PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

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Phil. Trans. R. Soc. Lond. B 2000 **355**, 419-431
doi: 10.1098/rstb.2000.0583

THE ROYAL SOCIETY

The structural basis of muscle contraction

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The myosin cross-bridge exists in two conformations, which differ in the orientation of a long lever arm. Since the lever arm undergoes a 60° rotation between the two conformations, which would lead to a displacement of the myosin filament of about 11 nm, the transition between these two states has been associated with the elementary 'power stroke' of muscle. Moreover, this rotation is coupled with changes in the active site (CLOSED to OPEN), which probably enable phosphate release. The transition CLOSED to OPEN appears to be brought about by actin binding. However, kinetics shows that the binding of myosin to actin is a two-step process which affects both ATP and ADP affinity and vice versa. The structural basis of these effects is only partially explained by the presently known conformers of myosin. Therefore, additional states of the myosin cross-bridge should exist. Indeed, cryoelectron microscopy has revealed other angles of the lever arm induced by ADP binding to a smooth muscle actin–myosin complex.

Keywords: muscle mechanism; actin; myosin; X-ray structure; kinetics

> . . . die Muskulatur ein kompliziertes, nur vom Standpunkt der reinen Mechanik verständliches Hebelsystem, welches die scheinbar verwickelsten Probleme mit den einfachsten Mitteln lößt.
>
> L. E. Boltzmann, Antrittsvorlesung as Prorector, Leipzig, 1900

1. INTRODUCTION

The cross-bridge theory for muscle contraction proposes that the myosin cross-bridge binds to the actin filament in an initial conformation and then undergoes a change of state which moves the actin filament past the myosin filament. This elemental event, which is part of a cycle driven by ATP hydrolysis, is known as the 'power stroke'. Each stroke of the cross-bridge leads to the hydrolysis of one ATP molecule. The cross-bridges were first visualized over 40 years ago (H. E. Huxley 1957) and a quantitative cross-bridge theory of muscle contraction with coupled ATP hydrolysis was proposed by A. F. Huxley (1957). The theory is supported by an impressive body of structural, physiological and biochemical data, but direct visualization of cross-bridge movement has proved difficult. However, to quote H. E. Huxley (1958) '. . . recent studies have made the problem more difficult by seeming to require that a movement of 100 Å in part of the muscle structure be the consequence of a single chemical event'.

In the past decade, X-ray crystallography has provided the atomic structures of actin and myosin. To date, two conformational states of myosin S1 have been revealed by X-ray crystallography. The myosin cross-bridge indeed appears to be a molecular machine that amplifies the small changes at the active site into the 100 Å changes needed to transport actin past myosin by means of a long lever arm. It now seems that we can describe the end states of the power stroke in structural terms and already have some understanding of the molecular mechanisms leading to this event. However, static structures need to be interpreted within a framework of biochemistry and physiology. Whereas the structural data consist of incontrovertible lists of coordinates, the job of analysing these into communicating functional units still provides scope for debate.

The myosin cross-bridge may be cleaved off the myosin molecule as a soluble fragment (myosin S1, *ca.* 120 000 D) (Margossian & Lowey 1973*a*,*b*), which contains three polypeptide chains, one heavy and two light. Myosin S1 is a fully competent actin-activated ATPase that transports actin in *in vitro* motility assays. Moreover, in the absence of nucleotide it forms a tight (rigor) bond to actin filaments. Structural investigations of myosin have therefore concentrated on myosin S1 as a minimal model for the cross-bridge. Furthermore, the complex between filamentous actin and S1 (decorated actin), which was the first macromolecular system to be investigated by electron microscopy image reconstruction (Moore *et al.* 1970) remains the best hope for getting structural data on the actin–myosin interaction.

Changes in the intrinsic protein fluorescence of myosin S1 have played a central role in signalling conformational states of myosin and linking these to catalytic events. The resulting mechanism of the myosin ATPase (often referred to as the Bagshaw–Trentham scheme (Bagshaw & Trentham 1973)) has aquired considerable importance. The crystal structures allow an identification of the residues involved in the intrinsic protein fluorescence changes, which enables the embedding of the structural results in the Bagshaw–Trentham mechanism. Moreover, the structural data also point to the need for a revision of this scheme.

The conformational change which embodies the power stroke is very likely to be correlated with a chemical state

hange. White (White & Taylor 1976) has shown that hosphate release is the relevant chemical change and hat this is coupled with force production. The two onformations of myosin show marked differences in the hosphate-binding pocket, which would result in very ifferent γ-phosphate affinities for the two states. Further- nore, the small changes in the active site are coupled ith large changes in the distal part of the molecule. It eems likely that the two myosin conformations represent he ends of the power stroke.

2. THE SWINGING CROSS-BRIDGE AND THE LYMN–TAYLOR CYCLE

Myosin is a product-inhibited ATPase that is strongly imulated by actin, which is a nucleotide exchange factor or myosin. This mechanism was first elucidated by Lymn Taylor (1971), who combined their results with the winging cross-bridge proposals of H. E. Huxley (Huxley 969) to produce the following cycle.

. In the absence of nucleotide the myosin cross-bridge binds tightly to the actin filament to form the 'strong' or 'rigor' complex (figure 1*a*).
. The binding of ATP to the ATPase site on the myosin cross-bridge rapidly dissociates the actomyosin complex; myosin then hydrolyses ATP and forms a stable myosin–products complex (ADP·Pi) (figure 1*b*).
. Actin recombines weakly with this complex (figure 1*c*).
. Strong actin binding releases the products, thereby forming the original strongly bound actin–myosin complex (figure 1*d*).

During this last process the cross-bridge undergoes a onformational change (the rowing-like 'power stroke', tages 3 to 4) which also allows the products of hydrolysis o be released so that the cycle can repeat.

It has in fact proved remarkably difficult to visualize a ridge during the swing. Finally, after many years of ffort involving the development of new X-ray sources see Huxley & Holmes (1997) for historical review), time- esolved X-ray fibre diagrams from contracting frog uscle provided evidence of cross-bridge movement Huxley *et al*. 1981; Irving *et al*. 1992).

3. THE LEVER-ARM HYPOTHESIS

On account of a number of spectroscopic and tructural observations (see review by Cooke 1986), the winging cross-bridge was modified into a swinging lever ypothesis in which the bulk of the cross-bridge was nvisaged to bind to actin with a more or less constant eometry. Large movements were envisaged as arising om the distal (C-terminal) part of the myosin cross- ridge moving as a lever arm (figure 2).

The crystal structure of the myosin cross-bridge (from hicken muscle) without nucleotide (and thus thought to epresent the end of the power stroke) was first deter- ined by Rayment and co-workers (Rayment *et al*. 993*b*). One remarkable feature of their structure was hat it showed the cross-bridge to consist not only of a ore or less globular 'head' or motor domain, which ontained the ATPase site and the actin-binding site, ut also a long C-terminal α-helical 'tail', which binds

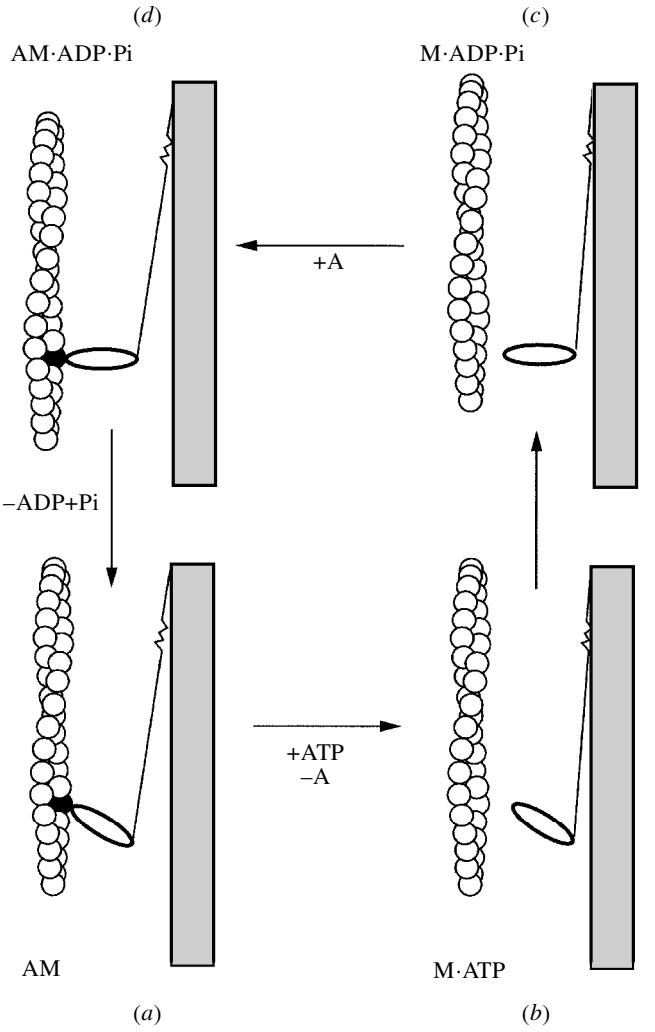
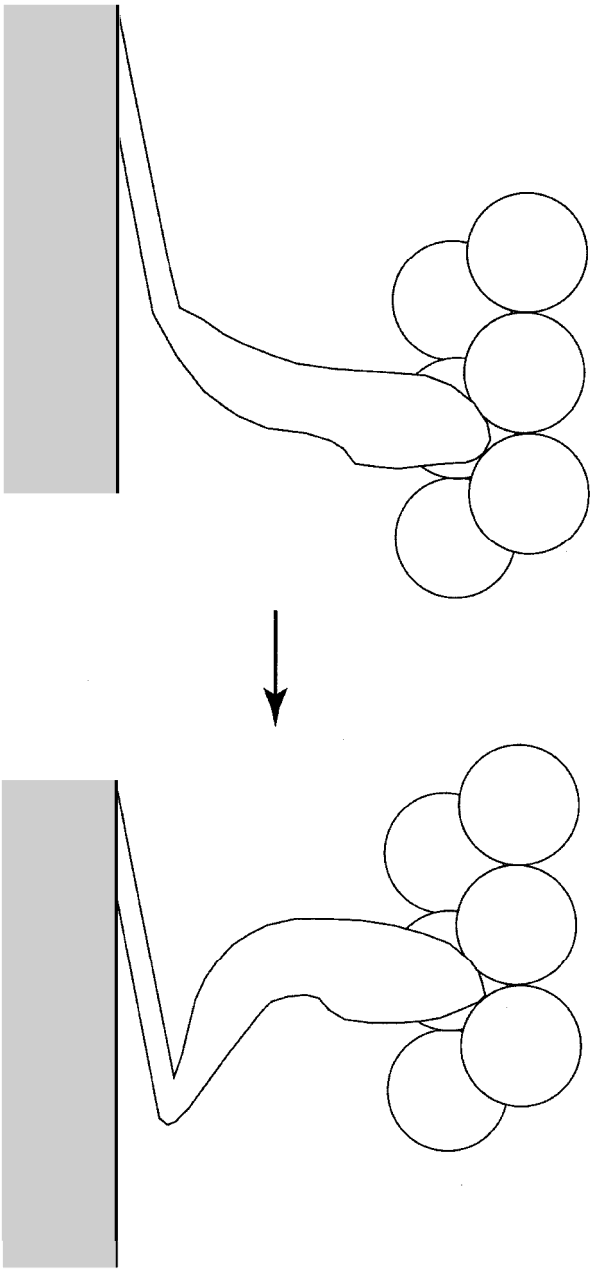


Figure 1. The Lymn–Taylor cycle (Lymn & Taylor 1971): the myosin cross-bridge is bound to actin in rigor 45° position—'down' (*a*). ATP binds, which leads to very fast dissociation from actin (*b*). The hydrolysis of ATP to ADP and Pi leads to a return of the myosin cross-bridge to the 90° 'up' position whereupon it rebinds to actin (*c*). This leads to release of the products and return to (*a*). In the last step actin is 'rowed' past myosin.

the two calmodulin-like light chains and which looked like a lever arm. By combining the crystal structures with cryoelectron microscopy of decorated actin, the cross-bridge was postioned on the actin filament, and it was found that the tail was indeed correctly orientated to be a lever arm (Rayment *et al*. 1993*a*).

In the case of smooth muscle and some other myosins (but not striated muscle myosins), the addition of ADP to decorated actin causes changes in the angle of the lever arm, which can be clearly observed by cryoelectron microscopy (Jontes *et al*. 1995; Whittaker *et al*. 1995). This effect seems to be limited to actomyosins with a relatively high affinity for ADP. These experiments are noteworthy for providing the first incontrovertible evidence of lever arm movement.

Fluorescence polarization measurement provides a method of measuring lever-arm orientation *in situ* if the probes can be attached in a way that hinders probe rotation and does not inhibit myosin function. Since the atomic structures of the light chains are known (Houdusse



igure 2. The major change in the cross-bridge is confined to ne distal part which moves as a lever arm (Cooke 1986).

t Cohen 1996; Rayment *et al*. 1993*b*; Xie *et al*. 1994), enetically engineered light chains can be manufactured vith sufhydril groups in suitable positions to allow difunc- onal chromophores to be securely attached. Furthermore, ne chemically modified light chains can be diffused into nuscle fibre and exchanged for endogenous light chains Irving *et al*. 1995). Corrie *et al*. (1999) have measured the olarized fluorescence from rhodamines attached via pecifically engineered SH groups. Novel is the two-point ttachment to reduce uncorrelated movement of the robe. Also a new method for removing the effects of fast bration of the probe is employed. This approach shows nat in contracting muscle fibres the light chains swing nrough angles (30–40°) that are quite consistent with the nolecular models for the swinging lever arm proposed bove, if one assumes that only a small proportion (15%) f the cross-bridges are active at any one time.

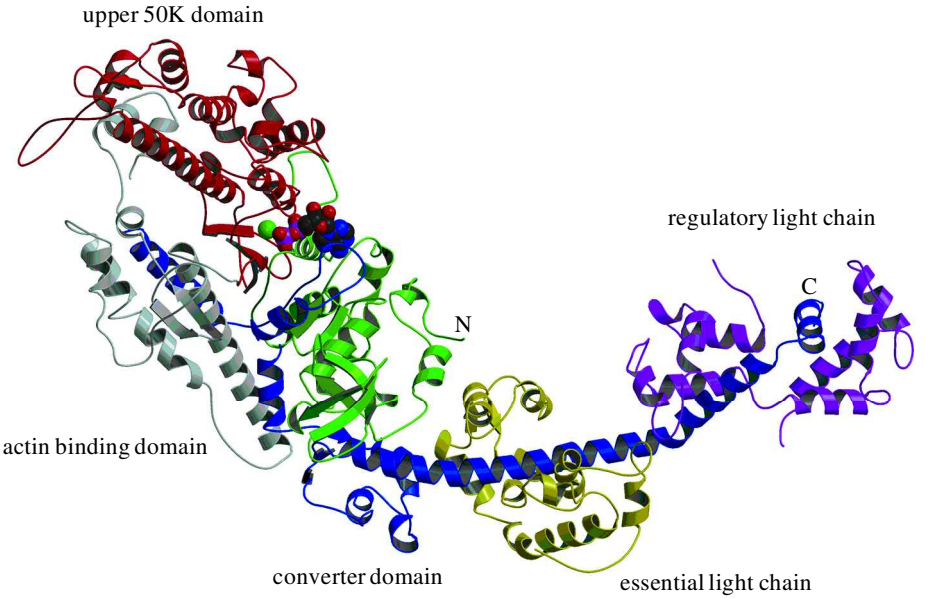
The length of the lever arm can be altered by inserting or deleting light-chain-binding sites (Uyeda *et al*. 1996; VanBuren *et al*. 1994). Alternatively an artificial lever arm fabricated from α-actinin repeating units can be added on to the myosin motor domain (Anson *et al*. 1996). Modified myosin was used in *in vitro* motility assays in which the speed of transport of actin filaments across a lawn of myosin heads is measured. It was shown that the ATPase rates of the constructs were unaffected by the alterations to the lever arm length, in which case the speed of transport would be expected to be proportional to the length of the lever arm. It was found that the speed of actin transport was indeed proportional to the length of the lever arm.

4. TWO MYOSIN CONFORMERS

(a) *Chicken skeletal S1*

In the following section we refer to the chicken skeletal myosin sequence. S1 comprises the first 843 residues of the heavy chain together with the two light chains. It is the morphological cross-bridge and contains the enzymatic activity of myosin. Further limited proteolysis breaks the S1 into three fragments named after their apparent molecular weights: 25 K (N-terminal), 50 K (middle), and 20 K (C-terminal) (Mornet *et al*. 1979). These fragments were thought to represent subdomains of the S1. The structure of S1 shows them rather to arise from the digestion of two flexible loops.

S1 is tadpole-like in form (Rayment *et al*. 1993*b*) (figure 3), with an elongated head consisting of a seven-stranded β-sheet surrounded by numerous α-helices, and a C-terminal tail. All three fragments (25 K, 50 K and 20 K) contribute to the seven-stranded β-sheet. The α-helices form a deep cleft extending from the nucleotide-binding site to the actin-binding site. The C-terminal tail, which, in the intact myosin molecule is connected to the thick filament, forms an extended α-helix which binds the two calmodulin-like 'light chains'. The proteolytic fragments are colour-coded 25 K (N-terminal) green; 50 K red; and 20 K (C-terminal) blue (figure 4). The 50 K fragment actually spans two domains which Rayment *et al*. (1993*b*) have called the 50 K upper domain and the 50 K lower domain (or actin-binding domain), which are separated by the cleft. The actin-binding domain is grey. The N-terminus lies near the start of the tail and the first 80 residues form a protruding SH3-like β-barrel (not present in all myosins). The rest of the 25 K fragment together with the 50 K upper fragment (81–486) form one large domain which accounts for six out of the seven strands of the β-sheet and which constitutes the bulk of the molecule. The ATP-binding site is in this large domain near the 25–50 K fragment boundary and contains a P-loop similar to that found in a number of ATPases and G-proteins (Smith & Rayment 1996*b*). The ATP-binding site is about 4.0 nm from the actin-binding site. The 50 K lower fragment (487–600) actually forms a well-defined domain which constitutes the major part the actin-binding site. This is followed by a large positively charged disordered loop (625–647), which is also involved in actin binding. The first part of the ensuing 20 K domain (648–689) is an integral part of the 25–50 K domain and consists of a long helix running from the



igure 3. Structure of myosin S1 from chicken skeletal muscle (Rayment *et al.* 1993*b*). S1 has an elongated head consisting of a even-stranded β-sheet and a C-terminal α-helical tail or 'neck', which carries two calmodulin-like light chains—the regulatory ght chain (magenta) and the essential light chain (yellow). The proteolytic fragments are colour-coded 25 K (N-terminal) reen; 50 K red; and 20 K (C-terminal) blue. The 50 K fragment spans two domains: 50 K upper domain and the 50 K lower omain or actin-binding domain. The actin-binding domain has been coloured grey. All three fragments (25 K, 50 K and 20 K) ontribute to the seven-stranded β-sheet. Numerous α-helices which surround the β-sheet form a deep cleft extending to the ctin-binding site. Figure prepared with Bobscript and Raster3D (Esnouf 1997; Merritt & Bacon 1997). Reproduced from Geeves & Holmes (1999), with permission.

ctin-binding site to a seventh strand of the β-sheet. This s followed by a turn and a broken helix containing two eactive thiols (SH1 707 and SH2 697). Comparison with ther structures (see § 4(d)) shows that the distal part of he SH1–SH2 helix forms the hinge for the ensuing lever. here follows a small compact domain called the converter' (Houdusse & Cohen 1996) that functions as a ocket for the C-terminal α-helical tail. The C-terminal ail with its associated light chains has been called the egulatory domain' or 'neck'.

(b) *Actomyosin*

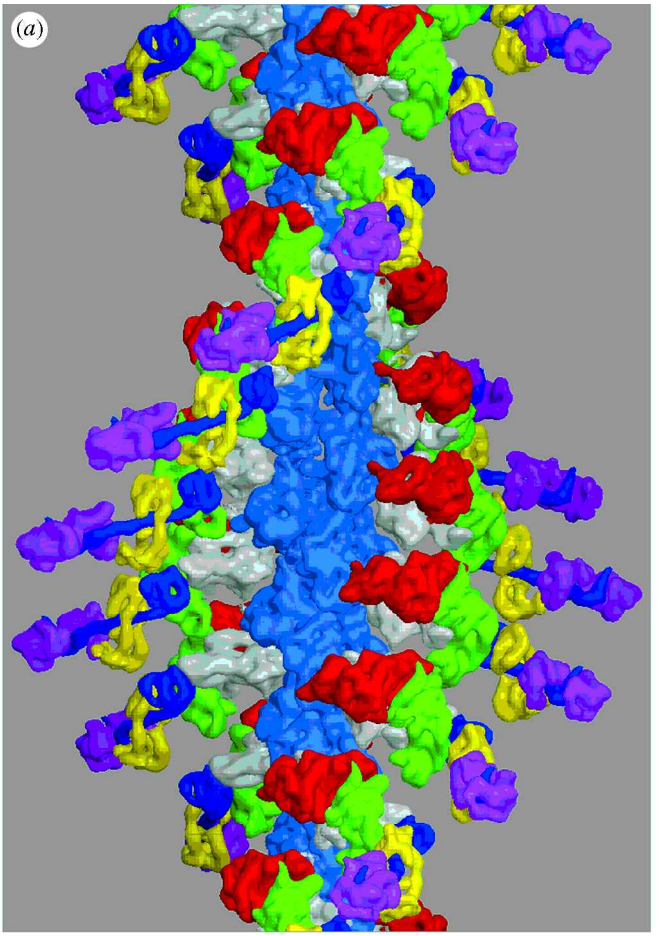
An atomic model of the actin–myosin complex figure 4*a*,*b*) was obtained by fitting the atomic structures f f-actin and S1 into three-dimensional cryoelectron nicroscope reconstructions of 'decorated actin' (Rayment *al.* 1993*a*; Schroeder *et al.* 1993). The chicken muscle S1 sed for the crystals was without nucleotide and therefore ould be expected to be close to the rigor configuration but see § 4(g)). The S1 binds to the lower side of one domain f actin but with a considerable contact to the subdomain 2 f the next actin molecule below. The actin-binding sites nd nucleotide-binding sites are on opposite sides of the -sheet and are separated by 40–50 Å. The cleft in myosin xtends from the ATP-binding site to the actin-binding site o that movements in this cleft could provide a physical link etween the ATP site and the actin-binding site.

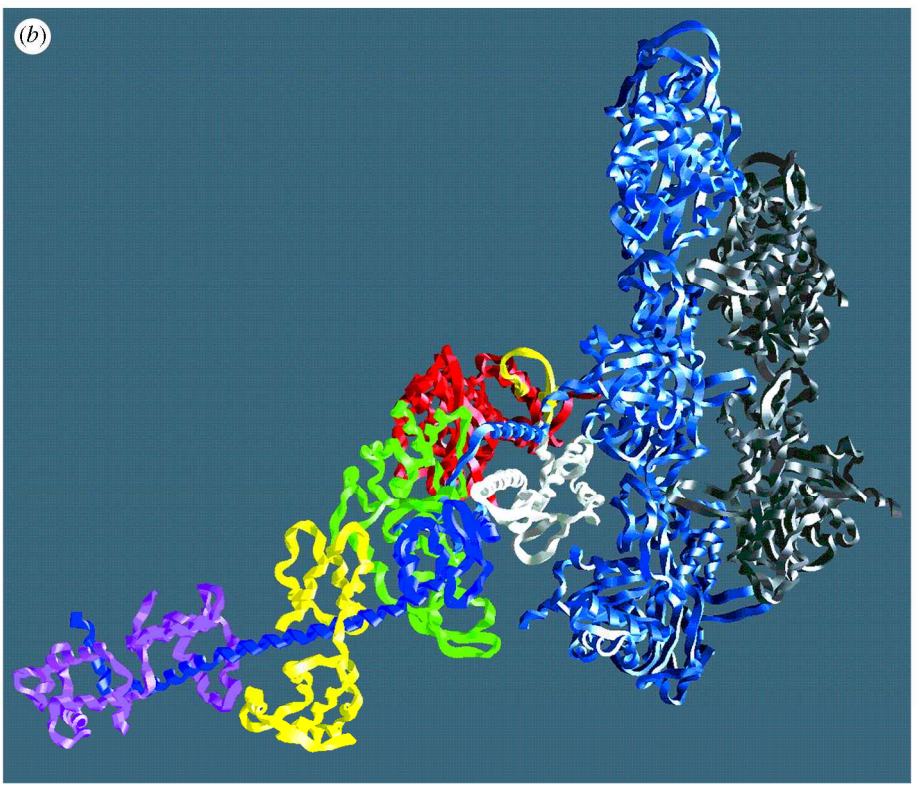
(c) *Truncated myosin II from smooth muscle and* Dictyostelium

Sequence comparisons of all known myosins show that he motor domain is highly conserved up to the point here the long C-terminal α-helix issues from the converter domain. This part of the myosin S1 has been called the 'core' (Cope *et al.* 1996). Proteolytic cleavage of chicken myosin close to the boundary produces a kinetically normal 'motor' (Waller *et al.* 1995), whereas experiments with shorter constructs of expressed *Dictyostelium* myosin II S1 show that damage to the converter domain leads to S1 constructs with modified ATPases (Kurzawa *et al.* 1997). However, the form truncated at 759 in *Dictyostelium* (corresponding to 780 in the chicken sequence) appears kinetically normal (Kuhlman & Bagshaw 1998). Rayment and co-workers have carried out extensive studies of a crystalline truncated fragment of *Dictyostelium* myosin II S1, which stops at 759 and therefore has no lever arm. Recently, studies from Cohen's laboratory of truncated chicken smooth muscle constructs with half of the lever arm present have yielded a wealth of new structural data (Dominguez *et al.* 1998).

(d) *'OPEN' and 'CLOSED'*

The crystal structures of the *Dictyostelium* myosin truncated at 759 have been determined in the presence of a number of ATP analogues, particularly ADP·BeF_x, ADP·AlF_4 (Fisher *et al.* 1995) and ADP·vanadate (Smith & Rayment 1996*a*). ADP·vanadate complexes are used as analogues of the transition state. While the ADP·BeF_3 state looks similar to chicken muscle without nucleotide (Rayment *et al.* 1993*b*), as do complexes with ADP and other ATP analogues (Gulick *et al.* 1997), the ADP·vanadate structure shows large changes in the S1 structure. These changes are also apparent with ADP·AlF_4. The γ-phosphate-binding pocket closes and there is a partial closing of the 50 K upper–lower domain cleft. These changes are in turn coupled to large movements in the





igure 4. (*a*) The structure of the actin–myosin complex ('decorated actin') based on the coordinates given by Rayment *et al.* (1993*a*) nd reduced to 10 Å resolving power. The actin molecules are shown blue. The components of the myosin cross-bridge are shown with ıe same colour-coding as in figure 3. (Program: K. C. Holmes, unpublished.) (*b*) Five actin monomers and one myosin cross-bridge in ribbon representation with the same colour-coding as in figure 3 are shown. The actin molecules are shown blue and grey. Note that ıe cross-bridge makes contact with two adjacent actin monomers. Figure prepared with GRASP (Nicholls *et al.* 1991).

C-terminal region. The 50 K upper domain and the actin-binding domain rotate a few degrees with respect to each other around the helix 648–666, which acts as a hinge, in a way that closes the nucleotide-binding pocket (figure 5); a movement of some 5 Å. At the same time, the outer end of the long helix (residues 475–509, which by analogy with the G-proteins is called the switch 2 helix), and its associated loop (509–519), bends out and twists. We will refer to these two states as OPEN and CLOSED.

(e) *The CLOSED form enables ATP hydrolysis*

Smith & Rayment (1996*b*) point out the similarity between the active site of CLOSED myosin and ras p21 and other G-proteins. The differences between the OPEN and CLOSED forms in the neighbourhood of the active site reside almost entirely in the conformation of the linker region (465–470), which connects the 50 K upper and lower domains. Smith & Rayment point out that this region is structurally equivalent to the switch 2 region in ras p21. The mutual rotation and closing of the 50 K upper–lower domains cleft causes movements of about 5 Å around the γ-phosphate-binding pocket. In the OPEN form, the switch 2 region is not part of the nucleotide-binding pocket. A similar movement of the switch 2 region depending on trinucleotide being bound is also found in the G-proteins (Bohm *et al*. 1997). A similar but even larger movement of the switch 2 region is found in the empty site of F1-ATPase compared with the ATP or ADP sites (Abrahams *et al*. 1994). Only in CLOSED (e.g. in S1·ADP·vanadate) can the hydrogen bond (invariant for G-proteins, kinesins and myosin but not present in F_1-ATPase) between the amide of G466 and the γ-phosphate be formed. Moreover, in CLOSED, the γ-phosphate is also coordinated by the Mg^{2+} ion, the invariant P-loop lysine and S181 (Kurzawa *et al*. 1997). Furthermore, through the formation of a salt bridge with R236, E468 comes close to the putative attacking water and may stabilize and polarize this molecule. In OPEN, E468 takes on a quite different orientation. It is difficult to see how hydrolysis can proceed in OPEN, which would therefore appear not to be an Mg·ATPase: the closing would appear to be essential for enabling hydrolysis. Smith & Rayment (1996*b*) proposed that the CLOSED form was brought about by the formation of the transition state complex with the γ-phosphate stabilized in the pentacovalent intermediate conformation. However, ADP·BeF_3 complexes can also produce this state.

(f) *ADP·BeF_3 can produce both OPEN and CLOSED states*

ADP·BeF_3 is thought to be an analogue of ATP. Fisher *et al*. (1995) solved the structure of *Dictyostelium* S1 with ADP·BeF_3 bound in the active site and found it to be remarkably similar to skeletal chicken S1 without nucleotide. Therefore it appears that the structure of the ATP state is OPEN, which is puzzling, since it would not be able to hydrolyse the ATP. Moreover, the attitude of the converter domain (and hence inferred position of the 'neck' or lever arm) is near to what we anticipate would be the end of the power stroke, which is also unexpected for the ATP state. More recently Schlichting *et al*. (2000) have solved the structure of an ADP·BeF_3 complex of truncated *Dictyostelium* S1 and find it to be very similar to the ADP·vanadate complex. The γ-phosphate-binding site is closed and the converter domain is in the rotated configuration. The construct used in this case was five residues shorter than that used by Fisher *et al*. (1995). For reasons which are not yet clear, the shorter construct binds ADP strongly (Kurzawa & Geeves 1996). It may be that the difference in binding energy of ADP·BeF_3 is adequate to favour the CLOSED structure.

Crystallographic studies on truncated forms of smooth muscle myosin (Dominguez *et al*. 1998) show the closed conformation with both ADP·BeF_3 and ADP·AlF_4 so that it appears that the CLOSED form can accommodate ATP both in its normal and pentacovalent forms. It therefore seems reasonable to assume that the CLOSED form could also accommodate ADP·Pi without further substantial alterations of the protein conformation.

(g) *The structure of nucleotide-free myosin may not yet be known*

From its structure, the initial chicken S1 structure is classified as OPEN. It is very similar to the structure obtained from truncated *Dictyostelium* myosin in the presence of ADP, AMPPNP or ATP-γS. One would expect there to be differences in the geometry of the nucleotide-binding pocket between nucleotide-free and ADP or ATP forms: in this respect chicken S1 and *Dictyostelium* OPEN are virtually identical. The great similarity between the two structures does not account for the expected differences in nucleotide affinity between the nucleotide-free form and the ADP bound form. The simplest way to resolve this conundrum is to suppose that OPEN is normally the state associated with ADP binding, and that the additional presence of the γ-phosphate leads to CLOSED (i.e. the difference between OPEN and CLOSED is solely associated with the presence or absence of the γ-phosphate). If this is the case, then the structure of myosin without nucleotide is yet to be revealed (one would argue that the chicken S1 structure had turned out atypically because of crystallization conditions or methylation; White & Rayment 1993). Moreover, as we will argue below, both the crystallographic forms are probably weak actin binders.

(h) *The switch 2 region should open for phosphate release*

The CLOSED structure appears to generate a tight coordination pattern for the oxygens of the γ-phosphate both before and after cleavage, which would explain the high phosphate affinity. This interaction in turn is important for stabilizing the closed form when γ-phosphate is present. Opening the switch 2 region destroys the γ-phosphate-binding pocket and the SkE468-R245 salt-bridge. These changes would appear to facilitate γ-phosphate release (a 'back-door enzyme' (Pate *et al*. 1997; Yount *et al*. 1995)). It seems very likely that actin binding favours the OPEN form and thereby facilitates phosphate release. Unfortunately, the structural basis of this important mechanism has yet to be revealed.

(i) *A conformational switch leads to a 100 Å displacement*

The movement of switch 2 in the CLOSED form has ther more far-ranging consequences, namely the rotation f the converter domain through about 60°. Holmes (1996, 997) used the coordinates of the chicken S1 structure to rovide the missing lever arm for Smith & Rayment's ADP·vanadate structure (Smith & Rayment 1996*a*) and roposed that the new orientation of the lever arm was ndeed the beginning of the power stroke. A model of this ew state is shown in figure 7. For comparison, we show ne OPEN state. The end of the lever arm has moved nrough 11 nm along the actin helix axis between OPEN nd CLOSED, which is about the expected magnitude of ne power stroke. This large change is driven by a small 0.5 nm) change in the active site. Therefore, it now seems ather likely that the myosin power stroke works by witching between these two conformations.

Smith & Rayment (1996*a*) had reservations about this nterpretation because they thought that the truncation of ne light chains might cause artefacts and also because in neir crystals the converter domain was rather mobile. Moreover, the changes did not explain the chemical ross-linking effects (Huston *et al*. 1988).

(j) *Smooth muscle myosin*

Such doubts have been allayed by the publication of the tructure of smooth muscle myosin from Cohen's labora- ory (Dominguez *et al*. 1998). Chicken smooth muscle nyosin truncated at 791 and 820 (smooth muscle equences that correspond with 761 and 790 in striated nuscle) has been expressed in insect cells using the Baculo- irus vector. The shorter construct stops at the end of the notor domain 'core' (Cope *et al*. 1996), the longer onstruct encompasses the essential light-chain-binding te, i.e. the first half of the lever arm, and is complete with ne essential light chain. The structures of both constructs ave been solved complexed with ADP·AlF_4 and ADP·BeF_3. All show the myosin cross-bridge in the CLOSED form (the putative pre-power-stroke orienta- on), with the converter domain in the rotated posi- on essentially identical to that obtained in the two *Dictyostelium* constructs discussed above. There are no ubstantial differences arising from ADP·AlF_4 as compared with ADP·BeF_3, apparently showing that the nature of the gand (ATP analogue or transition-state analogue) does ot control the protein conformation. Since all the smooth nuscle crystals display extensive non-crystallographic ymmetry, CLOSED has now been obtained 16 times in a arge variety of different environments. It seems that one an rule out the possibility that the CLOSED form is an rtefact of truncation or crystallization.

(k) *Molecular mechanism*

The coordinates of both an OPEN form (Rayment *et al*. 993*b*), chicken skeletal muscle with sulphate in the active te, and a CLOSED form (Dominguez *et al*. 1998), hicken smooth muscle with ADP·BeF_3 and ADP·AlF_4 in ne active site, are now available from similar chicken nyosin sequences. This allows a more detailed analysis of ne conformational changes between the OPEN and CLOSED forms, which are in point of fact limited to parts f the region 460–520 (switch 2 and switch 2 helix) and the SH2–SH1 hinge region (694–716). Other changes take place by rigid-body rotations of secondary and tertiary structure elements. The movement which closes the γ-phosphate-binding site is a rotation of the actin-binding domain with respect to the 50 K upper domain about the helix 648–666. This pushes the SH1 helix into the switch 2 helix. The switch 2 helix responds by breaking and twisting at residue 497. As this happens, the end of the switch 2 helix rotates through about 60° (figure 6) and carries the converter domain (711–781) with it. The fulcrum for the rotation of the converter domain is provided by residues at the distal end of the SH1 helix (707–711) each of which undergoes rather moderate changes in its Φ–Ψ angles. The angle between the two parts of the SH1–SH2 helix alters at G699. In these structures there is no evidence of a melting or disordering of the SH1–SH2 helices as had been suggested by chemical cross-linking experiments (Huston *et al*. 1988).

This comparison shows that the transition from OPEN to CLOSED is achieved by substantial movements in only a very few residues The largest Φ–Ψ changes are limited to the switch 2 loop (i.e. residues 509–519), which includes the invariant W510. In the *Dictyostelium* OPEN form, which we equate with the ADP form, the switch 2 loop is in fact disordered.

At this preliminary level of analysis the changes do not resemble a state change with a high activation energy (such as the R–T transformation of haemoglobin, with helices clicking from one position to another) but rather appear like the meshing of cogs and gears. Apart from changes in the γ-phosphate-binding pocket itself, which includes the making–breaking of a salt-bridge which looks rather discontinuous, it appears that the transformation might occur rather smoothly without an appreciable activation energy. The myosin structure, therefore, may be able to transform between the two states or take up any intermediate state in response to external forces (such as the effects of actin binding and tension on the molecule). This would be a very desirable property in a molecular machine.

5. ALLOCATION OF STATES TO THE KINETIC SCHEME

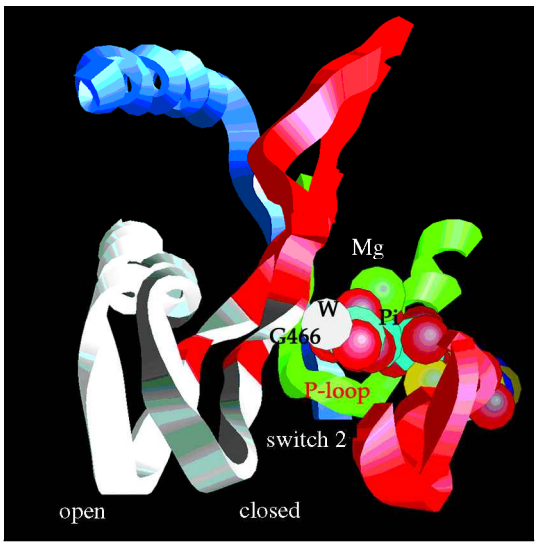
(a) *Bagshaw–Trentham scheme*

All myosins studied to date follow the same basic biochemical mechanism of ATP hydrolysis, which is known as the Bagshaw–Trentham scheme (Trentham *et al*. 1976), but with modifications in both the overall ATPase rate and the relative rates of individual steps (Jontes *et al*. 1997; Marston & Taylor 1980; Ostap & Pollard 1996; Ritchie *et al*. 1993):

$$\mathrm{M + ATP} \overset{1}{\Longleftrightarrow} \mathrm{M{\cdot}ATP} \overset{2}{\Longleftrightarrow} \mathrm{M^{*}{\cdot}ATP} \overset{3}{\Longleftrightarrow} \mathrm{M^{**}{\cdot}ADP{\cdot}Pi}$$

$$\overset{4}{\Longleftrightarrow} \mathrm{M^{*}{\cdot}ADP{\cdot}Pi} \overset{5}{\Longleftrightarrow} \mathrm{M^{*}{\cdot}ADP} \overset{6}{\Longleftrightarrow} \mathrm{M + ADP.}$$

In subfragment 1 from rabbit fast muscle, ATP binds in a two-step process, a diffusion-limited reaction (step 1) followed by a fast almost irreversible conformational change which results in an increase in the intrinsic protein fluorescence (step 2). Reversible ATP hydrolysis



igure 5. View of the ATP-binding site looking out from the ctin helix: P-loop (green); an MgATP molecule with the ase at the back and the three phosphate groups in the front carbon, yellow; nitrogen, blue; phosphate, light blue; oxygen, ed; magnesium, green); parts of the 50 K upper domain red), including the so-called 'switch 1' region (right); the witch 2 element in the open 'ADP' (white) and closed 'ATP' grey) conformations—the conserved glycine (466) is shown n grey or white—note that this residue moves about 5 Å etween the two conformations; and the helix (648–666), which acts as a fulcrum for the relative rotation of the 50 K pper and lower domains (blue). Figure prepared with RASP (Nicholls *et al.* 1991). Reproduced from Geeves & olmes (1999) with permission.

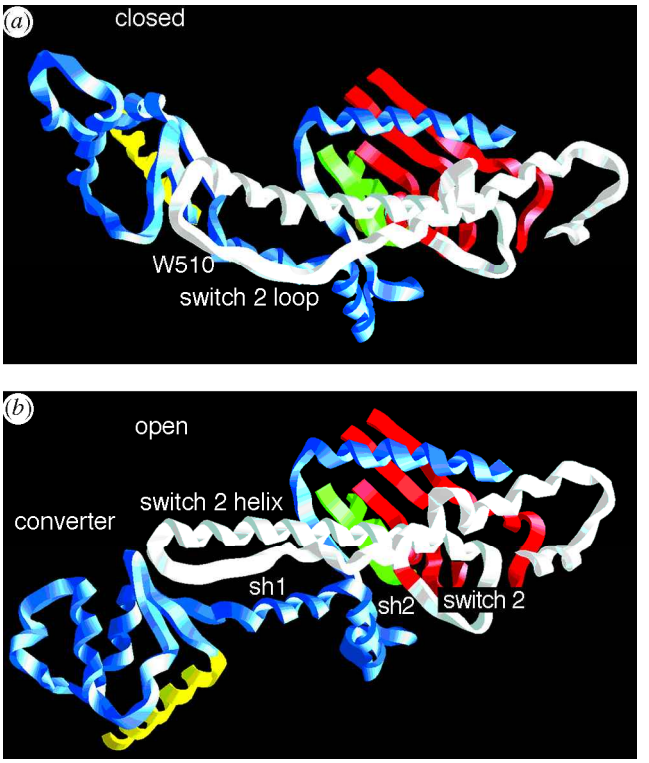


Figure 6. A view roughly at right angles to figure 5 with CLOSED (*a*) and OPEN (*b*) showing the large changes in the orientation of the converter domain (left) resulting from the inward movement of the switch 2 element (right), which results in a bending and twisting of the switch 2 helix and the associated switch 2 loop which carries the highly conserved W510. Figure produced with GRASP (Nicholls *et al.* 1991). Reproduced from Geeves & Holmes (1999) with permission.

hen occurs at a rate of $>100\,s^{-1}$ accompanied by a urther increase in fluorescence with an equilibrium onstant of *ca.* 10 (step 3). The products remain tightly ound to the protein and a slow ($0.05\,s^{-1}$) protein isomerzation is required (step 4) before Pi is rapidly released step 5). A fluorescence decrease accompanies step 4. ollowing Pi release, ADP dissociates in a two-step eaction which is essentially the reverse of steps 1 and 2 nd is associated with a further fall in fluorescence.

In terms of the above scheme, OPEN would most mply be assigned to the M^*N state since it is observed ith crystals containing ADP, AMP·PNP, ATPγS bound o the nucleotide site. The initial fluorescence change ccompanying nucleotide binding is most likely to be the esult of a perturbation of W113 and or W131 in the ucleotide-binding pocket, since myosins lacking these ryptophan residues show little fluorescence change on ucleotide binding (e.g. *Dictyostelium* and scallop; Ostap Pollard 1996; Ritchie *et al.* 1993). State CLOSED ould therefore correspond to M^{**}ADP·Pi or the transiion state of the hydrolysis reaction.

(b) *W510 signals OPEN to CLOSED*

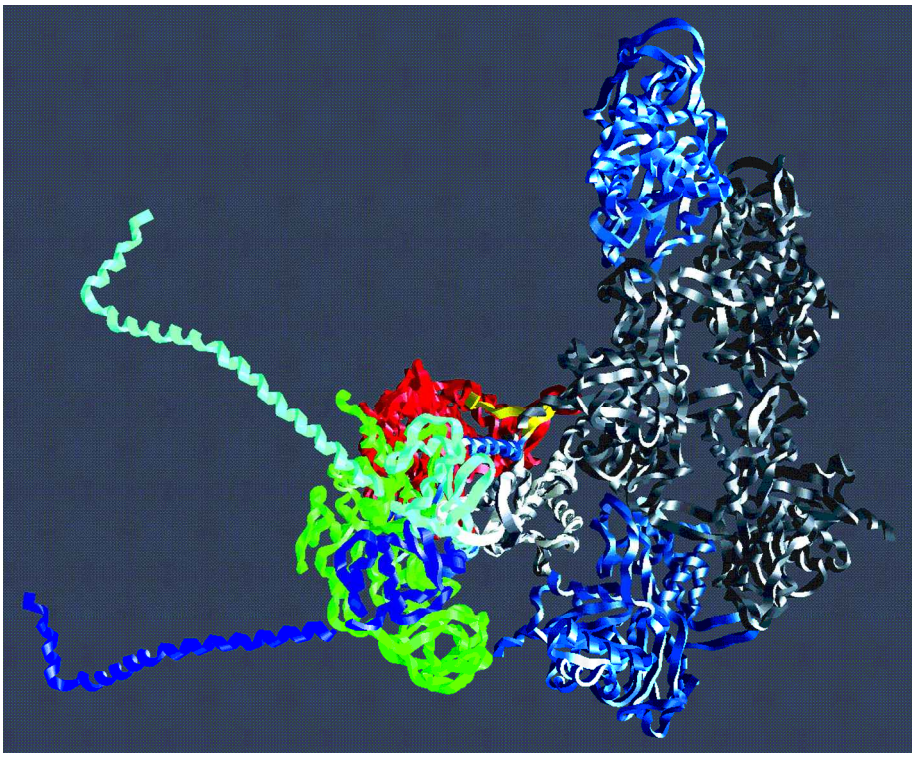
Both *Dictyostelium* and chicken myosin show a fluorescence change apparently accompanying the hydrolysis ep. Of the three tryptophan residues in the motor omain common to *Dictyostelium* and chickens, the local nvironments of W440 (Dicty 432) and W595 (Dicty 84) do not alter appreciably between OPEN and LOSED forms. Therefore they are not strong candidates for the intrinsic fluorescence signal. However, W510 (Dicty 501) sits close to the junction between the switch 2 helix and the converter domain and twists from a solvent-exposed to solvent-shielded environment between OPEN and CLOSED. Therefore it is *a priori* a strong candidate for the signal (see also Burghardt *et al.* 1998; Rayment *et al.* 1996). Thus the protein fluorescence change accompanying hydrolysis would arise from the perturbation of W510, which actually signals the movement of switch 2 and the lever arm.

(c) *An extra state*

However, OPEN is apparently not an ATPase. Thus the conformational change signalled by the change in intrinsic fluorescence must precede hydrolysis. We therefore need to consider step 3 as comprising two events: step 3a is the conformational change and step 3b is the hydrolysis step. The notion that hydrolysis should be fast and not associated with a large protein isomerization is novel. On the other hand, the acompanying isomerization (3a) is very significant since the converter domain and lever arm move a large distance as the phosphate-binding pocket shuts.

$$M^*ATP \overset{3a}{\Longleftrightarrow} M^{**}ATP \overset{3b}{\Longleftrightarrow} M^{**}ADP{\cdot}Pi.$$

In all measurements made to date, the second component of the fluorescence change and the hydrolysis step occur at the



igure 7. A reconstruction of the 'pre-power-stroke' state from the crystallographic data on the *Dictyostelium* construct truncated t 759 and complexed with ADP·vanadate (Smith & Rayment 1996*a*) with the same colour-coding used in figure 4*b*. Note the 0° rotation of the converter domain campared with figure 4*b* and that the lever arm is in an 'up' position. (The missing 'neck' or ever arm has been modelled from chicken S1 data by superimposing the converter domains—the light chains have been omitted or clarity). To establish the orientation with respect to the actin helix (right) the 50 K upper and lower domains have been uperimposed on the corresponding domains in the chicken S1 structure shown in figure 4. Also shown is the 'post-power-stroke' tate modelled as above from the crystallographic data on the *Dictyostelium* myosin motor domain truncated at residue 759 and omplexed with ADP·BeF_3 (Fisher *et al.* 1995). The end of the lever arm moves about 11 nm between the two states. Figure repared with GRASP (Nicholls *et al.* 1991).

ame observed rate. Therefore, hydrolysis must be very fast ollowing the conformational change, otherwise a signifiant lag of the hydrolysis behind the fluorescence change ould have been observed. The hydrolysis step is also eadily reversible ($K_3 = 10$), i.e. occurs with a very small ree-energy change and has fast forward- and reverse-rate onstants ($k_{+3} + k_{-3} = 100\,s^{-1}$) (Bagshaw & Trentham 973). The simplest assignment of the rate constants to the wo components of step 3 is therefore that $k_{+3a} = 100\,s^{-1}$ and $_{-3b} = 10\,s^{-1}$, with k_{+3b}, $k_{-3a} \gg 100\,s^{-1}$. Thus M**ATP is n unstable state and breaks down rapidly in either the orward or reverse direction and as such has many characeristics of a transition state.

The small free-energy change for step 3 is known to be omposed of large compensatory changes in enthalpy and ntropy so that although there is only a small net change n ΔG, energy is being converted and/or stored in this tep (Kodama 1985; Millar *et al.* 1987).

(d) *Isotope exchange studies*

^{18}O isotope exchange studies (Bagshaw *et al.* 1975; Webb & Trentham 1981) provide the evidence for the fast rates f reversal of the hydrolysis reaction while the assignment f an equilibrium constant of 10 comes from analysis of he breakdown products on acid quenching the complex Bagshaw & Trentham 1973). The possibility that the M**ADP·Pi complex is a transition state complex is not ruled out by these studies. Hackney's work (Dale & Hackney 1987) has demonstrated that while the oxygens on the γ-Pi are rapidly exchanged with solvent the position of oxygen isotopes on the γ-Pi do not change. Thus it remains possible that the β–γ bond is not broken on forming M*ADP·Pi and that the γ-Pi is pentacovalent, i.e. the attack by water has occurred but the ATP β–γ Pi bond is not broken. Step 3 could therefore represent the formation of the pentacovalent γ-Pi complex (step 3b) which occurs concomitant with the OPEN to CLOSED conformational change (step 3a). This is close to the views of Smith & Rayment (1996*a*).

6. THE ACTIN–MYOSIN INTERACTION

(a) *Kinetic data*

There clearly are structural changes at the actomyosin interface on docking but these are probably limited in magnitude. Kinetically, the docking of myosin on to an actin filament can be resolved into at least three events (Geeves & Conibear 1995). Initial complex formation, largely involving charge–charge interactions, is followed by two changes in conformation of the complex. The first may involve the formation of stereospecific hydrophobic interactions (Geeves & Conibear 1995), the second involves a major rearrangement of the actin–S1 complex, since fluorescence probes on both actin and the nucleotide

n the myosin pocket report the change simultaneously Taylor 1991; Woodward *et al*. 1991). The second isomeri- ation also appears to involve a large volume increase of he complex, which is normally assigned to displacement f a large amount of water (Coates *et al*. 1985; Geeves 991).

$$\underset{\text{collision complex}}{A + M(N) \overset{K_0}{\Longleftrightarrow} A \sim M(N)} \overset{K_1}{\Longleftrightarrow} \underset{\text{A-state}}{AM(N)} \overset{K_2}{\Longleftrightarrow} \underset{\text{R-state}}{AM(N)}$$

he three events are best documented for rabbit myosin n the absence of nucleotide. As we apparently do not ave a high-resolution structure of the nucleotide-free nyosin S1, it is not simple to predict what actin binding vill do to the S1 structure. However, the three events ppear similar (but with marked changes in rate and quilibrium constants) for M·ADP (Geeves 1989; Geeves t Gutfreund 1982) and it is suggested that M·ADP·Pi inds in the same three steps but that the equilibrium onstant for the last step is small (*ca*. 1, weak binding to ctin) until Pi is displaced from the myosin. The last of he conformational changes has been suggested to be losely coupled to the power stroke of the ATPase cycle Fortune *et al*. 1991; Geeves 1992; Geeves *et al*. 1984).

The key feature of this mechanism is that the isomeri- ation of step 2 results in a major strengthening of the ctin binding to myosin and simultaneously a weakening f the nucleotide binding to actin. The equilibrium onstant of this step varies for different nucleotides. Thus n the absence of nucleotide K_2 is large (>100), for ATP it s small ($K_2 \ll 1$) and ATP displaces actin. For other ucleotides and nucleotide analogues K_2 is intermediate nd whether the ternary complex or the binary complex vith either nucleotide or actin predominates depends on he concentrations used.

In the case of fast rabbit myosin, the formation of the R- tate reduces the affinity of the myosin head for ADP by nore than 100-fold (Geeves 1989; Siemankowski & White 984) and the rate of ADP dissociation is accelerated more han 500-fold. These factors vary widely for different nyosin types. For chicken smooth muscle myosin, for xample, the ADP affinity is reduced only fivefold while he dissociation rate is accelerated tenfold by actin (Cremo t Geeves 1998). Actin also induces acceleration of Pi issociation, typically more than 200-fold.

(b) *Correspondence with structural data*

The current evidence suggests that the formation of the A-state (probably involving stereospecific interactions in he actomyosin interface) has little influence on the ucleotide-binding pocket, since the labels on the nucleo- ide do not detect formation of the A-state. Nor does the ormation of the A-state significantly weaken the affinity f nucleotide for myosin. The formation of the A-state herefore appears to be limited to local changes at the ctomyosin interface. Therefore the binding of either the CLOSED or OPEN form to actin to make an A-state nay be expected to be similar in each case. The isomeri- ation to the R-state seems to require an empty γ-Pi site s no R-state has been detected for myosin complexed vith ATP, ATPγ-S or ADP and the Pi analogues (Geeves & Jeffries 1988). The R-state can be formed in the presence of either ADP ($K_2 = 10$) or pyrophosphate ($K_2 = 2$). Thus the A-state can be formed with any myosin complex but the isomerization to the R-state is inhibited by a tightly bound Pi. Formation of the R-state is likely to involve opening of the back door (allowing Pi to dissociate if not covalently bound) and then weakening of the nucleotide affinity. The order is important. Thus the A-to-R isomerization of the CLOSED-form–A-state occurs with opening of the back door and loss of Pi. Isomerization of the OPEN form depends on the state of the Pi site. With ATP, ATPγ-S or ADP·BeF_3 bound, the formation of the R-state will not be allowed. With no nucleotide or ADP bound, the isomerization to the R-state is highly favoured.

(c) *Present structures do not illuminate the mechanism of actin-induced ADP release*

To understand the complete ATPase cycle requires more detailed structural knowledge of the way in which actin reverses the CLOSED–OPEN conformational change.

Both OPEN and CLOSED have relatively high affinity for nucleotide so that actin-mediated release of nucleotide must involve more structural change than we presently see. The effect of actin would not be expected to be simply a reversal of the OPEN–CLOSED change since one expects a vectorial mechanism to drive the cycle which is provided by the requirement for γ-Pi release to precede ADP release. The missing part of the cycle is the mechanism whereby actin binds to the CLOSED form and induces a series of conformational changes which lead to γ-Pi release followed by ADP release. While the transition CLOSED to OPEN would lead to γ-Pi release, the structures give no indication of how actin induces ADP release nor how ATP binding induces actin dissociation. However, solution biochemical studies have provided insights into the docking of actin with myosin and its effect on nucleotide binding to myosin.

We suggest that both CLOSED and OPEN forms with the γ-Pi occupied are forms that can only interact weakly with actin to form the A-state and that we need at least one more conformation to complete the cycle. Initially, actin must bind to the CLOSED form to give a weakly bound actin (the A-state). The complex should then proceed to a third form which should have the following properties: the third state should bind strongly to actin (R-state) with high pyrene–actin fluorescence; the back door should be open and with very weak Pi affinity and weak nucleotide affinity (low mant–nucleotide fluorescence); the switch 2 should be open and the converter domain and lever arm in the post-power-stroke down position.

A difficult question is how the transition between the three states CLOSED (M·ADP·γ-Pi) to R-state (A·M or A·M·D) to OPEN (M^*ATP) to CLOSED occurs. The crystal structures allow us to propose that for OPEN to CLOSED the trigger is the moving in of switch 2 in response to the presence of γ-Pi. At present we have no indications as to how the other two transitions might function. However, solution biochemistry helps set the ground rules. Actin can bind the CLOSED to the $M^{**}ADP{\cdot}\gamma$-Pi to form the A-state with no major changes in conformation. The actin then produces a conforma- tional change in the complex which gives a stronger

ctin binding (R-state as sensed by pyrene on actin) and auses the switch 2 to move out (opens the back door), ·Pi dissociation, and the converter–tail to move down. ubsequent to this change actin causes a change in the ucleotide site to promote ADP release. This second hange can be seen in some slower myosins as an dditional tail movement upon ADP release (Coluccio *et l*. 1998; Jontes & Milligan 1997; Jontes *et al*. 1995; Whittaker *et al*. 1995). ATP binding to AM to return to ne open M*ATP-OPEN state reverses the A-to-R tran- tion but does relatively little to the converter–tail or to ne back door (or switch 2). The ensuing transition to CLOSED takes place after the release from actin has aken place.

7. CONCLUSION

The two myosin conformers presently available are onsistent with the scheme proposed by Lymn & Taylor n 1971. A major change in myosin conformation is ssociated with (or just precedes) the hydrolysis step. Moreover, this conformational change is a reversal (a riming) of the conformational change of the power troke (which is driven by actin binding). This is essen- ally the basis of figure 1.

However, there are still states to be discovered. We do ot yet understand how actin binding controls nucleotide ffinity and vice versa. New crystal structures now being orked on should provide futher insight. However, when ll is said and done, we expect these to support Boltz- mann's concept that muscle consists of simple mechanisms nked by molecular cogs and levers.

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