PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

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Phil. Trans. R. Soc. Lond. B 2000 **355**, 433-440
doi: 10.1098/rstb.2000.0584

THE ROYAL SOCIETY

Mechanics and models of the myosin motor

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In striated muscles, shortening comes about by the sliding movement of thick filaments, composed mostly of myosin, relative to thin filaments, composed mostly of actin. This is brought about by cyclic action of 'cross-bridges' composed of the heads of myosin molecules projecting from a thick filament, which attach to an adjacent thin filament, exert force for a limited time and detach, and then repeat this cycle further along the filament. The requisite energy is provided by the hydrolysis of a molecule of adenosine triphosphate to the diphosphate and inorganic phosphate, the steps of this reaction being coupled to mechanical events within the cross-bridge. The nature of these events is discussed. There is good evidence that one of them is a change in the angle of tilt of a 'lever arm' relative to the 'catalytic domain' of the myosin head which binds to the actin filament. It is suggested here that this event is superposed on a slower, temperature-sensitive change in the orientation of the catalytic domain on the actin filament. Many uncertainties remain.

Keywords: muscle contraction mechanism; myosin; molecular motors; motor proteins

1. GENERAL FEATURES

Most of what is now known about the physical processes involved in generation of tension or shortening of muscle has been deduced from experiments on two types of muscle, the skeletal muscles of vertebrates (mostly frog and rabbit) and to a lesser extent the highly specialized 'synchronous' flight muscles of certain of the Orders of insects, which are exceptionally regular in structure. Both of these are types of striated muscle, i.e. their fibres are crossed at intervals of a few micrometres by alternate bands of higher and lower refractive index, reflecting higher and lower total concentrations of protein. The bands with higher refractive index are known as the A bands because they are optically anisotropic, i.e. they are birefringent, with the slow direction along the long axis of the fibre, the intervening low-refractive-index bands being nearly isotropic and therefore known as the I bands. It is natural to suppose that the mechanism of smooth (unstriated) muscles, and of other forms of movement driven by other types of myosin, is essentially similar, but any such phrase serves chiefly to conceal our ignorance of the extent of the differences.

It was shown in 1953–1954 (H. E. Huxley 1953; H. E. Huxley & Hanson 1954; A. F. Huxley & Niedergerke 1954) that shortening of the muscle fibre takes place by relative sliding movement of two sets of filaments whose ends overlap, the high refractive index and birefringence of the A bands being due to the presence there of 'thick filaments', composed mostly of the protein myosin (Hasselbach 1953; Hanson & H. E. Huxley 1953), that interdigitate with 'thin filaments' composed mostly of actin. Tension is developed if shortening is prevented ('isometric contraction').

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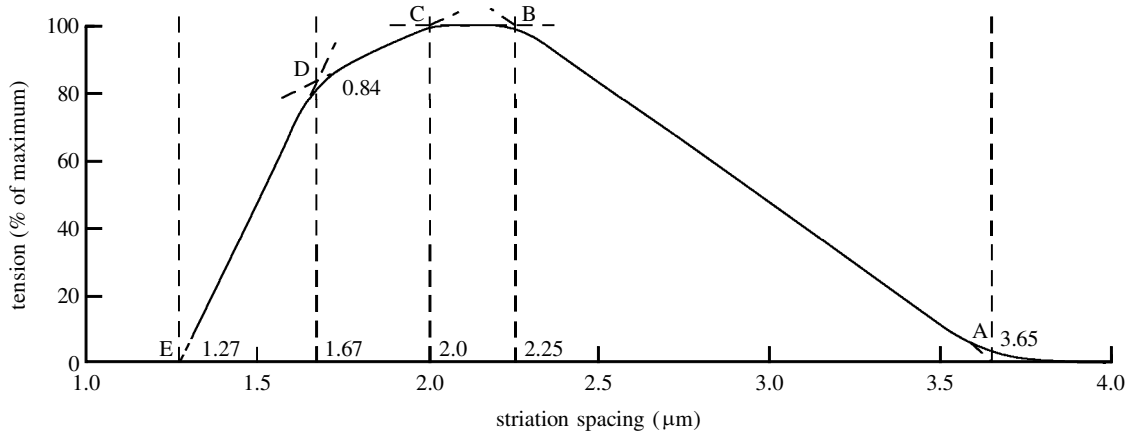
Two very general features of the mechanism of muscle contraction that are now almost universally accepted were suggested by observations that had been made long before the advent of the sliding-filament theory, as follows.

2. INDEPENDENT FORCE GENERATORS

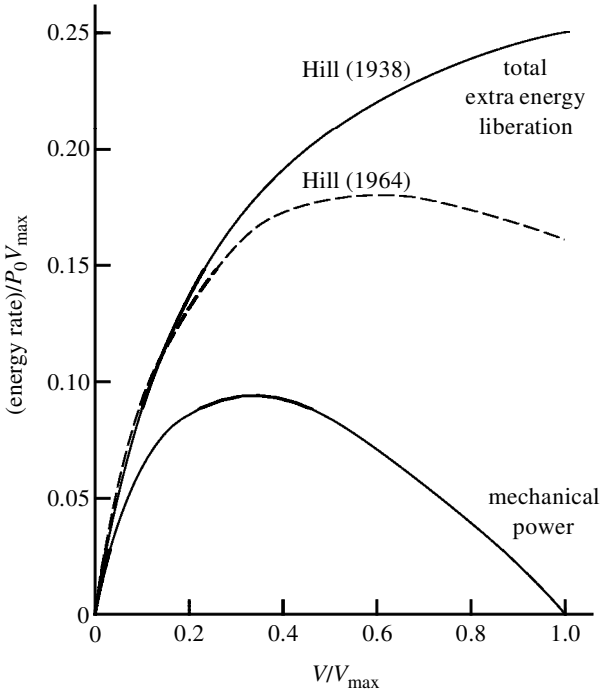
Experiments by Ramsey & Street (1940), in which intact isolated muscle fibres from the frog were stretched to various lengths and then stimulated, showed a roughly linear decline of active force with extension of the fibre beyond the length at which it gave maximum force (figure 1). This received a simple explanation on the sliding-filament theory, namely that contributions to force were provided by active sites, uniformly spaced along each zone where myosin and actin filaments overlap, and acting more or less independently, so that total force would be proportional to the extent of overlap (A. F. Huxley & Niedergerke 1954). The agreement with the relation expected from the lengths of the filaments was shown to be quantitative when precautions were taken to avoid complications due to non-uniformity of the stretching of a fibre (Gordon *et al.* 1966). Meanwhile, these active sites were identified, by electron microscopy, with 'cross-bridges' extending from the myosin filament to the actin filament in each zone where they overlap (H. E. Huxley 1957).

3. CYCLIC ACTION

Most, if not all, theories of muscle contraction before the advent of sliding filaments, and many since then, assumed that shortening was due to a progressive change from a long to a short state in the contractile material. As far as I know, the first suggestion of a cyclic, as opposed to progressive, mechanism was made by Dorothy



igure 1. Variation of active tension with overlap between myosin and actin filaments. Frog muscle: A, filaments just not verlapping; B, all myosin heads overlapped by actin filament; C, actin filaments collide at centre of A band: D, myosin laments collide at centre of I band. From Gordon *et al.* (1966). The point E where active tension is shown as falling to zero not well defined because, even at shorter lengths some tension does develop extremely slowly and the shortening is then reversible ('delta state', Ramsey & Street 1940).



igure 2. Rate of energy liberation above isometric rate, as a unction of shortening speed. Solid line: rectangular hyperbola om Hill (1938). Broken line: revised formula of Hill (1964). rom A. F. Huxley (1974).

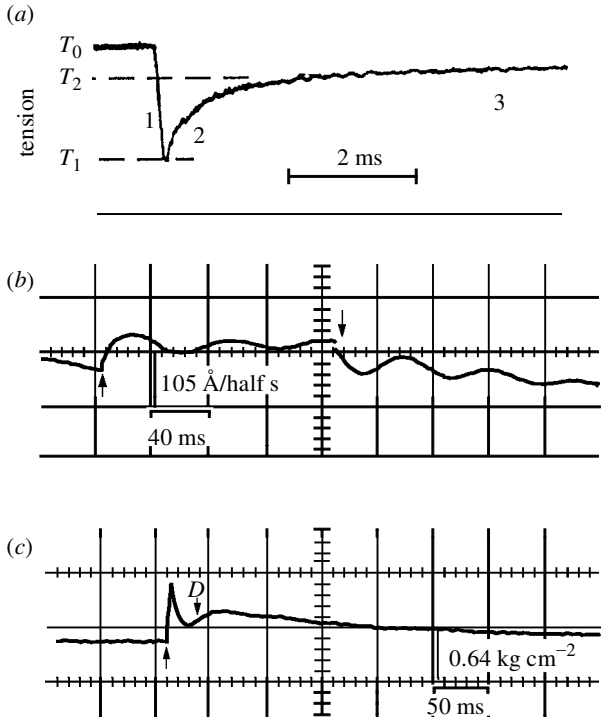
eedham (1950, p. 48), on the basis of the relationships etween load, speed of shortening and rate of heat roduction found by Hill (1938) in intact frog muscles, nd formulated by him in simple mathematical expres- ions. More explicitly, I remember her pointing out the nalogy between Hill's hyperbolic dependence of rate of nergy liberation (heat + work) on speed of shortening figure 2) on the one hand, and on the other the hyper- olic Michaelis–Menten dependence of the rate of an nzymic reaction on substrate concentration. (More ecent work has modified Hill's (1938) relationship.)

Such ideas, involving repeated operation of each active site during a single contraction, are incompatible with the notion, generally accepted at that time, that the active sites in a protein chain switch successively from a long to a short state during muscle contraction. On the other hand, they fit naturally with sliding filaments: the frequency with which a site on the myosin filament approaches a site on the actin filament with which it may interact is proportional to the speed with which the filaments slide past one another, i.e. to the speed of shortening, just as in an enzymic reaction the frequency with which substrate molecules approach the enzyme is proportional to substrate concentration. Practically all current theories of contraction that have been developed to a quantitative level are cyclic in this sense.

4. A. F. HUXLEY'S 1957 THEORY

I developed the idea of cyclic interactions in a theory that provided an adequate fit to Hill's equations (A. F. Huxley 1957). It was purely kinetic in character, i.e. it did not make specific postulates about the structural and biochemical events underlying the interactions between myosin and actin sites. Its essential features are as follows.

1. Each cross-bridge formed by an interaction between myosin and actin contains an elastic element, allowing Brownian movement before an interaction occurs and causing force to be produced when the cross-bridge is strained.
2. The rate constant for attachment is moderate when the separation between the two sites is within a certain range where attachment will cause positive tension but zero if attachment would cause negative tension.
3. The rate constant for detachment is small as long as the cross-bridge is exerting positive tension but becomes large as soon as shortening has brought the cross-bridge past the position where the force it exerts is zero.



igure 3. (*a*) Early part of tension response of a muscle fibre o a sudden shortening of 4.5 nm in each overlap zone (frog, bout 1 °C). Phase 1: initial drop. Phase 2: early tension ecovery, complete in about 2 ms. Phase 3: slowing (sometimes eversal) of tension recovery. Phase 4: the final roughly xponential approach to the original tension, takes about 00 ms and is not seen in this record. Adapted from A. F. Huxley (1974), fig. 8. (*b*, *c*) Contrast between oscillatory ength change in response to load step (*b*, load increased from .9 T_0 to T_0 between arrows) and limited sequence of phases n tension response to length step (*c*, stretch by 4.8 nm in each verlap zone, at first arrow). Phase 3 after a stretch shows up s a delayed rise of tension (*D*). (*b*) and (*c*) from Armstrong *et l.* (1966).

Simple functions leading to an explicit solution of the quations were chosen for the dependence of the rates of ttachment and detachment on extension of the cross-ridge, and the parameters were adjusted by trial and rror so as to obtain an adequate match to Hill's quations.

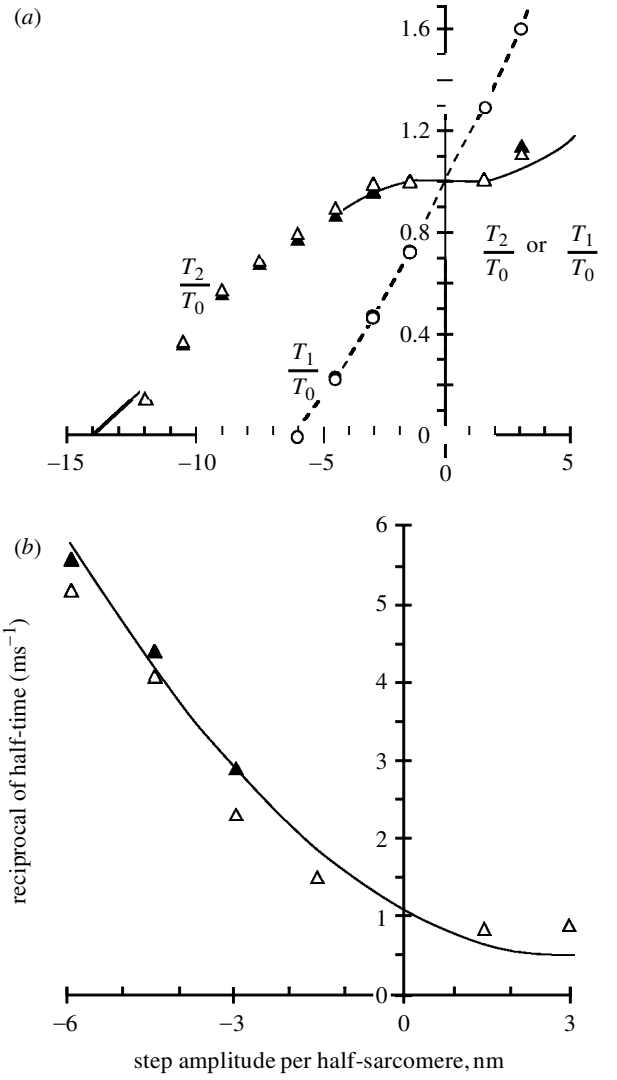
The theory is certainly incomplete. For example, it ssumes that force generation occurs instantaneously hen a cross-bridge is formed, and it therefore fails to xplain the rapid changes of tension that were sub-equently found to follow when the length of a muscle bre is suddenly changed during contraction. Some of its uantitative features are also wrong; for example, the etachment rate while a cross-bridge is exerting positive orce was chosen to match the rapid exponential phase of elaxation at the end of a period of stimulation, whereas is now known that this phase is due to rapid elongation f particular parts of the muscle fibre, usually near its nds. Later work by Hill (1964) showed that the rate of nergy liberation did not continue to increase with short-ning speed throughout the range but declined when the naximum velocity was approached (figure 2); explana-ons for this have been suggested (A. F. Huxley 1973; Barclay 1999) but remain speculative. On the other hand, the 1957 theory was successful in predicting the dependence of longitudinal stiffness on speed of shortening, which had not previously been measured. Most, if not all, current cross-bridge theories have features qualitatively similar to those numbered 1–3 above.

It is nowadays supposed that most or perhaps all of the tension produced by a cross-bridge is generated by transitions that occur after attachment. It is, however, uncertain whether the first attached state rigid enough to transmit force does exert force and if so whether its contribution is positive or negative. A small negative contribution might be the origin of the 'latency relaxation', the small drop in tension after a stimulus before tension begins to rise.

5. MECHANICAL TRANSIENT RESPONSES

Attempts at measuring the responses of muscle to sudden changes of load or length during contraction had been made before the Second World War (e.g. Gasser & Hill 1924), but had been inconclusive, largely because the recording instruments were not fast enough. The first useful measurements were made by Podolsky (1960) on a small frog muscle; he recorded the time-course of the length changes that followed when the load on the muscle was suddenly reduced, and found that the steady speed of shortening was approached through a damped oscillation. Almost all the experiments on transient responses in my laboratory have used the converse type of experiment in which length, not load, is altered suddenly. In this case (figure 3*a*), there is a simultaneous change of tension (phase 1) followed by approach to the original tension through a sequence of three more phases, each with a roughly exponential decline. Phase 2 is a rapid (of the order of 1 ms in frog muscle near 0 °C) recovery towards the initial tension; during phase 3 the rate of recovery is greatly reduced or actually reversed (of the order of 10 ms); and in phase 4 there is a roughly exponential recovery to the original tension (of the order of 50 ms). The contrast between the oscillatory response to load change and the small number of exponentially decaying phases in response to length change is brought out in figure 3*b*,*c*, reproduced from Armstrong *et al.* (1966). I did in fact show (unpublished data) that these are two different expressions of the same properties, by superposing numerically the tension responses to a sequence of small length steps whose amplitude and direction were chosen so that the overall tension change was a step, and found that the length steps added up to an oscillatory change indistinguishable from the experimental length change following a small step change of load. The fact that the response to length change is composed of first-order delays while that to load change is oscillatory implies that the molecular events are directly affected by longitudinal displacement of the filaments rather than by the tension in them.

Over most of the range where it can be measured, the tension change during phase 1 is nearly proportional to the imposed length change, implying a roughly linear compliance in the muscle structure. On present evidence, about half of this appears to be actually within the cross-bridges and half in the filaments. It is still uncertain to



igure 4. Nonlinearities in (*a*) extent, and (*b*) rate, of early ension recovery (phase 2) after a step change of length. ines: summary of experimental results of Ford *et al.* (1977). T_0, T_1 and T_2 defined in figure 3*a*. Symbols: simulation based n an updated version of the theory of A. F. Huxley & immons (1971); filled symbols taking account, and open ymbols not taking account, of detachment of cross-bridges. rom A. F. Huxley & Tideswell (1996).

hat extent the cross-bridges can exert negative tension hen relatively large shortening is imposed.

During phase 2, there is little change in stiffness of the uscle, suggesting that few cross-bridges detach or attach uring this phase. The tension change is therefore usually ttributed to events happening in cross-bridges that were lready attached before the length step was imposed, i.e. his phase is thought to represent the actual 'working troke' of attached cross-bridges.

Phase 3 is not well understood. In small releases, ension actually falls, so it is natural to suppose that cross-ridges are detaching. In a small stretch, this phase shows p as a delayed rise in tension, as in figure 3*c*; it may be ue either to an increase in the rate of attachment of yosin heads previously free or a decrease in the rate of etachment of heads previously attached (or both). uring this phase, the events underlying the rapid

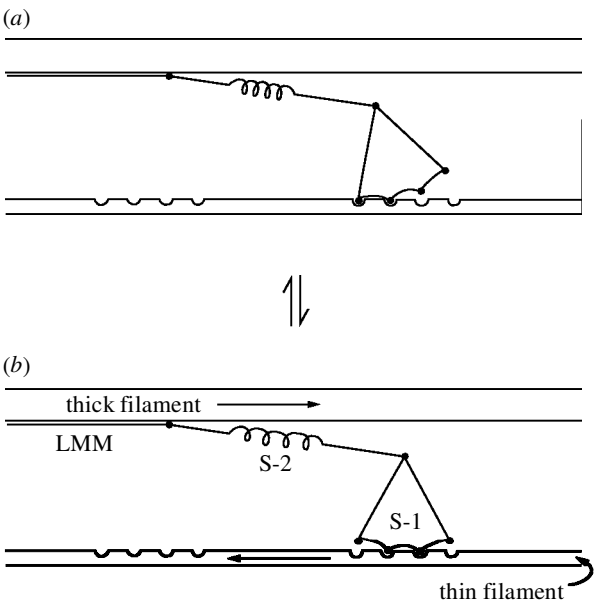


Figure 5. Schematic representation of theory of A. F. Huxley & Simmons (1971). Tension is generated by clockwise rotation of the myosin head, stretching the spring in the connection to the myosin filament. This rotation goes in a small number of steps. In going from state A1 (*a*) to A2 (*b*), the bond that is formed is stronger than the one that is broken. The diagram is not meant to imply anything more than the stepwise progression and the presence of an elastic element within the cross-bridge, e.g. the stepwise change may occur at a hinge where a 'lever arm' is attached to the part bound to actin (as in figure 6) and the elastic element may reside in bending of this lever arm. Adapted from A. F. Huxley (1974).

regeneration of the power stroke (Lombardi *et al.* 1992; see p. 439) take place.

During Phase 4, presumably bridges are detaching and reattaching further along the actin filament.

As regards the origin of the force generated by a muscle, phase 2 is the most informative. It shows two striking nonlinearities, respectively in the extent (figure 4*a*) and in the speed (figure 4*b*) with which tension approaches the value before the imposed step. A semi-quantitative explanation for both these nonlinearities was given (A. F. Huxley & Simmons 1971) by assuming that during steady contraction, each cross-bridge could exist in one or other of two (or more) states (as shown schematically in figure 5), and that there was an equilibrium in which the bridge switched from one to another of these states at intervals of time comparable to the duration of phase 2 (of the order of 1 ms). Denoting these attached states by A1, A2, etc., the bonds holding the bridge in state A2 would be stronger than those holding it in state A1, creating a tendency to switch from state A1 to A2, stretching the elastic element and therefore causing an increase in tension. This increase in tension creates a tendency for the cross-bridge to revert to state A1, leading to an equilibrium that would be disturbed when the muscle fibre is stretched or released because this would alter the force on the cross-bridge; phase 2 of the transient represents the re-establishment of equilibrium. At any instant, the total tension depends on the relative numbers of cross-bridges in states A1 and A2. The range

f sliding movement over which this process can occur is mited by the extent of movement corresponding to witching between the states in question; this would be he explanation of the nonlinearity in the amount of edevelopment of tension after a sudden length change. The nonlinearity of the speed of the recovery would arise ecause the work done in stretching the elastic element onstitutes part of the activation energy for the switch rom A1 to A2 and will vary according to the force in the lastic element.

6. NUMBER OF ATTACHED STATES

The number of these attached states, and the amount f movement corresponding to each step from one to the ext, are still very uncertain. Estimates of the total mount of movement based on X-ray structures of rystallized fragments of myosin have ranged between nm (Rayment *et al*. 1993) and 10–12 nm (Dominguez *et l*. 1998); these structures have given no indication whether there is more than one step. The curve in gure 4*a* reaches zero tension with a release of about 3 nm but some 2 nm of this is accounted for by the lament compliance; simulations (A. F. Huxley & ideswell 1996) required two steps with a total movement f about 10 nm. The reason we assumed two steps is that he theory of A. F. Huxley & Simmons (1971) leads to an nstability if the extent of the step from A1 to A2 is reater than the interval between sites on the actin fila- ent where a myosin may attach (presumably 5.5 nm, he spacing between actin monomers in each of the rands of the filament). However, this argument has een undermined by a simulation by Duke (1999), with a ngle step of 11 nm; instability is present but results only n slow asynchronous relative movements of adjacent laments and the overall shortening of the fibre does not now any instability.

Using a single-molecule technique, Veigel *et al*. (1999) ave recently found that the working stroke of certain ow, non-muscle myosins consists of two well-separated teps, each of about 5 nm. When they used subfragment 1 of skeletal muscle, they could not fully resolve two teps in the attachment but showed that it occupied some ms as against 1 ms for detachment, suggesting strongly hat it too consists of more than one step. There is no vidence yet to show whether these represent two events while the myosin is attached to a particular actin nonomer or whether the second step is due to detach- ent with immediate attachment at the next actin, as eems to be the case in another type of experiment Kitamura *et al*. 1999).

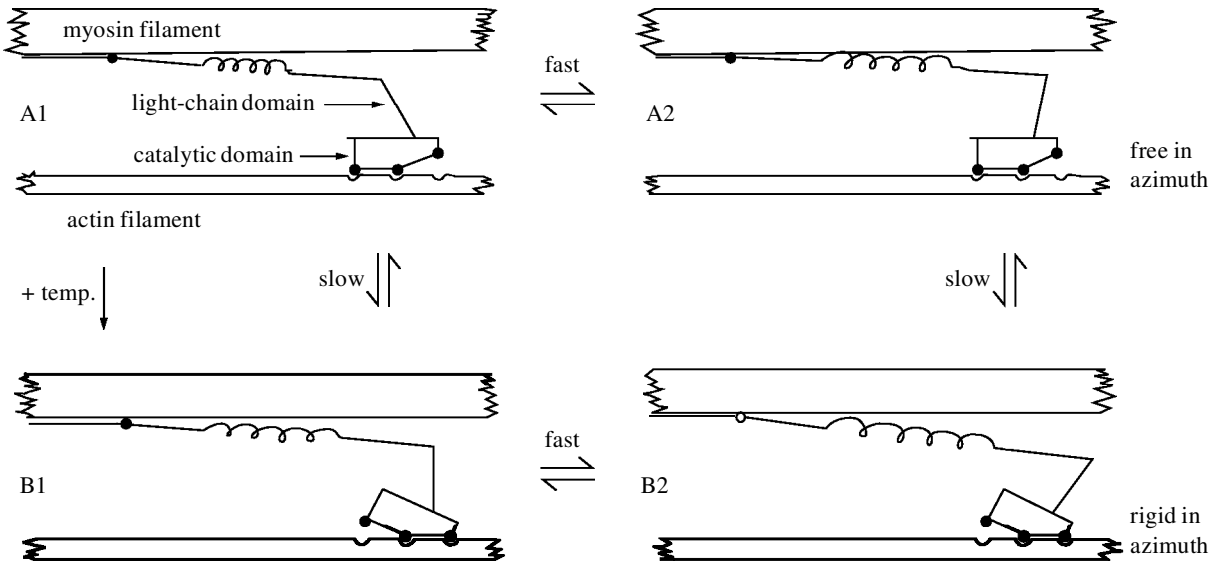
7. STRUCTURAL CHANGES

The first indication of a structural change underlying he working stroke was given by Reedy *et al*. (1965). Their lectron micrographs of asynchronous insect flight muscle howed that the long axes of the cross-bridges were oughly perpendicular to the fibre axis in resting muscle ut at about 45° in rigor. This difference was in the direc- on that would correspond to shortening of the fibre if, as to be expected, the resting state resembles that at the tart of the working stroke and rigor resembles that at the end. The idea that this change of orientation was the event which caused shortening or production of force was developed by H. E. Huxley (1969) and has long been generally accepted. It was supposed at first that the cross-bridge tilted as a whole, driven by a change in the angle at which it is attached to the actin filament, but the atomic structure of the myosin head (S1 fragment) has shown a probable hinge between the catalytic domain (which binds to the actin filament) and the long α-helix to which the light chains are attached, and it is now usually supposed that the latter part of S1 is a 'lever arm' which tilts relative to the catalytic domain (Rayment *et al*. 1993; Holmes 1997). It is now clear, from measurements by resonance energy transfer between luminescent or fluorescent probes attached on either side of the hinge, that adding ATP to an appropriate myosin fragment causes a bend at the hinge in the direction corresponding to reversal of the working stroke (Getz *et al*. 1998; Suzuki *et al*. 1998).

It is not, however, excluded that tilt may occur at both places, i.e at the attachment of the catalytic domain to the actin filament as well as at the hinge, and Schmitz *et al*. (1997) and Taylor *et al*. (1999) have proposed that this is likely on the basis of three-dimensional reconstructions from electron micrographs of cross-bridges in asynchronous insect flight muscle. Such a rolling movement of the catalytic domain along the actin filament might be one of the steps proposed by Diaz Baños *et al*. (1996) on the basis of detailed calculations of the forces between atoms in the myosin head and in actin monomers.

8. TEMPERATURE JUMP EXPERIMENTS

A recent observation of a quite different kind has also given support to this idea. When the temperature of a fibre during active contraction is raised within a fraction of a millisecond, tension rises relatively slowly (of the order of 1–100 ms depending on the initial and final temperatures). Bershitsky & Tsaturyan (1989) showed that there is no accompanying increase of stiffness, indicating that the tension change is not due to an increase in the number of bridges attached and contributing to tension. It might be supposed that this tension rise is due to a shift in the equilibrium between two of the attached states postulated to explain phase 2 of the tension transient, but Bershitsky & Tsaturyan (1992) and Davis & Harrington (1993) showed that there is no component with a time-course similar to the early part of phase 2 at the temperature reached in the jump, though the later part of phase 2 may well be due to the same event as the tension rise after a temperature jump. These observations show that different transitions must be involved in the two cases. Further, Bershitsky *et al*. (1997) and Tsaturyan *et al*. (1999) have shown that the slow rise of tension after a temperature jump is accompanied by a striking change in the X-ray diffraction pattern. It was already well known that the first layer line related to the long actin helix (spacing 36 nm) becomes strong when a muscle goes into rigor, but during contraction at low temperature it is hardly stronger than at rest (H. E. Huxley *et al*. 1982). Bershitsky *et al*. (1997), however, found that during the rise of tension following a temperature jump, this actin layer line becomes stronger, implying that the azimuthal



igure 6. Schematic diagram illustrating possible superposition of two different force-generating transitions. Rapid regeneration f tension after sudden shortening would be the result of transition from A1 to A2 or from B1 to B2 (tilting of the light-chain omain (the lever arm) relative to the catalytic domain) while the rise of tension following a temperature jump would be the esult of transition from A1 to B1 and/or from A2 to B2 (rocking of the catalytic domain on the actin filament). In the A-states he catalytic domain is free to rotate about the fibre axis but in the B-states it is not, i.e. the B-states represent stereospecific inding while the A-states do not. The rise of tension on going from A-states to B-states would drive the tilting of the lever arm in he direction from A2 to A1 and from B2 to B1.

rientation of the myosin heads attached to actin comes o follow the long helix of the thin filament. Furthermore, saturyan *et al*. (1999) estimate that the fraction of cross-ridges labelling the actin helix increases from about 5% at 5–6 °C to about 60% at 30 °C (permeabilized rog fibres). The absence of an increase of stiffness ollowing the temperature jump shows that the heads ust have been already attached to actin but disorient-ted in azimuth. An increase in the intensity of the sixth nd seventh actin layer lines (5.9 and 5.1 nm) had been bserved during the rise of tension when muscle in rigor heated to about 50 °C (Rapp & Davis 1996), again ndicating an increase in stereospecific binding with emperature.

It is easiest to imagine that this change takes place at he attachment of the catalytic domain of the myosin ead to the actin filament, as shown schematically in gure 6. Since the change is accompanied by a rise in ension, it must involve tilting of the whole myosin head o as to increase the force transmitted by the elastic lement, and it must be a change from a state free to otate in azimuth to one that is rigid in that direction. his would be the situation if, for example, the attachment oints shown in figure 6 are all single-point attachments hile in the B-states there is also an attachment displaced n the direction perpendicular to the diagram. The fact hat the B-states are favoured by a rise in temperature uggests that one of the attachments in the B- but not the -states is the hydrophobic link, as in the change from tate 3 to state 4 in fig. 7 of Diaz Baños *et al*. (1996).

The change in orientation of the lever arm shown in gure 6 on going from A1 to A2 or B1 to B2 would not rise if the elastic element resides in bending of the lever rm.

In any case, it is necessary to suppose that the two steps can occur more or less independently of one another in order that the time-constants of early recovery after a stepwise release and after a temperature jump should be different, as is found even when the step is applied during the tension rise following a temperature jump (S. Y. Bershitsky and A. K. Tsaturyan, personal communication). Changes in attitude at the two hinges would, however, interact with one another, especially under isometric conditions (no relative sliding permitted), since any tendency for a change in attitude at one hinge to cause sliding would have to be counteracted by an opposite change in attitude at the other. The mean torques generated at the two hinges must produce the same value of sliding force, equal to the tension transmitted by the elastic element of the cross-bridge. The equilibria of both steps would be altered by a change in tension, as was discussed above in relation to phase 2 of the response to a length step.

9. DETACHMENT AND REATTACHMENT WITHIN ONE ADENOSINE TRIPHOSPHATE CYCLE

Many experiments have suggested that a cross-bridge may remain attached while the filaments slide past one another for distances too long for a particular myosin head to remain attached to the same actin monomer, although only one adenosine triphosphate (ATP) molecule was used (e.g. Higuchi & Goldman 1995; Kitamura *et al*. 1999). The simplest explanation would be that the cross-bridge detaches and reattaches immediately at another site on the thin filament. Two situations where there is good evidence for this are (i) the experiment of Kitamura *et al*., where most of the movement took place in steps closely equal to

ne spacing between adjacent monomers in each strand of ctin in the thin filament, and (ii) stretch of a muscle fibre uring contraction. In the latter case, the tension exerted y the fibre undergoes a number of striking changes. A etailed explanation of these was given by Piazzesi *et al*. 1992) on the basis that a cross-bridge torn off by the xcess tension imposed by the stretch was in a state capable f reattaching with a rate constant two orders of agnitude greater than when detached by the normal rocess of binding a fresh ATP molecule. The chemical tate of a myosin head detached in this way is not known, hough it seems likely that it has lost the terminal phos- hate group of the ATP molecule that was bound to it hen it attached. Another phenomenon that may be xplained by detachment during sudden shortening and eattachment in a time of the order of 10 ms (Piazzesi & ombardi 1995) is 'repriming', the rapid regeneration of ne power stroke (Lombardi *et al*. 1992). The phenomenon s that, for a given total amount of shortening, the tension T_2 reached in the early tension recovery is greater if ne shortening is divided into two steps separated by about –20 ms than if it is applied as a single step or as two steps eparated by less than 2 ms. There are, however, at least wo other explanations for the phenomenon (A. F. Huxley Tideswell 1997).

10. UNCERTAINTIES

(a) *Force generated by one cross-bridge*

It will be evident from what I have said that there are ill great uncertainties in our knowledge of the mechan- cal aspects of the contraction process. For instance, I ave not mentioned a value for the amount of tension ontributed by a single cross-bridge: it is clearly of the rder of a few piconewtons (I usually assume 4 pN) but ll methods of estimating it are subject to uncertainty. My wn work has been entirely on intact, isolated muscle bres in which it is reasonable to assume that, unlike in *in itro* experiments, the cross-bridges are in their normal tuations, but there is much controversy as to the propor- on of the myosin heads that form active cross-bridges at ny one time (e.g. it is not clear whether both heads of a yosin molecule can contribute to tension, nor how much f the length of each half-turn of the thin-filament helix s available for myosin atachment), and there is consider- ble variation in the total tension per unit cross-sectional rea given by different fibres.

Another uncertainty is whether the compliance in a ross-bridge is in the link to the myosin filament as shown chematically in figures 5 and 6, or in bending of the ever arm, or in flexibility at the hinge.

(b) *Single-molecule experiments*

Ideally, the impressive single-molecule experiments ow being carried out in many laboratories should give a irect answer to questions such as the amount of force per ross-bridge, but the results from different laboratories ary widely. There are many sources of possible error, uch as: most laboratories use myosin molecules, intact or agmented, stuck down in a layer of nitrocellulose in nknown orientation (an honourable exception being the aboratory of T. Yanagida, where the usual preparation is synthetic thick filament in which only a few of the myosins still have heads capable of attaching to actin); errors are introduced by the compliance of the actin filament and particularly by its attachment to a bead held in a light trap; much Brownian noise is always present; and the time resolution is not good enough to tell exactly when attachment occurs or to observe transient responses. Skinned or permeabilized fibres have the immense advantage over intact fibres that they make it possible to vary the concentrations of solutes at will, but such preparations generally give low values for the tension per unit area and show less tension recovery in phase 2 of the response to sudden shortening than intact fibres; further, the sarcomeres are less regular and it is not clear how much local shortening happens while the total length is held constant and the preparation is activated.

Apart from such quantitative uncertainties, there is always a possibility—indeed, a probability—that our present concepts are seriously incomplete or even wrong. For example, it is still usually assumed that force is generated entirely by the lever arm tilting about a single axis, whereas it seems to me that there is now a real possibility that tilting can occur at the attachment to actin as well as at the hinge. And the suggestion by Harrington (1979) that contraction may be due to melting of the α-helix in the S2 portion of myosin has not been excluded.

The ideas about the events following a temperature jump arose in conversation with Dr Bershitsky and Dr Tsaturyan. I am also grateful to them and to Dr Vincenzo Lombardi for helpful criticisms of the manuscript.

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Discussion

L. Cruzeiro-Hansson (*Department of Mathematics, Heriot-Watt University, Edinburgh, UK*). I would like to suggest that there may be no distinction between linear and rotary motors in the sense that conformational changes in linear motors may also be due to rotation of α-helices. In this sense, all motors may be rotary.