The swinging lever-arm hypothesis of muscle contraction

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The molecular mechanism of muscle contraction is a problem that has exercised biophysicists and biochemists for many years. The common view of the mechanism is embodied in the 'cross-bridge hypothesis', in which the relative sliding of thick (myosin) and thin (actin) filaments in cross-striated muscle is brought about by the 'cross-bridges', parts of the myosin molecules which protrude from the thick filaments and interact cyclically with the actin filaments, transporting them by a rowing action that is powered by the hydrolysis of ATP. This hypothesis is, however, rather vague on the molecular details of cross-bridge movement and, in the light of the recently determined crystal structures of myosin and actin, it has evolved into the more precise 'swinging lever-arm hypothesis'.

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Introduction

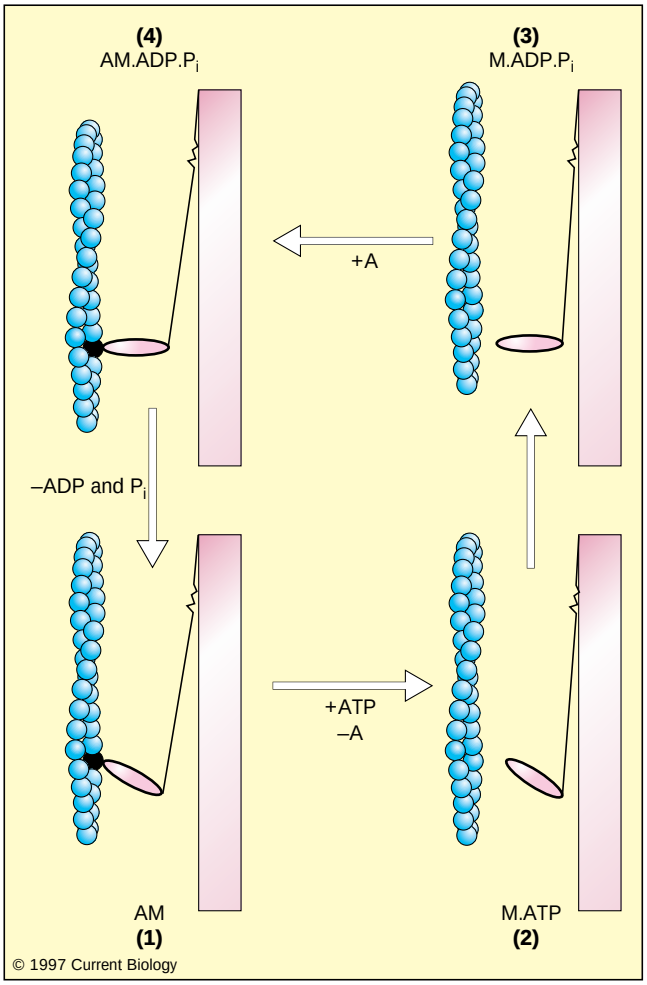
Although the swinging myosin cross-bridge hypothesis of muscle contraction had become the textbook norm by 1971 [1–3], it has proved remarkably difficult to catch a bridge *in flagranti delicto* (reviewed in [4]). Nevertheless, the swinging cross-bridge hypothesis provides by far the best framework for correlating and explaining the vast muscle literature. The hypothesis is most succinctly expressed by the Lymn–Taylor [2] cycle (Fig. 1), as follows: ATP rapidly dissociates the actin–myosin complex by binding to the ATPase site on the myosin cross-bridge; myosin then hydrolyzes ATP and forms a stable myosin–products complex (ADP.Pi); actin recombines with this complex and dissociates the products, thereby reforming the original actin–myosin complex. After recombining with actin, the cross-bridge undergoes a conformational change leading to the rowing-like stroke, sometimes referred to as the power stroke; during this process the products of ATP hydrolysis are released (Fig. 1).

However, there were indeed problems with this hypothesis — the swinging cross-bridge needed an update. In fact, the hypothesis was never very clear about how the cross-bridge moved on actin, and has been discretely modified over the years into a swinging lever-arm hypothesis, in which the bulk of the cross-bridge is envisaged to bind to actin with a more or less fixed geometry, and only the distal (carboxy-terminal) part of the myosin molecule moves [4] (Fig. 2). A swinging lever-arm explains why substantial changes in the cross bridge orientation were not visible: only a small fraction of the cross-bridge mass moves during the cycle. Furthermore, it has gradually become clear that the proportion of cross bridges taking part in a contraction at any one time is only a small fraction of the total, making the registration of active cross-bridge movement doubly difficult.

Structures

In the last seven years, atomic structures have been determined of, first, actin [5–7] and then myosin [8], and these have provided a renaissance for muscle research. In particular, the crystal structure of the myosin subfragment 1 (see below) [8] endowed the myosin cross-bridge with an extended carboxy-terminal tail which looks like a lever arm; moreover, the tail is in the correct orientation and position actually to function as a lever arm [9]. In the last year, a number of independent experiments have given results that are in excellent accord with the idea that the carboxy-terminal tail functions as a lever arm and, indeed, they have provided evidence that

Figure 1



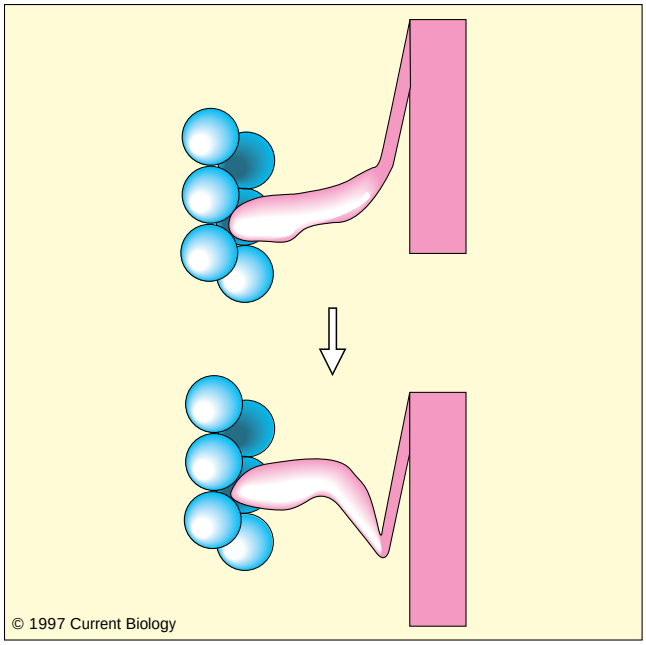
Lymn–Taylor cycle and swinging cross-bridge model. The helices of blue spheres represent the actin (A) filament, the shaded red areas the myosin (M) thick filament. The myosin cross-bridges are shown in their conventional conformations at 90 ° and 45 ° to the actin filament. Initially the nucleotide-free cross-bridge is bound to the actin filament in the 45 ° conformation (state 1, rigor). The binding of ATP brings about rapid release from actin of the cross bridge (state 2), still in the 45 ° position. The subsequent hydrolysis of ATP puts the cross-bridge into the 90 ° conformation (state 3), whereupon it rebinds to actin (state 4). The binding to actin brings about the release of products of hydrolysis and the cross-bridge returns to its initial state, thereby 'rowing' the actin past the myosin.

the tail can move. Furthermore, new crystal structures [10,11] with bound analogues of ADP.Pi appear to show an alternative orientation of the lever of the anticipated kind and, moreover, they give insight into the mechanism whereby ATP hydrolysis is turned into mechanical work.

The myosin molecule, which is made up of two heavy chains and four light chains, has a long (140 nm) tail and two heads, each of 120 000 kDa. The carboxyl termini of the heavy chains form extended α-helices, which dimerize to form the long coiled-coiled tails. The tails, in turn, aggregate to form the myosin thick filament. The amino-terminal fragment of myosin known as subfragment 1 (S1) comprises the head or cross-bridge, which also contains the ATPase active site. S1 is tadpole-like molecule [8], with an elongated head, containing a seven-stranded β sheet and numerous associated α-helices that form a long narrow cleft. The carboxy-terminal tail, which binds two light chains, is in the form of an extended (80 Å long) α-helix. The carboxyl terminus of the tail joins onto the thick filament through a (presumed) flexible link. In the description that follows, we refer to the chicken skeletal myosin sequence, although much of the new data has been obtained with *Dictyostelium* myosin (for a sequence comparison, see [12]). Proximal and distal refer to the relationship to the actin helix in 'decorated' actin (see below).

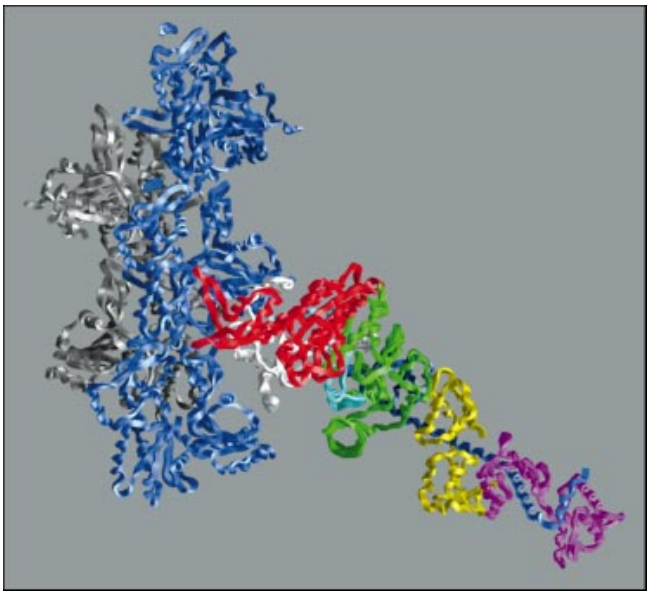
Proteolytic digestion breaks myosin S1 into three pieces: the 25 kDa, 50 kDa and 20 kDa fragments, which were thought to be discrete domains [13]. The structure determination showed that the fragments actually arise from the positions of exposed loops, rather than at domain boundaries. However, the names remain and are used as the basis of a standard colour-coding for portions of the S1 polypeptide chain [8]: 25 kDa (amino-terminal), green; 50 kDa (middle), red; and 20 kDa (carboxy-terminal), blue

Figure 2



Numerous experiments indicated that the scheme shown in Figure 1 needed revision: as illustrated here, only the distal part of the myosin cross-bridge moves, acting like a swinging lever-arm [4].

Figure 3



The structure of the actin–myosin complex. This ribbon diagram shows, on the left, five actin molecules in an actin helix (blue and grey to distinguish the two strands) and, on the right, a myosin cross-bridge. The portion of myosin shown corresponds to an S1 fragment, with the various domains highlighted in different colours, as follows: 25 kDa domain, green; 50 kDa upper domain, red; 50 kDa lower domain, white; first part of the 20 kDa domain, including the SH2 helix up to residue 699, light blue; SH1 helix, converter domain and carboxy-terminal helix, dark blue; regulatory light chain, magenta; and essential light chain, yellow. The diagram was prepared with GRASP [27] using S1 coordinates [8] PDB–1MYS. The coordinates used in this figure are available from holmes@mpimf-heidelberg.mpg.de.

(Fig. 3). It transpires that the 50 kDa fragment actually spans two domains, which Rayment *et al.* [8] have called the 50K upper fragment and the 50K lower fragment.

The S1 amino terminus lies distal to actin, and the first 80 residues form a protruding β-barrel domain that is rather like a Src-homology 3 (SH3) domain, but of unknown function (this is not present in all myosins [12]). The rest of the 25K fragment and the 50K upper fragment together (residues 81–486) form one domain, which accounts for six of the seven strands of the β-sheet. The ATP-binding site is in this domain, near the 25–50 kDa fragment boundary, and contains a characteristic P-loop similar to that found in some other ATPases and GTP-binding (G) proteins. The ATP-binding site is about 4 nm distal to the actin-binding site. The 50 kDa upper fragment provides about half the actin-binding site. The 50K lower fragment (residues 487–600) actually forms a well-defined domain which constitutes the other half of the actin-binding site. A large positively-charged loop (residues 625–647) follows, which is involved in actin binding.

The first part of the following 20 kDa domain (residues 648–689) is an integral part of the 25–50 kDa domain, and consists of a long helix running distally from the actin-binding site to a seventh strand which is inserted in the β sheet. This is followed by a turn and a broken helix containing two reactive thiols (SH1 707 and SH2 697). The SH1-containing helix may form part of the hinge for the ensuing lever (see below). Distal to this, and apparently rigidly connected to the long α-helical tail, is a small compact domain (residues 711–781) which has been termed the 'converter domain' [14]. This apparently functions as a socket for the carboxy-terminal α-helical tail, which carries the two light chains and has been called the 'regulatory domain' or 'neck'. The main function of the regulatory domain or neck, however, appears to be as a 'lever arm' to amplify rotational movements experienced by the converter domain during ATP hydrolysis.

Fitting of the F-actin and S1 atomic structures into three-dimensional cryoelectron microscope reconstructions yielded an atomic model of the actin–myosin complex [9,15] (Fig. 3). This showed that the cleft between the 50 kDa upper and lower domains extends from the ATP-binding site to the actin-binding site, and that the opening and closing of the cleft may provide the physical link between the ATP- and actin-binding sites. Actin binding is thought to favour the closed-cleft form. The actin-binding and ATP-binding sites are on opposite sides of the β sheet and are separated by about 40 Å. Furthermore, the very extended carboxy-terminal α-helical tail or neck of S1, which lies distal from the actin helix, is ideally placed and correctly orientated to be a lever arm [9].

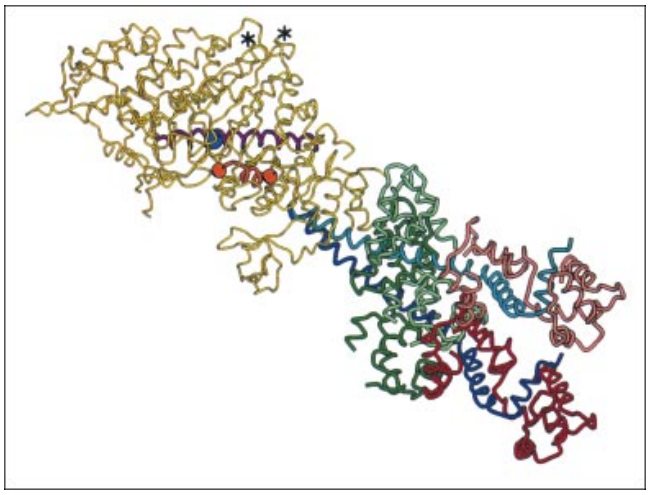
The swinging lever arm

Electron microscopy

According to the Lymn–Taylor cycle, in the presence of the products of ATP hydrolysis, one would expect myosin to be at the beginning of the power stroke. A suitable system for observing such a conformation would be 'decorated actin', an actin filament in which an S1 fragment is bound to each actin. The structure of this complex can be observed by electron microscopy, particularly by cryoelectron microscopy. Such studies have provided us with the best data on the rigor complex — that is, without ATP — of actin and the myosin cross-bridge [16], and were used for assembling the actin–myosin complex from the atomic models of its components [9]. The rigor state is assumed to be at the end of the power stroke and gives us a reference point.

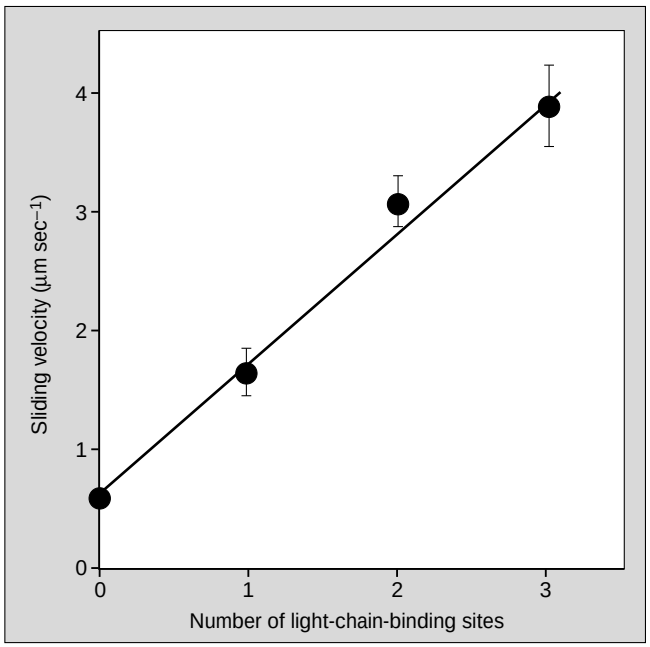
Unfortunately, corresponding data in the presence of ATP is difficult to get, because the proteins dissociate rapidly on adding ATP. Milligan, Sweeney and coworkers [17,18] therefore investigated the effects of adding ADP, which does not produce such a large reduction in actin–myosin affinity and which in suitable muscles would be expected

Figure 4



The cross-bridge lever arm moves 35° on removing ADP [18]. The model of the S1 fragment shown has been derived by fitting crystallographic data to cryoelectron microscopic images of actin decorated with smooth muscle myosin S1. The elevated position (paler colours) of the neck (lever arm) of smooth muscle S1 is found in the presence of ADP; the lower position (darker colours) corrsponds to nucleotide-free (rigor) S1. (Reproduced from [18] with permission.)

Figure 5



The speed of transport of actin filaments across a lawn of myosin heads attached to a cover slip and irrigated with ATP, as a function of the length of the S1 neck region [19]. The neck region of S1 was shortened or lengthened by inserting or deleting light-chain-binding sites. The observed speed is linearly related to the number of light-chain-binding sites in the lever arm. The position of the intercept for zero velocity allows an estimate of the position of the hinge, which is close to the SH1 group. (Reproduced from [19] with permission.)

to lead to a partial reversal of the power stroke. Indeed, cryoelectron micrograph reconstructions of actin decorated with brush border myosin or with smooth muscle myosin by Jontes *et al.* [17] and Whittaker *et al.* [18] do show a 30–35° rotation of the lever arm away from rigor on binding ADP (Fig. 4). These experiments provide the first direct demonstration of the anticipated lever-arm swing. The hinge is close to the SH1 region.

Lengthening the lever

Spudich and coworkers [19] have been able to test the effects of altering the length of the neck in myosin S1 fragments. They used S1 of myosin II from the slime mold *Dictyostelium discoideum*, which is homologous to vertebrate skeletal myosin, and altered the neck length by adding or subtracting light-chain-binding regions. An *in vitro* motility assay, in which actin filaments are transported across a lawn of S1 molecules irrigated with ATP, showed that the speed of actin transport was linearly related to the number of light-chain-binding sites on the S1 molecules (Fig. 5). Furthermore, the intercept of the graph at zero velocity corresponds to the position of the SH1-containing helix, again indicating the possible function of this region as a molecular hinge.

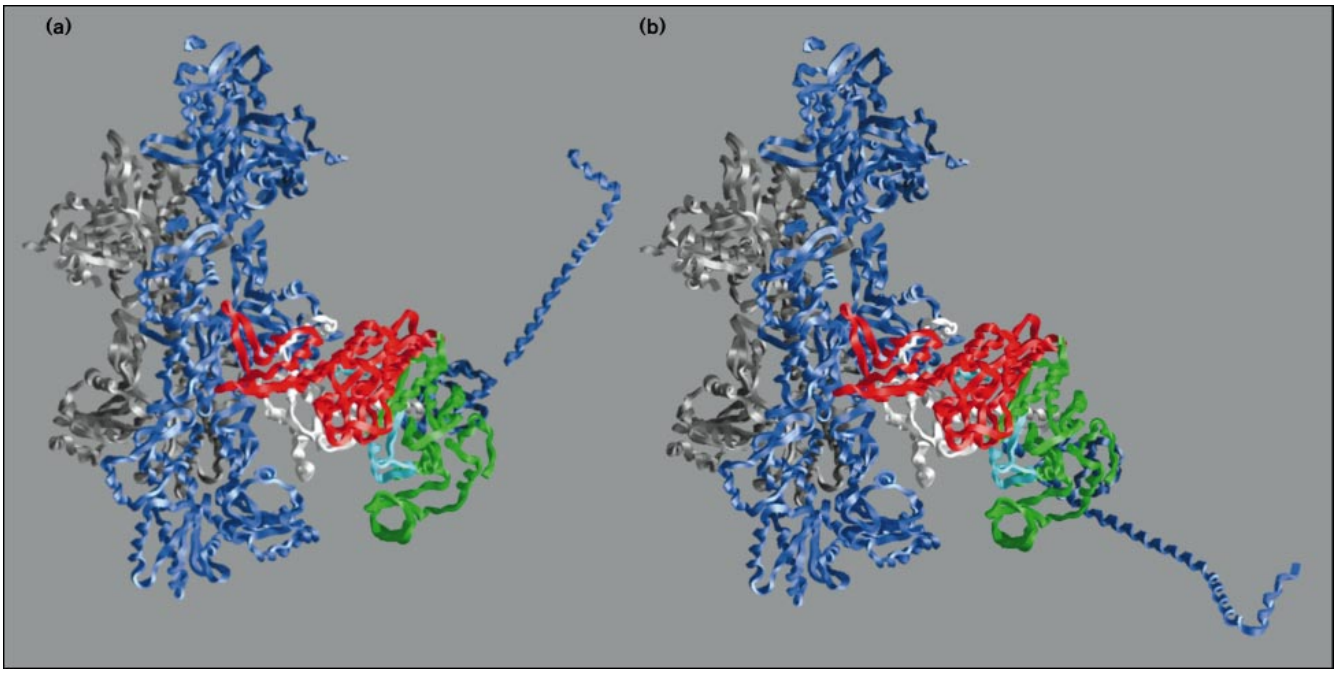
In a similar vein, Manstein and coworkers [20] have replaced the myosin neck region with artificial lever arms consisting of one or more α-actinin repeats (a triple α helix motif) and have tested the engineered myosin in *in vitro* motility assays. These authors also found that the speed of transport is proportional to the length of the lever arm. Kinetic measurements showed that the variation in speed of transport of actin could not be accounted for by alterations in the ATPase activities, as these were essentially unaffected by the presence or number of α-actinin repeats. Therefore, they conclude that the length of the lever arm controls the velocity of transport.

Mutations and markers

Mutagenesis studies have also indicated that the SH1-containing helix is important in controlling movement of the myosin lever arm. Patterson and Spudich [21] have reported that a mutation of either of the two glycines that flank this helix (residues 697 and 707) produces a *Dictyostelium* cell that is unable to undergo cytokinesis at 13°C. Moreover, substitution of the glycine at position 699 by alanine slows myosin transport of actin 100-fold [22].

By attaching specific fluorescent markers onto the 'regulatory' light chain, it has been shown that this region of myosin undergoes a small angular movement on contraction [23]. If the 'lever arm hypothesis' is true, one would have predicted a larger rotation, of ~60°. However, this discrepancy can be explained if only a fraction

Figure 6



Reconstructions of the actin–myosin complex at the beginning and end of the power stroke (the light chains have been omitted for clarity). **(a)** The 'beginning' of the power stroke, based on the truncated S1–ADP.vanadate coordinates (PDB-1VOM) [11]. The missing lever arm has been restored using the chicken S1 coordinates (PDB-MYS) with an appropriate rotation. The break in the chain at the beginning of the lever-arm marks the extent of the fragment of S1 used in the crystal structure analysis [11]. **(b)** The 'end' state, or rigor complex (as in Fig 3 with the light chains removed). Note that the end of the lever arm moves about 12 nm between the two states. Diagram prepared with GRASP [27].

(~10 %) of the cross-bridges in active muscle take part in contraction at any one time, as the magnitude of this apparent rotation can be proportionally scaled up towards the expected value.

All these experiments are consistent with view that myosin motility involves a rotating lever arm with a hinge near the thiol SH1. Moreover, chemical cross-linking studies on the reactive thiol groups of SH1 and SH2 have indicated that this region is sensitive to the presence and state of the bound nucleotide [24] and may be close to the putative force-generating 'motor'.

Crystallographic studies

Rayment and colleagues [10,11] have studied a crystalline S1 fragment of *Dictyostelium* myosin II, which has been truncated after residue 761. The truncation eliminates the neck and the associated light chains, but the 'converter' domain is still present. The expressed fragment corresponds to the myosin 'core' which has been shown by sequence comparisons to be common to all myosins [12] — many myosins have quite different neck regions to myosin II, but all have very similar cores. Experiments with shorter constructs [25] have shown that damage to the converter domain leads to unusual ATPase activity; however, the form truncated at 761 appears kinetically normal (C. Bagshaw, personal communication). The crystal structures of the 761 construct have been determined with bound ATP analogs: ADP.BeF_x [10], ADP.AlF_4 [10] and ADP.vanadate [11]. It is revealing to interpret these structures in terms of the lever-arm hypothesis.

Dictyostelium S1–ADP.BeF_x

The crystal structure reported for chicken myosin S1 [8] apparently represents the end of the power stroke, with no bound nucleotide. The crystal contains a sulphate ion bound in the active site, which could lead to small movements, but the S1 structure fits excellently into the electron micrograph reconstructions [9] of nucleotide-free actin–myosin. The crystal structure [10] of truncated *Dictyostelium* S1 with bound ADP.BeF_x, thought to be an analogue of ATP, shows a close similarity with chicken S1, as expected from the Lymn–Taylor scheme (Fig. 1) if the chicken S1 structure does indeed represent the rigor state. The absence of the neck region and light chains appears to have little effect on the structure of the myosin core.

Dictyostelium S1–ADP.AlF_4

ADP.AlF_4 is thought to be an analogue of the transition-state complex. The crystal structure of the complex with truncated *Dictyostelium* S1 [10] shows that, on binding

$ADP.AlF_4$, the cleft that extends from the nucleotide-binding site to the actin-binding site half closes. This is achieved by movements of the 50 kDa lower domain, which lead to substantial changes in the nucleotide-binding region as well as alterations in the actin-binding site (described below). This structure appears to have an active catalytic site.

Smith and Rayment [26] have pointed out that within the nucleotide-binding region there is great similarity between the structures of the closed form of myosin S1 and the 'switch 2 region' in the small GTPase Ras. Comparing *Dictyostelium* S1–$ADP.AlF_4$ and chicken S1, the region corresponding to the Ras switch 2 region moves away from the nucleotide-binding site by 4-5 Å in chicken S1 — which we take to be rigor-like — thereby removing an important hydrogen bond to the γ-phosphate, which probably facilitates phosphate release. There are also substantial changes in the distal part of the molecule, including a 10° rotation of the SH1-containing helix. These changes could not be described further because the structure is disordered, but they become apparent in the complex with ADP.vanadate.

Dictyostelium S1–ADP.vanadate

ADP.vanadate is used as an analogue of the ATPase transition state, or possibly of the ADP.Pi state. The *Dictyostelium* S1–ADP.vanadate structure [11] shows, with higher resolution and completeness, features already indicated by the S1–$ADP.AlF_4$ structure. Compared to the chicken structure, there is a closing of the 50 kDa upper and lower domain cleft, and movements in the SH1/SH2 region. The 50 kDa lower domain rotates a few degrees around the helix 648–666, so that the inner end closes the nucleotide-binding pocket. The outer end of the long helix (475–507) bends out 24° at valine 497; this allows the converter domain (711–781), which would carry the carboxy-terminal lever arm, to rotate 70 ° in the plane containing the long actin filament axis, by swinging around the glycines at the ends of the SH1-containing helix Using the coordinates of rigor-state chicken S1 to complete the missing light-chain-binding region, we find that this rotation moves the carboxy-terminus of the tail vertically by about 12 nm from the position found from the chicken S1 coordinates in the rigor state (Fig. 6).

It seems, therefore that the structure of the complex between S1 and ADP.vanadate could indeed be a model for the beginning of the power stroke. However, Smith and Rayment [11] themselves urge caution in this interpretation, on the grounds that the distal part of the S1 fragment shows considerable disorder, and the movement seen could be an artifact arising from the missing light chains. However, the fact that these results fit so nicely with other experiments supporting the lever-arm hypothesis points to their significance. Moreover, the expressed fragment of S1 contains the full core of myosin and appears biochemically to be normal.

Conclusions

After some lean years, research on the molecular mechanism of muscle contraction is making real progress. A number of experiments reported in the last year are strongly supportive of the lever-arm mechanism. Crystal structure analysis is highly consistent with this model, and has probably given us a view of the elusive 'beginning-of-the-power-stroke' state. It is also possible to glimpse the mechanism whereby the hydrolysis of ATP and binding to actin bring about the movements of the lever: the coupling is brought about by a rotational movement of the 50 kDa lower domain, induced by ATP hydrolysis, which in turn allows the lever to rotate about a fulcrum close to the SH1-containing helix. The return from this state to the rigor state is the power stroke. The position of the 50 kDa lower domain is also controlled by binding to actin.

References

1. Huxley HE: The mechanism of muscular contraction. *Science* 1969, 164:1356–1366.
2. Lymn RW, Taylor EW: Mechanism of adenosine triphosphate hydrolysis of actomyosin. *Biochemistry* 1971, 10: 4617–4624.
3. Huxley AF, Simmons R: Proposed mechanism of force generation in striated muscle. *Nature* 1971, 233: 533– 538.
4. Cooke R: The mechanism of muscle contraction. *CRC Crit Rev. Biochem* 1986, 21:53–118.
5. Kabsch W, Mannherz HG, Suck D, Pai EF, Holmes KC: Atomic structure of the actin:DNase I complex. *Nature* 1990, 347:37–44.
6. Holmes KC, Popp D, Gebhard W, Kabsch W: Atomic model of the actin filament. *Nature* 1990, 347:44–49.
7. Lorenz M, Popp D, Holmes KC: Refinement of the F-actin model against X-ray fiber diffraction data by the use of a directed mutation algorithm. *J Mol Biol* 1993, 234:826–836.
8. Rayment I, Rypniewski WR, Schmidt-Base K, Smith R, Tomchick DR, Benning MM, Winkelmann DA, Wesenberg G, Holden HM: Three-dimensional structure of myosin subfragment-1: a molecular motor. *Science* 1993, 261:50–58.
9. Rayment I, Holden HM, Whittaker M, Yohn CB, Lorenz M, Holmes KC, Milligan RA: Structure of the actin–myosin complex and its implications for muscle contraction. *Science* 1993, 261:58-65.
10. Fisher AJ, Smith CA, Thoden JB, Smith R, Sutoh K, Holden HM, Rayment I: X-ray structures of the myosin motor domain of ***Dictyostelium discoideum*** complexed with MgADP.BeFx and MgADP.AlF4$^-$. *Biochemistry* 1995, 34:8960–8972.
11. Smith CA, Rayment I: X-ray structure of the magnesium(ii).ADP.vanadate complex of the ***Dictyostelium-discoideum*** myosin motor domain to 1.9 resolution. *Biochemistry* 1996, 35:5404–5417.
12. Cope M, Whisstock J, Rayment I, Kendrick-Jones J: Conservation within the myosin motor domain — implications for structure and function. *Structure* 1996, 4:969–987.
13. Mornet D, Pantel P, Audemard E, Kassab R: The limited tryptic cleavage of chymotriptic S1: an approach to the characterisation of the actin site in myosin heads. *Biochem Biophys Res Comm* 1979, 89:925–932.
14. Houdusse A, Cohen C: Structure of the regulatory domain of scallop myosin at 2Å resolution: implications for regulation. *Structure* 1996, 4:21–32.
15. Schroeder RR, Manstein DJ, Jahn W, Holden H, Rayment I, Holmes KC, Spudich JA: Three-dimensional atomic model of F-actin decorated with ***Dictyostelium*** myosin S1. *Nature* 1993, 364:171–174.
16. Milligan RA, Flicker PF: Structural relationships of actin, myosin, and tropomyosin revealed by cryo-electron microscopy. *J Cell Biol* 1987, 105:29–39.

17. Jontes JD, Wilson-Kubalek EM, Milligan RA: The brush border myosin-1 tail swings through a 32° arc upon ADP release. *Nature* 1995, 378:751–753.
18. Whittaker M, Wilson-Kubalek EM, Smith JE, Faust L, Milligan RA, Sweeney HL: Smooth muscle myosin moves 35Å upon ADP release. *Nature* 1995, 378: 748–751.
19. Uyeda T, Abramson PD, Spudich JA: The neck region of the myosin motor domain acts as a lever arm to generate movement. *Proc Natl Acad Sci USA* 1996, 93:4459–4464.
20. Anson M, Geeves MA, Kurzawa SE, Manstein D: Myosin motors with artificial lever arms. *EMBO J* 1996, 15:6069–6074.
21. Patterson B, Spudich JA: Cold-sensitive mutations of ***Dictyostelium*** myosin heavy-chain highlight functional domains of the myosin motor. *Genetics* 1996, 143:801–810.
22. Kinose F, Wang SX, Kidambi US, Moncman CL, Winkelmann DA: Glycine 699 is pivotal for the motor activity of skeletal muscle myosin. *J Cell Biol* 1996, 134:895–909.
23. Irving M, Allen TS, Sabidodavid C, Craik JS, Brandmeier B, Kendrick-Jones J, Corrie JET, Trentham DR, Goldman YE: Tilting of the light-chain region of myosin during step length changes and active force generation in skeletal muscle. *Nature* 1995, 375:688–691.
24. Huston EE, Grammer JC, Yount RG: Flexibility of the myosin heavy chain: direct evidence that the region containing SH1 and SH2 can move 10 Å under the influence of nucleotide binding. *Biochemistry* 1988, 27:8945–8952.
25. Kursawa SE, Manstein DJ, Geeves MA: ***Dictyostelium discoidium*** myosin II: characterisation of functional myosin motor fragments. *Biochemistry* 1997, in press:
26. Smith CA, Rayment I: Active site comparisons highlight structural similarities between myosin and other P-loop proteins. *Biophys J* 1996, 70:1590–1602.
27. Nicholls A, Sharp KA, Honig B: Protein folding and association: Insights from the interfacial and thermodynamic properties of hydrocarbons. *Proteins* 1991, 11:281–296.