-wagging idea which represented the fallback position at the end of the 1980's (Rayment et al., 1993b). This time around, however, the model was a bit more specific and had a firm basis in structure that could lead, in principle, to

definitive tests of functional relationships. Proteolytic susceptibility had long ago been used to identify three distinct fragments of the S1 heavy chain polypeptide, named for their sizes: the 50 kDa, 25 kDa, and 20 kDa regions (Figure 1). Actin binding is mediated by the 50 kDa domain, the ATPase site spans the 50-25 kDa domain interface, and the 20 kDa domain binds the two light chains. A striking feature of the crystal structure is that the 20 kDa domain consists almost entirely of an exceptionally long, uninterrupted α helix, comprising 70+ amino acids, that is presumably prevented from spontaneous collapse (i.e., rigidified) by its interactions

with the two light chains, which envelop it along most of its ~ 9 nm length. The immediate possibility suggested by this feature is that it might somehow serve as a “lever arm” to drive the rest of the molecule forward when rotated at its base through some hydrolysis-induced angle (see Spudich, 1994). Clearly, such a mechanism could mechanically amplify smaller motions in the head. But can it explain molecular steps believed to be ~ 10 nm, perhaps greater? And are such large-amplitude motions, in fact, required? Without substantial rearrangements, the crystal structure only seems to admit to motions of 5 or 6 nm (Rayment et al., 1993b), and at least one report of myosin step size falls within this range (Molloy et al., 1995).

The original structure of chicken myosin S1 had sulfate, as opposed to ATP or ADP, in the enzyme active site. This raised the question whether the crystallized form reflected the shape of the native protein before, or after, the hydrolytic event postulated to produce conformational changes—or perhaps something else again. Put simply, would myosin crystallized with different substrates have different shapes? To address this question, Rayment’s group has crystallized and solved a series of shorter myosin heavy chain fragments from *Dictyostelium* with various bound nucleotide and transition-state analogs, including Mg.ADP.BeF_x, Mg.ADP.AIF₄⁻, Mg.ADP.vanadate, and Mg.PPi (Fisher et al., 1995; Smith and Rayment, 1995, 1996). To obtain these crystals, it was necessary to work with protein fragments too abbreviated to carry the light chains (~ 730 – 740 amino acids), so positions of the “lever arm” were not determined. Broadly speaking, the results fall into two classes. Structures with bound ADP-beryllium fluoride, Mg-pyrophosphate, and sulfate are similar to one another, and would seem to correspond to an “ATP-like”, prehydrolysis form. The structures with bound ADP-aluminum fluoride or ADP-vanadate form a second class. These are again similar to one another, but display various structural changes distinct from the first class, and would seem to be candidates for a “transition-state” form. Although large-scale structural changes were seen in certain “transition-state” structures near the carboxy-end of the structures where the lever arm would emerge, these occur in a part of the molecule that may not be structurally trustworthy, by virtue of the polypeptide being unnaturally lopped off near that point for crystallization purposes. It seems fair to say that the lever arm hypothesis has not yet been corroborated by crystallographic work, although there are tantalizing hints in the structural data that a subdomain of the molecule near the 25 kDa–20 kDa interface, optimistically dubbed by some the “converter” region, might undergo substantial changes.

Evidence for Shape Changes

There is ample reason to believe that major rearrangements must take place during the myosin mechanochemical cycle. There are two reactive sulfhydryl groups located on cysteines in skeletal muscle S1, designated as SH-1 and SH-2. These sulfhydryls (Cys707 and Cys697, respectively) are found on consecutive regions of α helix joined by a short turn, and spatially

separated in the chicken myosin structure by ~ 1.8 nm (Rayment et al., 1993b). However, they can be cross-linked by a variety of bifunctional reagents that span distances as short as 0.3 nm (Burke and Reisler, 1977). Moreover, crosslinking of SH-1 and SH-2 results in the trapping of nucleotide, in ADP form, at the active site of the enzyme (Wells and Yount, 1979). Clearly, some structural alteration must occur concomitant with hydrolysis that shortens the distance from SH-1 to SH-2: one not yet reported for the crystal structures.

Indeed, structural evidence for certain large-scale motions of myosin S1 has emerged recently, but this has raised more questions than it answers. Actin filaments decorated with either the S1 fragment of smooth muscle myosin II or brush border myosin I were bathed in solutions containing high levels of MgADP, to generate complexes containing the ADP-bound form, as opposed to the nucleotide-free form of rigor (Whittaker et al., 1995; Jontes et al., 1995). 3D cryoelectron microscope reconstructions of such filaments showed heads bound with the characteristic “arrowhead” pattern seen for skeletal muscle myosin. In the main, the head shapes were roughly similar to those previously observed with skeletal muscle myosin in rigor (Milligan and Flicker, 1987), but with a twist: the tail portions of these molecules had undergone extensive rotations with respect to the rigor forms: $\sim 23^\circ$ for smooth muscle S1 (corresponding to a displacement of ~ 3.5 nm at the end of the tail) and $\sim 35^\circ$ for brush border myosin I (corresponding to a displacement of 5.0 to 7.2 nm at the end of the tail). Could this be the smoking gun? Probably not. Conventional models of force generation (Spudich, 1994) don’t place the power stroke in the part of the cycle corresponding to ADP release. Also, the free energy change associated with ADP release is rather small (although the large energy drops elsewhere in the sequence might suffice, in principle, given the cyclical nature of the reaction scheme). Finally, these changes simply are not seen in skeletal myosin. In follow-up work with EPR spectroscopy, Cooke and colleagues placed spin probes on the regulatory light chain of smooth muscle myosin. When the labeled chains were exchanged for native ones, changes in the mean angles of probe orientation of up to 20° were found in muscle upon addition of ADP. Conversely, similar experiments with skeletal muscle myosin failed to produce any significant change in the mean orientation angle (Gollub et al., 1996). If not a power stroke, to what, then, does the ADP-induced shape correspond? Milligan, Sweeney, and colleagues speculate that it might be the so-called “latch-bridge” state, which is the smooth muscle analog of the catch-bridge state of molluscan myosins, whereby muscle fibers are able to lock up in contracted forms and sustain loads without a continual need to burn ATP (Whittaker et al., 1995). Assuming this interpretation is correct, it raises the specter that there may be a multitude of structural forms associated with the mechanochemical cycle.

A collaboration among several labs in the U.S. and U.K. has used fluorescence polarization spectroscopy to identify orientational changes of the light chains during muscle movement. Chicken gizzard light chains were expressed in *E. coli*, labeled with a single reactive rhodamine fluorophore at Cys108, and exchanged into rabbit

skeletal muscle (Irving et al., 1995). Measurements of polarization states at rest, during active muscle contraction, and under stretch were consistent with a tilting of the light chain “lever arm” region. However, the inferred angular change was disappointingly small: just $\sim 3^\circ$, even assuming that all probes in the ordered fraction responded. One explanation might be that the real angular change is much larger, but that only a tiny fraction of heads in the muscle fiber bear force and respond to length steps, and there is some support for this view from *in vitro* studies. The same group is now attaching fluorescence probes that bind to two reactive thiols and thereby cannot rotate about the attachment point, resolving angular ambiguities inherent in the original approach. The use of two or more such light chain probes oriented (nearly) orthogonally to one another should provide unprecedented resolution of molecular changes in real time.

Tests of the Lever Arm Model

If the 20 kDa region truly functions as a kind of lever arm, then changes in the lever arm length might produce corresponding changes in the myosin step size. This line of thinking has been pursued actively by Spudich and coworkers, who genetically engineered mutant *Dictyostelium* myosins with different sizes of lever arm, altered by changing the number of light chain binding regions (Uyeda et al., 1996). Three variants were created. The first was deleted for both light chain binding sites, the second was deleted for the regulatory chain binding site, while the third carried a tandem repeat of the essential light chain binding site along with the normal regulatory site, endowing it with three light chains. The three mutant constructs, together with the wild type and its twin light chain binding sites, constitute a series with 0, 1, 2, or 3 light chains of increasing length. The four proteins were expressed in cells, purified, and scored for motility *in vitro* and for ATPase activity. All four moved actin *in vitro*, at average sliding velocities that were found to increase monotonically with the number of binding sites. Not only did the shorter lever arm constructs move correspondingly slowly, but importantly, the one with an additional light chain site moved even faster than the wild type. In fact, the sliding velocities were in strict linear proportion to the lengths of the putative lever arms: a result almost too good to be true! On the assumption that the sliding velocity is proportional to the step size, this linear relationship permits the data from *Dictyostelium* to be extrapolated back into the (nearly identical) chicken myosin structure to locate the approximate fulcrum point of the lever, which turned out to be at the very base of the 20 kDa region, near the location of the α helices bearing the reactive thiols SH-1 and SH-2.

The underlying assumption in this work is that the sliding velocity of filaments *in vitro*, v , identically reflects the myosin step size, d . This will only be true when the step timing is exactly the same for each of the different myosins, since $v = d/\tau$, with τ being something like the time taken per step. Unfortunately, τ as just described is ill-defined. Does one take for τ the time required for a complete ATPase cycle (i.e., the reciprocal of the turnover rate)? If so, then the “lever arm interpretation” of

the experiment fails, since ATPase rates for the constructs differ from wild type by factors of ~ 2 . Spudich and company argued, with some justification, that the relevant time to consider is not the turnover time, but rather a time corresponding to that fraction of the cycle during which myosin and actin are tightly bound and can develop force, i.e., the strong-binding time, τ_s . This time is significantly shorter than the overall cycle time, occupying $\sim 5\%$ or less of the cycle in wild type. But, then, are the strong-binding times identical in wild type and all the mutant constructs? That remains to be demonstrated. A separate appendix to this paper, coauthored by Spudich and Howard, explored the theoretical consequences of relaxing the rigid lever assumption. If the lever arm were elastic instead, and had a flexural compliance typical of α -helical coiled-coil structures, then such an arm might provide a natural site for the well-known series elastic compliance of muscle fibers, assigning the stiffness to crossbridge flexibility. Moreover, the force produced by myosin under load would be inversely proportional to the *square* of the lever arm length, and not to the inverse lever arm length, as would be the case for a rigid system. The ultimate test of all this will not come from indirect determinations of velocity, but from direct, single molecule measurements (presumably underway at Stanford and elsewhere). For now, the question is whether the molecular steps taken by the different sized constructs are linearly proportional to their arm lengths, and if so, how the force is related to those lengths. In principle, the technology exists to do definitive experiments, using optical traps combined with nanometer-scale measurements (Simmons et al., 1996; Svoboda and Block, 1994), but these pose a daunting challenge for the future. It is noteworthy that up to this point, virtually all tests of the lever arm hypothesis have been conducted under near-zero load conditions.

Others are busy subjecting the lever arm concept to similar tests. A collaboration between the labs of K. Trybus and D. Warshaw has begun to characterize expressed smooth muscle myosins that are either neckless, wild type, or carry an additional essential chain binding site (dubbed “giraffe”). These smooth muscle species carry additional mutations designed to relieve them of regulation by phosphorylation. This group is not only measuring ATPase rates and velocities *in vitro*, but also using optical trapping technology to measure unitary steps and forces. However, earlier work by these (and other) investigators has raised a caution: perturbations of the neck region, near the interface of the essential light chain and motor domain, can have profound effects on the kinetics of the crossbridge cycle, despite the large distance between this region and the ATP binding site (VanBuren et al., 1994). Clearly, tinkering with any part of the myosin molecule may produce both kinetic as well as mechanical effects, so rather extensive characterization of mutants may be required before reaching conclusions (Sweeney and Holzbauer, 1996).

If the role of the 20 kDa α helix and associated light chains were simply to act as a mechanical lever, then replacing this region with an arbitrary domain of comparable size and rigidity might do the trick. Amazingly, this works! Dietmar Manstein, Michael Geeves, and coworkers grafted an “artificial lever arm” to the *Dictyostelium*

motor domain in place of the normal sequence beyond residue Arg-761 (this corresponds to Lys-782 in the chicken myosin sequence, a point right where the long helix enters the globular head domain). The artificial arm was manufactured from either one or two repeat segments coded by portions of the Dictyostelium α -actinin gene. The repeats are ~ 120 residues in length and predicted to form coiled-coils consisting of three α helices (a spectrin-like repeat), producing rigid domains ~ 6 nm long. When care was taken to attach motors stereospecifically to a glass surface, using anti-His tag antibodies, both constructs successfully moved actin filaments in vitro at speeds comparable to, and even in excess of, wild type (Anson et al., 1996). Detailed biochemical characterization of these mutants, using both steady state and transient kinetics, found no remarkable changes from the wild type for those rate constants determined (ATP hydrolysis rate, ATP binding rate, ATP-acto-motor affinity, ADP-acto-motor affinity). In this study as well, a clear correlation was observed between velocity in vitro and lever arm length, although perhaps not as strict a proportionality as reported by the Spudich group.

The lever arm hypothesis is attractive for a number of reasons. Not only does it suggest a means of mechanical amplification as well as provide a plausible site for the series elastic compliance in actomyosin, but it offers potential insight into the mechanism of regulation and evolutionary variation. If the structural integrity of the arm is maintained through association with the light chains, then phosphorylation of these peptides by light chain kinase could conceivably regulate directly myosin's ability to produce force. The myosin family is known to consist of twelve or more distinct classes of motor (Mooseker and Cheney, 1995). Certain classes differ notably in the lengths of the "lever arm" region and the numbers of associated light chains. Chicken brain myosin V, for example, has an exceptionally long region that binds up to six light chains per head (Cheney et al., 1993), and it moves more speedily than other unconventional myosins. Could myosin V have evolved the molecular equivalent of "seven league boots"? Time will tell.

A Converter?

If the lever arm is just that, and can functionally be replaced by unrelated protein constituents, then attention turns to the part of the motor responsible for driving the lever arm itself, near the base of the lever and around the ATP binding site: the secrets of mechanochemical coupling must lie here. Of long-standing interest has been the amino-terminal segment of the 20 kDa domain carrying the two short α helices bearing reactive thiols SH-1 and SH-2 (residues 687-714), discussed above. This region lies in close proximity to a curious β -sheet motif in the 25 kDa domain, as well as to the ATP binding pocket. Located in the short turn joining the α helices is a single glycine residue (Gly699) that is absolutely conserved across the gene myosin family. Gly699 lies approximately at the pivot point of the lever arm, as inferred from other work. Reasoning that the mobility of the two helices might be critical to function, Winkelmann and colleagues mutated this residue of the chicken skeletal myosin gene to alanine, and developed a unique

system to express the mutant protein as a mouse/chicken chimeric form in a mouse myogenic line that forms contractile myotubes (Kinoshita et al., 1996). This single conservative mutation dramatically affected myosin activity, resulting in a >100 -fold reduction in speed. By doing mixing experiments with varying amounts of wild-type and mutant myosin, and assaying actin filament velocities driven by these in vitro, it was concluded that the mutant myosin had a dominant effect on slowing the speeds of the mixtures, well beyond any anticipated reduction due to the difference in ATPase rates. One explanation for this may be that the mutant has an altered duty cycle, spending much more time bound to actin. This can happen, for example, if the strong-to-weak binding transition in actomyosin (induced by ATP rebinding to the motor while attached to actin) is inhibited, perhaps through a slowing of the ADP release step that precedes the rebinding, which is generally rate-limiting. Perhaps ADP release is directly coupled to a mechanical rearrangement involving both SH-1 and SH-2 helices? Clues such as this may provide important new insights into the coupling of chemistry and mechanics. Many labs are contemplating experiments specifically designed to address structure-function relationships in the so-called "converter" region of myosin at the base of the lever.

Motors Galore: A Perspective

Recently, crystal structures for the motor domains of two more mechanoenzymes, kinesin and ncd, were determined by Robert Fletterick's laboratory (Kull et al., 1996; Sablin et al., 1996). Although kinesin and ncd move along microtubules—not actin—and have heads just half the size of myosin, a surprising similarity was discovered: the α -carbon backbones of both microtubule-based motor domains (which, incidentally, are nearly identical to one another) are nearly superposable on the central region of the myosin S1 structure! This, despite the lack of any significant sequence identity at the amino acid level, except perhaps in the immediate ATP-binding region, which is common to several families of kinase and phosphatase. The unmistakable structural similarity between the myosin and kinesin motor domains suggests that these two proteins may share a common mechanism, not to mention a common ancestry. Nothing corresponding to a "lever arm" was seen in either kinesin or ncd structures, but this was not unexpected, since the domains crystallized were truncated at 349 or 366 residues, respectively, somewhat shy of the regions that would be homologous to the long α helix of myosin. Moreover, the final ~ 30 C-terminal amino acids of the kinesin motor domain were not resolved in the crystal structure. However, circular dichroism of kinesin peptides formed from amino acids 330-370, corresponding to the lever arm region and beyond, suggests that it may form a coiled structure. Could kinesin and ncd also employ the equivalent of a lever arm? If yes, there are obvious problems with this idea. First, kinesin's lever arm seems too short: the predicted sequence should only be able to produce a step in the neighborhood of 2.5 nm (Kull et al., 1996), yet kinesin molecules appear to advance in increments of 8 nm (Svoboda et al., 1993).

How might such a big step be accommodated? Second, kinesin's light chains bind to the C-terminus of the molecule, distal from the motor domain, and are involved in binding cargo, not in stiffening the neck (their complete elimination has no effect on kinesin movement, in any case). Without anything to wrap it, what mechanism would prevent the collapse of the lever arm?

All is not lost, however. It seems possible that kinesin's "lever" may not so much resemble a pry bar as a leash, allowing one free kinesin head to advance freely while tethered to the other, which remains bound to the microtubule. Current thinking is that the two kinesin heads may walk "hand-over-hand" (Block, 1995; Peskin and Oster, 1995; Duke and Leibler, 1996). The translocation event, therefore, may more closely resemble biased diffusion than leveraged displacement. The length of the leash could be extended the requisite distance by some degree of melting of the α -helical coiled coil forming the kinesin tail. Alternatively, the actual coiled-coil region might begin closer to the C-terminus than is conventionally thought. Moreover, the mechanical integrity of the hypothetical lever/leash could be buttressed (or otherwise stabilized) by transient interactions between itself and the head domain during the mechanochemical cycle. So there would still seem to be ample room for maneuver, and researchers working on actin- and microtubule-based motors will doubtless have much to discuss in the years to come.

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