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Lever Arm Model of Force Generation by Actin−Myosin−ATP†

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My goal is to provide the reader with a narrowly focused perspective on the current working hypothesis, the lever arm model, for the mechanism of force production from chemical energy by muscle motor proteins. Recent data on large-scale domain rearrangements and their underlying atomic level structural changes are presented and critically evaluated. The emphasis is on what additional information is needed to formulate and test the lever arm model as a molecular mechanism. To simplify the presentation of the hypothesis, I have tried to limit severely the number of references, and to use the most contemporary ones when possible. In many cases, I do not cite the earliest publications of important observations or ideas. I hope that my colleagues will be forgiving. To obtain a more integrated historical picture of discoveries in the field, one can work backward from the recent articles cited here or consult a review (*1*).

Lever Arm Mechanics

The current working hypothesis for the mechanism of force generation by the muscle proteins myosin and actin postulates the rotation of a lever arm as the primary mechanical component of the power stroke (Figure 1A). The lever arm, also called the regulatory domain (*2*), is an ∼8 nm stretch of myosin heavy chain, mostly α-helix, to which two myosin light chains are bound (Figure 1B). It is located between a catalytic domain, which binds actin and hydrolyzes ATP, and a fibrous rod domain, which connects the lever arm to the thick filament. The combined catalytic and regulatory domains constitute the motor domain (*3*), designated as M in this article and also called myosin subfragment 1, or S1. Myosin contains two motor domains (Figure 1C), but a single domain can generate force (*4*). In the simplest lever arm model of force production, the average orientation of the catalytic domain does not change significantly, while rotation of the lever arm about a fulcrum site changes its orientation with respect to both the catalytic and rod domains (Figure 1A). A single motor domain power stroke moves actin by 5−12 nm (*5*−*7*).

Catalytic Cycle

During the hydrolysis of an ATP molecule by actomyosin, the motor domain spends part of the cycle bound to actin and part of it free (*8*). Using a minimal scheme to depict the coupling of the mechanical and hydrolytic cycles, ATP binding causes the motor domain to dissociate from actin as M·MgATP (Figure 2). While the motor domain is free from actin, hydrolysis is hypothesized to induce lever arm rotation, which produces a more compact M·MgADP·P_i. This complex binds actin, A, to form A·M·MgADP·P_i, in which the motor domain is weakly bound and perhaps loosely tethered about some average orientation. According to the model, force is then produced by a reverse rotation of the lever arm, as the ATP hydrolysis products are released (step 4 in Figure 2, or see Figure 1A). Orthophosphate is released first, perhaps through an opening that is created, a back door, by the lever arm rotation (*9*). Consistent with this model, which has rotation coupled to the M·MgATP → M·MgADP·P_i step, if rotation is inhibited by mechanically constraining the motor domain, ATP binds to M but hydrolysis does not occur (*10*). Product release increases the standard free energy for the binding of the motor domain to actin by 3 orders of magnitude, and rigidifies motor domain segmental and internal motions (*11*). In muscle, the motor domains cycle asynchronously, and during shortening, only a small fraction of the actin molecules in a filament have a motor domain bound at any time.

Motor Domain Crystal Structures

Lever arm rotation is clearly shown in high-resolution motor domain crystal structures. In one structure, M has no

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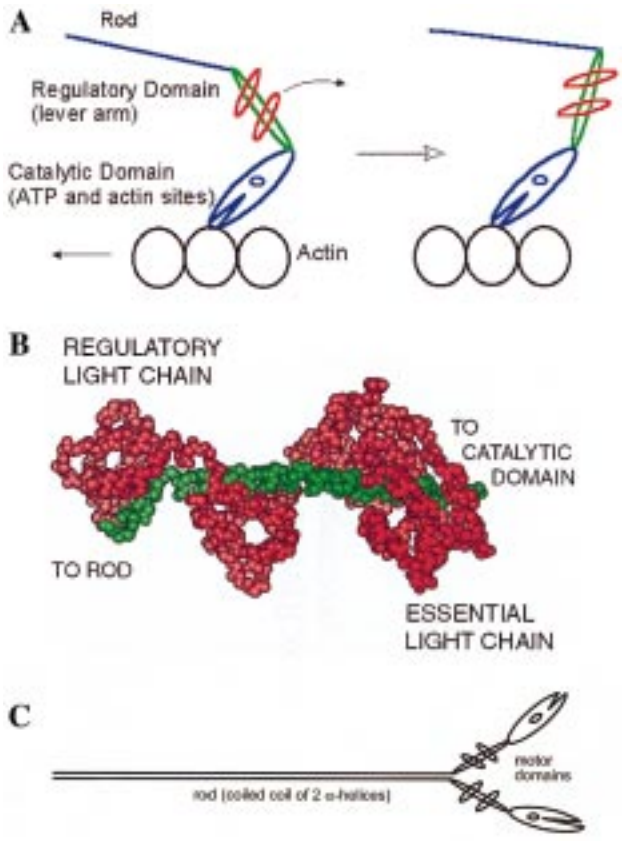

FIGURE 1: Lever arm mechanics. (A) A schematic representation of lever arm rotation as it is thought to occur during force production by a single myosin motor domain bound to actin. The myosin motor domain comprises a catalytic domain and a regulatory domain. The catalytic domain (purple) contains an ATP site and an actin binding site, which has two parts separated by a cleft. The lever arm is the regulatory domain, which is a stretch of myosin heavy chain (green) bound by two light chains (red), an essential light chain near the catalytic domain and a regulatory light chain. The lever arm rotates about a fulcrum site near the center of the motor domain, as the ATP hydrolysis products (not shown) are released. The average orientation of the actin-bound catalytic domain (blue) does not change during lever arm rotation. The flexibly attached rod portion of myosin connects the motor domain to the thick filament. Rotation of the lever arm slides the actin filament to the left, relative to the thick filament position. (B) The regulatory domain, called the lever arm, of scallop muscle myosin consists of essential and regulatory light chains (red) bound to an ~8 nm α-helical segment of the heavy chain (green). Other muscle myosin regulatory domains have similar structures. The figure, with the backbone structure rendered as van der Waals surfaces, was derived from Brookhaven Protein Data Bank file 1scm (*2*). (C) A schematic representation of a myosin molecule. There are two motor domains attached to a coiled coil α-helical rod domain. Each catalytic domain (on the right side) has an ATP site (the circle) and an actin binding site, which includes a cleft (the v shape). The lever arm is attached flexibly to the catalytic domain and to the rod domain. In muscle, the rod segments aggregate to form the thick filament.

nucleotide bound (Figure 3A), and its lever arm is in a position that creates a more elongated conformation (*3*). In another structure, M is bound to MgADP·AlF_4 (Figure 3B), and its lever arm (a shorter structure that has only one light chain) is rotated by 70° to form a more compact conformation (*12*). The observed rotation is sufficient to move actin 10 nm. In addition to lever arm rotation, a domain near the fulcrum site, named the converter domain, moves 2.5 nm (*12*). The more elongated structure, M, is often assumed to have the lever arm in the position that it has in both

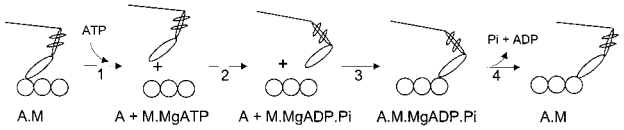

FIGURE 2: Chemomechanical cycle. An actin monomer (A) is depicted as a circle. Myosin is depicted with a single motor domain (M), described in more detail in Figure 1A. A·M is the rigor complex that exists after the ATP hydrolysis products have dissociated. MgATP binds A·M, and M·MgATP dissociates (step 1). ATP hydrolysis rotates the lever arm, producing a more compact M·MgADP·P_i (step 2). M·MgADP·P_i binds to an actin monomer further along the thin filament to form A·M·MgADP·P_i (step 3). The lever rotates back to its elongated position, releasing the hydrolysis products and forcing actin to move to the left (step 4). The amount of rotation that occurs for the individual P_i and ADP dissociation steps may vary for myosin from different sources.

M·MgATP and A·M (Figure 2). More recently, the solution of a third structure that has M bound to MgADP was reported (*13*). In M·MgADP, the lever arm is rotated so that the conformation is more elongated than M. The MgADP complex is also the first myosin crystal structure that has the region of residues 697−707 disordered rather than α-helical (*13*). This observation is of interest because a disordered region of residues 697−707 in the presence of ADP, at least as a transient species, is needed to explain the results from a great number of solution chemical cross-linking studies with skeletal muscle myosin (*14*). However, one should bear in mind that correlation of the structures of motor domain−ligand complexes observed in crystals to structural and kinetic data on motor domain−ligand complexes in solution is not straightforward, as is discussed in detail below.

An additional mechanical feature of the motor domain structure is a cleft in the catalytic domain (Figure 1A). The open end of the cleft appears to bifurcate the actin binding site (*3*), and its innermost part is close to one of two conformational switches that abut the ATP site (*15*, *18*). It has been hypothesized that the degree of closure of the cleft changes with nucleotide and actin binding (*16*). In the crystal structure of M, the cleft has a more open conformation (*3*), and in those of M·MgADP·AlF_4, it is more closed (*12*). These data suggest that nucleotide may control both lever arm rotation and cleft closure. Because the cleft is part of the actin binding site, its conformation may also be actin-dependent. Interactions between actin binding, cleft closure, nucleotide binding, and lever arm position are the mechanical components of most current hypotheses for the mechanism of force generation by actomyosin.

A series of investigations were carried out to determine high-resolution structures of several nucleotide and nucleotide analogue complexes of a truncated catalytic domain of the *Dictyostelium discoideum* myosin motor domain (*17*−*20*). The lever arm is not present, but correlations between cleft opening and bound nucleotide were found. To summarize, for these catalytic domain complexes, if MgATPγS, MgAMP-PNP, MgADP·BeF_x, $MgPP_i$, MgADP, or no nucleotide is bound, the structures are nearly identical and the cleft is in the more open conformation. If MgADP·AlF_4 or MgADP·V_i is bound, the cleft is in the more closed conformation. The motor domain conformation for the former group, with the open cleft conformation, was interpreted to mimic that of M·MgATP, and that of the latter group was interpreted to

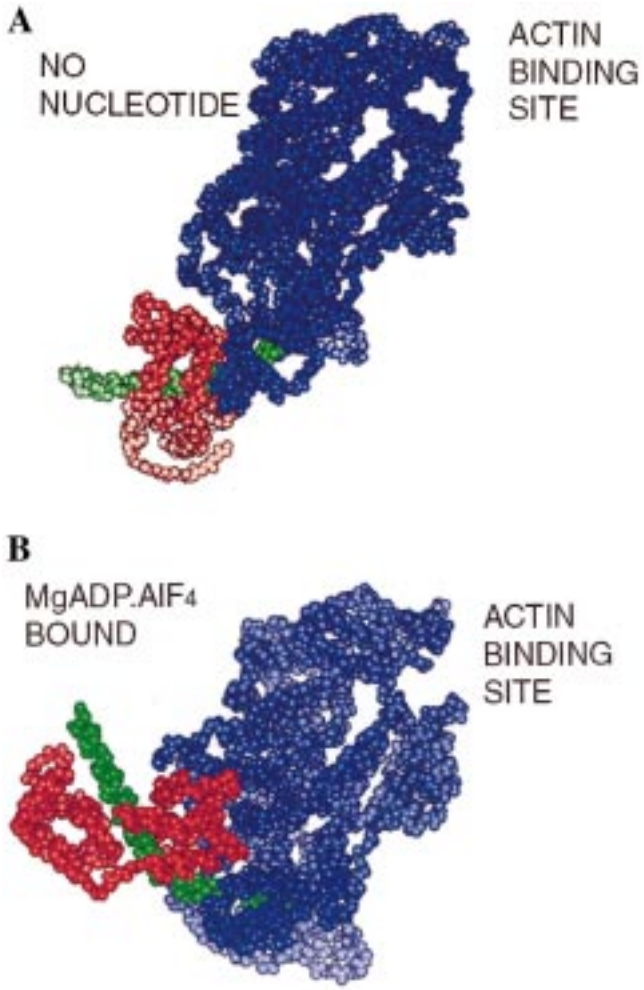

FIGURE 3: Motor domain crystal structures. Myosin motor domains with the lever arm in different positions. The catalytic domain (purple) is attached to a truncated lever arm consisting of an essential light chain (red) bound to a stretch of heavy chain (green). (A) Chicken skeletal muscle myosin motor domain with no nucleotide bound (although probably sulfate), derived from file Brookhaven Protein Data Bank file 2mys (*3*) by deleting the regulatory light chain portion and rendering the backbone structure as van der Waals surfaces. (B) Chicken smooth muscle myosin motor domain, with MgADP·AlF_4 bound, derived from Brookhaven Protein Data Bank file 1br1 (*12*) by rendering the backbone structure as van der Waals surfaces. The nucleotide is not shown. Using skeletal muscle myosin sequence numbers, compared to M with no nucleotide, shown in panel A, the lever arm has rotated by ~70° about fulcrum residues 699 and 710 when M·MgADP·AlF_4 is formed, shown in panel B (*12*). The increase in mass below the fulcrum in panel B is due to movement of the converter domain (*12*). The orientation that is shown is not suitable for evaluating the cleft conformation.

mimic the motor domain of M·MgADP·P_i or the transition state leading to M·MgADP·P_i (*20*).

The high-resolution crystal structures of myosin motor and catalytic domains have revolutionized the field of muscle contraction. However, when all the data are considered, it is not completely unambiguous as to which motor domain−nucleotide analogue complexes correspond to which actual motor domain nucleotide intermediates of the catalytic cycle. One reason for this ambiguity is that removing the lever arm can change the relationship between the nucleotide site and the cleft conformation. When a *D. discoideum* catalytic domain that is extended by several amino acids at the C-terminus (where the lever arm would be attached) is used to crystallize the MgADP·BeF_x complex, the cleft conformation changes from open to closed (*21*). The closed cleft conformation of the complex made with the extended catalytic domain is consistent with the closed conformation observed with M·MgADP·BeF_x (or M·MgADP·AlF_4) which has a lever arm attached (*12*).

It also may be relevant, with regard to the nucleotide dependence of both the cleft conformation and the lever arm position, that the structures that have been crystallized thus far do not contain motor domains from the same muscle type or with the same number of light chains present. The compact motor domain conformation in M·MgADP·AlF_4 (Figure 3B) and M·MgADP·BeF_x is chicken smooth muscle myosin heavy chain with a lever arm that has only the essential light chain present (*12*). The elongated motor domain conformation in M is chicken skeletal muscle myosin heavy chain with a lever arm that has both the essential and the regulatory light chains present (*3*). It has been shown that it is possible for the lever arm position to be different for the same motor domain−nucleotide complex if the motor domains come from different muscle types, as will be discussed below. More data are needed to clarify the current ambiguities about the cleft conformation and lever arm position dependencies on muscle type and on light chain constituency.

Lever Arm Dynamics in Solution

Low-resolution structures of motor domain complexes in solution derived from measurements of hydrodynamic size (*22*) and radius of gyration (*23*, *24*) also indicate that lever arm position is nucleotide-dependent. The various data are consistent with regard to the effect of nucleotide analogues and nucleotides on motor domain conformation. To summarize, analogues of unhydrolyzed ATP (ATPγS, AMPPNP, and ADP·BeF_x) produce the most elongated motor domain. Analogues of ADP·P_i (ADP·V_i and ADP·AlF_4) produce the most compact structure. For ADP or no nucleotide, the lever arm appears to be at an intermediate position. These assignments of lever arm position, based on low-resolution structural data, are consistent with the scheme shown in Figure 2. Comparable data are obtained whether the skeletal muscle myosin motor domain has only the essential light chain or has both the essential and the regulatory light chains bound.

In a related experiment, the lever arm of a *D. discoideum* motor domain was replaced by green fluorescent protein and its rotation monitored by fluorescence resonance energy transfer to blue fluorescent protein fused to another location on the catalytic domain. Consistent with the results described above for native motor domains, the mutant lever arm moves to make a more compact conformation when ATP is hydrolyzed and it moves back to the more elongated conformation when orthophosphate dissociates (*25*).

Solution measurements also allow the dynamic flexibility of the motor domain complexes to be estimated. In the submicrosecond and microsecond range, transient electric birefringence (*22*) and electron paramagnetic resonance (*26*) measurements indicate the ligand-free M and myosin have intrinsic segmental flexibility. These motions are probably due to tethered rotational diffusion of the lever arm. Submicrosecond segmental flexibility is maintained when the average position of the lever arm has rotated to form the more compact M·MgADP·V_i conformation (*27*), suggesting

that the lever arm in M·MgADP·P_i remains flexibly attached to the catalytic domain. The static disorder reported for electron paramagnetic resonance and cryoelectron microscopy measurements suggests that lever arm flexibility is retained when M·MgADP·P_i binds to actin (*28*, *29*). As will be discussed below, the nature and degree of lever arm flexibility in the A·M·MgADP·P_i complex bear on the mechanism of force production.

Actin-Bound Motor Domain Conformations

The above data indicating that myosin motor domains can exist in more than one conformation, corresponding to varying degrees of lever arm rotation, were all obtained in the absence of actin. No crystals of actin−motor domain complexes have been characterized yet, but there is evidence that lever arm rotation can occur when a motor domain is bound to actin. Comparison of images reconstructed from electron micrographs of actin filaments saturated with smooth muscle myosin motor domains with and without ADP bound suggests a substantial rotation of the lever arm occurs when ADP dissociates (*30*). Orientation changes observed using electron paramagnetic probes attached to the regulatory domain confirm that MgADP causes rotation of the lever arm of smooth muscle myosin motor domains bound to actin (*31*). These data are low-resolution and do not reveal the exact conformation of the lever arm or of the degree of cleft opening. But they support the idea that at least two motor domain structures, one bent and one more elongated, exist when myosin is bound to actin, as well as when it is free.

Interestingly, for the skeletal muscle myosin motor domain, no change in the orientation of the regulatory domain is observed when MgADP is removed (*31*). On one hand, this result is reassuring because for skeletal muscle, it is thought that lever arm rotation is coupled to the phosphate release step. On the other hand, different lever arm positions for skeletal and smooth muscle myosin M·MgADP suggest that the coupling of lever arm rotation to particular product release steps is different for skeletal and smooth muscle, and that the rotational increments for particular kinetic steps may be muscle type-specific in general. Unfortunately, it appears that the reasonable, convenient, and often-made assumption that all myosins have the same mechanochemical coupling is not valid.

Muscle Fibers

The ultimate test for the lever arm model is measuring lever arm rotation in a contracting muscle fiber. Achieving this goal is made difficult, in part, by the complexity of fiber structure. The signals from some methods include contributions from the many proteins present in addition to myosin, which make it difficult to resolve signal changes that are caused by lever arm rotation. A second problem incurred in fiber studies arises from the fact that the motor domain force producing cycles are asynchronous during shortening. One strategy for reducing extraneous signals when investigating fibers is to use orientation probes attached to specific sites on the motor domains. It is possible to exchange a regulatory light chain, which has a probe covalently attached, with the native regulatory light chain myosin. One can use the signal to measure selectively the orientation of the probe and thereby of the lever arm to which it is attached.

When light chains modified with an electron paramagnetic probe are exchanged into muscle fibers, the data for both relaxation and contraction are consistent with the bound motor domains having lever arms that are distributed among two orientational states separated by 36° (*28*). In one state, the motor domain is bound weakly to actin. This pre-power-stroke state corresponds to the structure on the left in Figure 1A. The lever arm orientation is more ordered than that of an unbound motor domain, but it retains substantial disorder around its average orientation (*28*, *58*). Disorder of the motor domains at the beginning of the power stroke has been confirmed by cryoelectron microscopy (*29*). The nature of the disorder is not completely clear. There may be contributions from intrinsic lever arm rotation, and/or reversible ATP hydrolysis-coupled lever arm rotation, and/or flexibility at the site of motor domain attachment to actin. The other state (on the right in Figure 1A), corresponding to the post-power-stroke conformation, is strongly bound and highly ordered. When the fiber changes from relaxation to contraction, the population distribution of the two states is shifted to favor the post-power-stroke orientation, indicating that lever arm rotation occurs during contraction (*28*). Rotation is not observed directly, but is deduced from the shift in the distribution of orientation states.

Contributions from local motions of orientation probes can pose problems when interpreting probe data. One approach for minimizing these local motions is to have the probe attached covalently at two sites. When such bifunctional fluorescent probes are attached to the regulatory domain in skinned fibers, the average probe orientations are similar for relaxation and contraction (*32*). This similarity for the average signal is due largely to the fact that the motor domains cycle asynchronously. One way to synchronize motor domain mechanics is to impose rapid length changes on an isometrically contracting fiber. Shortening allows lever arm transitions from the pre-power-stroke to the post-power-stroke states to occur. Stretches pull lever arms back toward the pre-power-stroke orientation. When fibers with fluorescent probes attached to the regulatory domain are rapidly released or stretched, rotation of the lever arms is observed (*32*). The size of the observed rotation is small and not adequate to explain the power-stroke displacement, but analysis of the nonlinear response of probe orientation after rapid shortening suggests that a fraction of the lever arms may be making much larger rotations (*32*).

In the simplest model of lever arm force production, the average orientation of the catalytic domain does not move. Both electron paramagnetic probes (*33*) and fluorescent probes (*32*) have been used to show that the catalytic domain does not rotate during stretches or force generation, although there is not complete agreement on this point (*34*).

Single-Molecule in Vitro Assays

A potential way to circumvent the problem of having many active motor domains at different stages of the contractile cycle in a muscle fiber is to measure properties of a single motor domain bound to actin, using an in vitro assay. Precise measurements of motor domain displacement, force, and stiffness can be taken using laser tweezers to position an actin filament over a single motor domain (*5*, *7*, *35*). It is also possible to use fluorescent probes to measure kinetic parameters for single substrate turnover events (*36*). The technically more difficult orientation measurements with

individual motor domains during force production are in progress, but the results are not available yet.

What Needs To Be Resolved?

In summary, the various structures of free and actin-bound motor domains with the regulatory domain in different positions provide strong evidence that the myosin lever arm can rotate and that nucleotide-dependent rotation is part of the force-producing cycle. The direct detection of lever arm rotation during force generation in fiber or in vitro assays remains somewhat elusive, although probably not for long. The molecular mechanism of force production by lever arm rotation is another matter. The goal of knowing what is happening at the atomic level to move the motor domain lever arm is far from met.

Actin-Free Intermediates. Several high-resolution crystal structures of actin-free contractile cycle intermediates already exist. In principle, these myosin motor and catalytic domain crystal structures should provide the atomic level structural details needed to determine the role of ATP binding and hydrolysis in rotating the lever arm. But, as discussed above, determining the precise nucleotide-coupled structural changes for the catalytic cycle intermediates has not been straightforward. One problem, at least for the MgADP·BeF_x complex, is that increasing the length of the catalytic domain heavy chain, or adding the essential light chain portion of the lever arm, changes the cleft conformation (*12*, *19*, *21*). This observation raises the possibility that the addition of the remaining regulatory light chain portion of the lever arm to the complex may also have affects on the conformation of the cleft or on the position of the lever arm.

A further complication is that the crystal and solution structures of M·MgADP·BeF_x and M·MgADP·AlF_4 appear to be inconsistent. In the crystal structures, both complexes have the lever arm rotated to produce the least elongated motor domain conformation (*12*). In solution, M·MgADP·AlF_4 assumes the least elongated conformation, but M·MgADP·BeF_x is among the most elongated motor domain conformations (*23*, *24*, *27*). There may in fact be no discrepancy as the crystal structure is of smooth muscle M·MgADP·BeF_x and the solution structures are of skeletal muscle M·MgADP·BeF_x. For smooth and skeletal muscle motor domain−ADP complexes bound to actin, the lever arm positions are different (*31*). The positions may be muscle type-dependent in the absence of actin as well.

A way to eliminate the ambiguities introduced by motor domain light chain and muscle type variations is to obtain high-resolution crystal structures of motor domain nucleotide complexes that are derived from the same muscle and that have both light chains present. The lever arm positions in the crystals could be confirmed for the same motor domain−nucleotide complexes in solution using low-resolution techniques. The solution studies would also characterize the dynamic flexibility of the actin-free intermediates. What is obvious to do is not necessarily easy to do, especially with regard to crystallizing motor domain complexes. A crucial first step may be identifying a muscle type for which the motor domain with both light chains bound will crystallize with different nucleotides bound.

Another contribution to the currently ambiguous correlation between the bound ATP analogue and the observed protein conformation may actually come from intrinsic properties of the myosin motor domain. The lever arm is designed to move. During contraction, at least when actin is bound and perhaps in other states, the lever arm orientation is maintained against forces in the 1−5 pN range. Lattice forces can be greater than this, and may be able to dictate which conformation is crystallized. This is not to suggest that the conformations observed in crystals form randomly. The bound nucleotide affects motor domain conformation in ways besides determining lever arm orientation, for example, surface charge distribution and hydrophobicity. These surface properties will contribute to and interact with the lattice forces. This speculation that lattice forces may in some cases determine which of several possible protein conformations will crystallize is unlikely to be important for globular enzymes which do not undergo large conformational changes that include segmental motions. But it may be relevant for motor proteins such as myosin because of their more asymmetric structures that include movable parts.

It should be possible to evaluate whether a conformation is determined by nucleotide or by lattice forces. The allosteric coupling of ATP site occupancy and lever arm position suggest that if lattice forces dictate lever arm position, the nucleotide interactions with the ATP site should be changed. Thus, one could confirm that a lever arm position is not due to lattice forces by ascertaining that the nucleotide has the same active site interactions as it has in the absence of lattice forces. This approach was useful in investigating aggregated M (*10*).

Actin-Bound Intermediates. High-resolution structures of motor domain−actin complexes are needed to understand the role of actin binding in product dissociation and in lever arm rotation. A comprehensive picture will require structural details of the actin−motor domain interface, and of the allosteric linkage from the interface to the ATP site and the fulcrum site. Structures of the actin−motor domain interface have been proposed, but they are based on assumptions that need to be verified. One reason assumptions are necessary is that actin filaments have yet to be crystallized. The F-actin structures that are available are models based on the crystal structures of actin monomer cocrystallized with another protein (*37*, *38*). These models continue to be enormously useful. But they are based on the assumption that structural changes at the myosin binding site on actin are not produced by actin polymerization, or by binding of the cocrystallized protein, or both. A second assumption is that high-resolution structural information about the actin−motor domain interactions can be obtained by fitting static actin and motor domain structures together. This assumption is necessary because there are no crystals available of any form of actin bound to a myosin motor domain. Models of complexes of actin-bound motor domains have been obtained by visual docking of a motor domain structure onto the modeled actin filament structure (*16*). The structure of a complex is then refined by computational techniques that optimize the short-range noncovalent interactions at the binding interface and the fit of the motor domain high-resolution structure into topographical envelopes determined from electron micrograph image reconstructions (*39*, *40*).

This approach is valuable for estimating the orientation of the motor domain on actin, including the lever arm position. However, the current limitations, with respect to the goal of obtaining high-resolution structural information

about the actual interface and its allosteric connection to the ATP and fulcrum sites, are severe. The docking experiments bring together two static structures that must change when they bind. These binding-induced structural changes at the interface may not be subtle. In addition, the structural changes are transmitted almost 10 nm away from the interface into the motor domain to increase the rate of product release, coordinate the rotation of the lever arm, and rigidify the motor domain. In principle, if the existing structures of actin and myosin motor domain were used in computer simulations that could optimize all the interactions, actin−actin, actin−motor domain, and motor domain−nucleotide, one would obtain a correct high-resolution structure. But for several reasons, such a complex process is not feasible at this time.

A powerful approach for probing the structure of the actin−motor domain interface and motor domain interior is to introduce mutations at specific locations. Structural changes at the atomic level can be correlated to functional changes. One approach has been to make mutations of the lever arm itself. Using mutant myosin motor domains that have variable length lever arms, it was shown that the rate of actin sliding in an in vitro assay increases with lever arm length (*41*). Mutations have also been made at specific sites of the actin−motor domain interface. Changes in the location and in the amount of electric charge on actin (*42*) or myosin (*43*) have indicated that for binding and force production the amount of electric charges at the interface is critical, but that the precise location is not. This surprising conclusion begs for high-resolution structures of actin−motor domain complexes for use in interpreting the data further. The ATP site has also been probed by mutation. The importance of specific residues in the active site (*44*−*46*) and in the switch regions near it (*47*, *48*) has been evaluated by replacement and exchange mutations. The allosteric interactions between the regulatory light chain and the actin binding site have to be modulated by mutations of the light chain (*49*−*51*) and of the actin binding site on the catalytic domain (*52*). The use of mutation to investigate structure−function relationships in motor proteins is still developing rapidly. The results are often provocative, but to reap the full benefit of these experiments, one needs high-resolution structures of at least some of the complexes.

It is important that high-resolution actin-bound structures obtained from crystals, or using actin filaments with the myosin binding sites saturated with motor domains, are corroborated by structures for which lattice and other forces are absent. Just as crystal lattice forces may affect lever arm position and therefore motor domain structure, when actin is saturated with bound motor domains, packing-induced motor domain interactions, either binding to one another (*52*) and/or sterically interfering with one another (*53*), may affect lever arm position and motor domain structure. Comparison of results from solution studies, in which the occupancy of binding sites on actin is low, to results from other studies, in which the occupancy is high, will allow contributions to the motor domain conformation from the close packing on actin, if they exist, to be evaluated. Such experiments will verify that the lever arm is in the same position, and also provide information about motor domain and lever arm dynamics.

Lever Arm Dynamics. The dynamics of lever arm rotation is an important element of the mechanism of force production. In solution, there are nucleotide-determined motor domain conformations, characterized by transient electric birefringence (*22*, *27*) or X-ray and neutron diffraction (*23*, *24*) to be elongated and compact. The solution conformations are consistent with motor domain crystal structures, which show the lever arm can be in different positions (*3*, *12*). It is also apparent from the transient electric birefringence data that in solution both conformations have flexibly attached segmental domains, and that the energy required to move the segments is less than *kT* (*26*, *27*). If it is assumed that the observed segmental motion is lever arm rotation, there are two interpretations of the intrinsic flexibility. One possibility is that each conformation has only one lever arm orientation, and the observed flexibility is due to limited local rotation about that orientation. A second possibility is that in solution each conformation has both orientations populated, but to different degrees depending on the bound nucleotide, and the observed flexibility is due to exchange between the two orientations. With regard to the second possibility, ^{31}P and ^{19}F NMR measurements indicate that in solution, with or without nucleotide, M exchanges between two discrete conformations (*55*, *56*). These conformations cannot be assigned lever arm positions or degrees of flexibility, but they differ in standard free energy by only about 1 kcal/mol, and exchange at rates greater than the rate of ATP turnover (*56*).

Taken together, these energetic and structural dynamic data suggest that M and M·MgADP·P_i analogues have conformations, with different lever arm orientations, which are separated by a small energy barrier. In either conformation, the lever arm can rotate freely to some degree, perhaps exchanging between the two orientations. In the absence of actin, the population distribution of the two orientations is determined by the bound nucleotide. When actin binds to from A·M·MgADP·P_i, the complex appears to retain its segmental flexibility (*28*, *29*), which may be required for accommodating the asynchronous motor domain cycling during shortening. But after product dissociation, the M in A·M is rigidified (*11*), consistent with maintaining the force-induced change in filament position. The energy barrier between the two lever arm orientations in A·M has not been measured, but presumably it is large.

If the actual events resemble those hypothesized above, the energy barrier and degree of rotation need to be quantitatively determined and correlated to changes of the internal structure of the motor domain in the absence and presence of actin. The largest gap in knowledge is the structure of actin−motor domain complexes, and the rigor complex, A·M, is the most likely both to be solved first and to have the greatest structural differences compared to the actin-free motor domain species. Structural changes other than lever arm position, which have been identified in the absence of actin, include the degree of cleft closure (*15*, *17*−*20*), the movement of the converter domain located near the fulcrum site (*12*), and the melting of a mostly α-helical structure at the fulcrum site (*13*, *14*). Helix melting is the most studied. Actin minimizes and unhydrolyzed ATP maximizes the extent of helix melting, suggesting that if its melting has a role it may be to enable a motor domain−ATP complex to dissociate from actin (*57*). This α-helical

region is likely to be intact in A·M (*14*, *57*). Less is known about cleft conformation and converter domain location. The cleft conformations in the crystal structures are not optimal for binding to actin (*16*, *39*, *40*), but it is not clear if the cleft should be more or less open when bound. In either case, any mobility in the cleft conformation would presumably be eliminated by actin binding. Because of its location near the lever arm fulcrum, and the fact that it moves when the lever arm moves (*12*), the converter domain location may be the structural feature most closely related to holding the lever arm in an elongated conformation in A·M, and to rigidifying motor domain internal motions. The converter domain changes location with little change in conformation in the actin-free M, M·MgADP·AlF_4, and M·MgADP·BeF_x structures (*3*, *12*). If its location is in fact key to rigidifying M in A·M, the converter domain location will be different than it is in actin-free structures, as they appear to be flexible and internally mobile (*11*, *22*, *26*, *27*) while M in A·M is not.

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