Acta Physiol Scand 1998, **163**, 307–323

Muscle contraction as a Markov process I: energetics of the process

C. E. SCHUTT[1] and U. LINDBERG[2]

1 Department of Chemistry, Princeton University, Princeton, NJ, USA
2 Department of Cell Biology, The Wenner-Gren Institute, Stockholm University, Sweden

ABSTRACT

Force generation during muscle contraction can be understood in terms of cyclical length changes in segments of actin thin filaments moving through the three-dimensional lattice of myosin thick filaments. Recent anomalies discovered in connection with analysis of myosin step sizes in *in vitro* motility assays and with skinned fibres can be rationalized by assuming that ATP hydrolysis on actin accompanies these length changes. The paradoxically rapid regeneration of tension in quick release experiments, as well as classical energetic relationships, such as Hill's force–velocity curve, the Fenn effect, and the unexplained enthalpy of shortening, can be given mutually self-consistent explanations with this model. When muscle is viewed as a Markov process, the vectorial process of chemomechanical transduction can be understood in terms of lattice dependent transitions, wherein the phosphate release steps of the myosin and actin ATPases depend only on occurrence of allosteric changes in neighbouring molecules. Tropomyosin has a central role in coordinating the steady progression of these cooperative transitions along actin filaments and in gearing up the system in response to higher imposed loads.

Keywords actin, chemomechanical transduction, Fenn effect, myosin, tropomyosin

Received 28 October 1997, accepted 23 February 1998

MYOSIN MOTORS IN MUSCLE CONTRACTION

In the cross-bridge theory, catalytic myosin heads projecting from myosin thick filament rods pull relatively rigid actin thin filaments towards the centre of the sarcomere during a contraction. The nature of the mechanism by which myosin 'motor' molecules might work has been the subject of intense investigation (Huxley & Kress 1985, Spudich 1994, Block 1996). There are two main types of cross-bridge theories proposed for chemomechanical transduction: (i) power-stroke models, where conformational changes in the myosin cross-bridges are coupled to ATP-dependent free energy changes (Huxley 1969, Huxley & Simmons 1971, Eisenberg & Hill 1985, Rayment *et al.* 1993), and (ii) thermal ratchets, where the free energy of ATP hydrolysis is used to bias the selection of directed components of Brownian motions (Harrington 1971, Mitsui & Ohshima 1988, Vale & Oosawa 1990, Cordova *et al.* 1992, Leibler & Huse 1993). However, cross-bridge theories fail to predict the fall-off in the ATPase rate at high shortening velocities (Pate & Cooke 1989, Leibler & Huse 1993), and predict efficiencies of free energy transduction (Irving *et al.* 1992, Higuchi & Goldman 1991, 1995) far below those observed experimentally (Kushmerick & Davies 1969). To these has been added the difficulty that actively contracting muscle fibres can regenerate tension in 15 ms, much faster than the observed ATP-splitting reaction of 100 ms, after being subjected to multiple tension-reducing shortening steps of around 5 nm (Lombardi *et al.* 1992, Irving *et al.* 1992, Linari *et al.* 1997).

Observations indicating that actin filaments account for ≈50% of sarcomeric elasticity (Huxley *et al.* 1994, Kojima *et al.* 1994, Wakabayashi *et al.* 1994, Higuchi *et al.* 1995), where an upper limit of 10% had been assumed in the classical theory (Ford *et al.* 1981), and conflicting measurements for the length of the myosin power stroke (Finer *et al.* 1994, Saito *et al.* 1994, Higuchi & Goldman 1995, Molloy *et al.* 1995), also raise doubts about the uniqueness and simplicity of the

Correspondence: Dr Clarence E. Schutt, The Henry Hoyt Laboratory, Department of Chemistry, Princeton University, 08544 Princeton, New Jersey, USA.

classical myosin cross-bridge theory as an explanation for muscle contraction. In fact, when filament extensibility is taken into account, cross-bridge models of muscle contraction are difficult to reconcile with the theory of independent force generators (Schutt & Lindberg 1993, Mijailovich *et al.* 1996).

Cross-bridge theories are cast in terms of the attachment and detachment rates of myosin on actin as the filaments slide by each other (Huxley 1957, 1974). Studies in solution have shown a specific requirement for actin to stimulate phosphate release from myosin to complete the ATPase cycle (Lymn & Taylor 1971). Thus, the myosin ATPase product release steps are chained to the movement of actin filaments which are assumed to be stiff. Energized myosin heads attach for a period, during which they either generate an active force (producing work) or contribute a passive drag (resulting in heat) to the motion of the actin filament before detaching. The product of the active force times the distance over which it acts is the work done by a single myosin motor. Recent experimental results imply a low efficiency of chemomechanical transduction for all such tightly coupled theories (Higuchi & Goldman 1991, 1995, Irving *et al.* 1992, Molloy *et al.* 1995); the forces are not only too small, but also act over too short a distance compared with drag distances.

In the face of these developments, the kinetic schemes associated with the myosin cross-bridge theory have taken on a number of different forms, such as multiple power strokes per ATP molecule hydrolyzed (Higuchi & Goldman 1991, 1995, Irving *et al.* 1992, Molloy *et al.* 1995), or distinct transduction pathways for different populations of cross-bridges (Chen & Brenner 1993, Cooke *et al.* 1994, Thomas *et al.* 1995, Piazzesi & Lombardi 1996, Huxley & Tideswell 1997, Linari *et al.* 1997). The variety and complexity of these kinetic schemes, the difficulty of relating them self-consistently to plausible structural changes in myosin and actin (Irving *et al.* 1995), while at the same time accounting for the high thermodynamical efficiencies observed in shortening muscle fibres (Kushmerick & Davies 1969), constitute the thematic origin of the present crisis in the field of muscle contraction.

ACTIN AS THE GENERATOR OF TENSION

We have proposed a model of muscle contraction (Schutt & Lindberg 1992, 1993, Schutt *et al.* 1995a) based on the observed organization of actin molecules into polymeric 'ribbons' in profilin:actin crystals (Schutt *et al.* 1989, 1993, Chik *et al.* 1996). This model explains the classical mechanical and X-ray observations on contracting muscle fibres, but differs from cross-bridge theories in one important respect: contraction forces are developed by myosin-induced extensions and contractions within actin filaments themselves. Instead of motor activity performed by the myosin cross-bridges, waves of structural transitions along actin filaments, directly coupled to an ATPase activity on actin, propagate progressively towards the centre of the sarcomere. The role of the myosin cross-bridge ATPase is to activate these transitions in thin filaments by catalyzing nucleotide exchange on actin.

In this model (Fig. 1), structural transitions in the overlap zone propagate vectorially along actin thin filaments towards the centre of the sarcomere, resulting in the transfer of momentum to the Z-disk via tropomyosin and the actin filaments in the I-band (Schutt & Lindberg 1992). The theory is fundamentally 'Markovian' in character (Cox & Miller 1965), as the state of each molecule in the overlap zone depends on the state of its immediate neighbours. Thus, an actin monomer in the ribbon state remains so until the neighbouring monomer on its I-band side undergoes the transition to the helical state. Similarly, the transition to the ribbon state for an actin subunit requires that its immediate neighbour has also done so. A detached myosin head, after it has hydrolyzed ATP (a fast step), rebinds only when a correctly oriented actin monomer moves into position, an event whose timing is also controlled by the orderly sequence of events occurring in actin filaments. Thus, all of the biochemical rates scale to the velocity of the waves of structural transitions travelling through the overlap zones of the sarcomeres.

Our explanation for the Fenn effect (Fenn 1924, Hill 1938), the classical observations that imply a two-way relationship between the mechanics of muscle contraction and the release of energy from biochemical sources, leads to a new interpretation for the mechanical role of tropomyosin in regulating muscle contraction. The role of tropomyosin is to transmit the load on the muscle fibre directly to the ribbon ↔ helix transition front(s). The load thereby directly regulates the rate of progression of events along thin filaments towards the centre of the sarcomere. In this model, work is produced as each actin monomer, upon rebinding tropomyosin and releasing inorganic phosphate, contracts 0.83 nm in going from the ribbon (r-actin) to the helical (h-actin) state. The transition from the relaxed to the contracting state is known to be regulated by the binding of calcium ions to the troponin complex (Tn-C, Tn-T, Tn-I, for a recent reference see Schaertl *et al.* 1995). However, in contrast to the 'steric blocking mechanism', in which the troponin complex shifts the equilibrium position of tropomyosin in response to calcium so as to expose myosin binding sites on actin, in the present model troponin-T responds by strengthening the head-to-tail 'lap-joints' between adjacent tropomyosin molecules, thereby establishing

strong mechanical linkages between the force generators (Schaertl *et al.* 1995).

We conclude that a lattice-dependent vectorial Markov process is required to achieve the high efficiencies observed over a wide range of load and speed in shortening muscle fibres (Kushmerick & Davies 1969). In a theory based on length changes along the direction of thin filament movement, the analysis of chemical free energy changes is simple; mechanical work becomes the force times the integrated length change within moving actin filaments as they pull on tropomyosin filaments. The efficiency of the process results from the smooth coordination of movements in the sarcomere and the direct transfer of force produced by conformational changes in actin to the tropomyosin molecules in direct mechanical connection with the load via the Z-disks. There is little wasted motion compared with the classical cross-bridge theory (Huxley 1957), where negative work on attached myosin heads being dragged is an ineliminable feature of tightly coupled models.

The proposition that ATP hydrolysis takes place on actin filaments requires reconsideration of the role of the myosin ATPase in muscular contraction. The question hinges on the pitfalls of extrapolating from solution studies on regulated acto-S1, where mass action and diffusion are the controlling processes, to the behaviour of actomyosin in the highly structured environment in filament lattices, where macromolecular motions coordinated by tropomyosin (under tension) might activate an actin ATPase as well. Myosin cross-bridges in the tightly bound state serve as traction points on the thick filaments for the reptating actin thin filaments. When the continuous process of tension development is interrupted, as when a quick shortening step is applied to contracting muscle fibres to reduce tension (Irving *et al.* 1992), the elastic energy stored in the myosin heads is released, whereas the free energy in metastable actin ribbon segments remains undiminished (Schutt & Lindberg 1992). Tension can be restored quickly (compared with the myosin ATPase rate) by the rapid conversion of a small number of ribbon monomers into helical ones, a process which rebends the myosin bridges while tension is being restored.

This paper extends our earlier work (Schutt & Lindberg 1992, 1993, Schutt *et al.* 1995a) by demonstrating that the ATPase rate calculated from the ordered sequence of biochemical changes in local cyclically contracting segments of myosin-activated actin filaments agrees with a wide variety of classical and recent measurements on the energetics of contracting muscle fibres. The ribbon-to-helix conjecture is based on the idea that actin subdomain rotations about hinge points can bring about a transition from the crystallographically observed ribbons to helical F-actin. We have recently determined the structure of an 'open state' of the actin monomer (Chik *et al.* 1996) which, when compared with the previously determined 'tight-state' conformation (Schutt *et al.* 1993), reveals where these hinge points in actin lie. A preliminary F-actin model, based on these ideas, gives a satisfactory picture of how gelsolin and other actin-binding proteins might bind to the filament (Schutt *et al.* 1997). The current 'atomic model' (Lorenz *et al.* 1993) of F-actin is incompatible with the ribbon-derived F-actin model. This controversy has been extensively discussed elsewhere (Schutt *et al.* 1995b), and the analysis of the energetics of muscle contraction, as presented here, adds weight to the argument in favour of a ribbon-based F-actin model.

FORCE DEVELOPMENT

A premise of the tight coupling hypothesis of free energy transduction in biological systems is that work is produced at the point where the products of nucleotide hydrolysis are released into solution (Eisenberg & Hill 1985). In the actin power-stroke model, product release occurs at two places (Fig. 1): (i) from myosin heads when they bind to actin to initiate the helix $\rightarrow$ ribbon transition in actin subunits, the 'initiation' step, and (ii) from individual r-actin monomers shortening the segment as they revert to the h-actin form and rebind to tropomyosin, the 'work-producing' step. The theoretical force developed by an actin segment undergoing a reversible length change is given by:

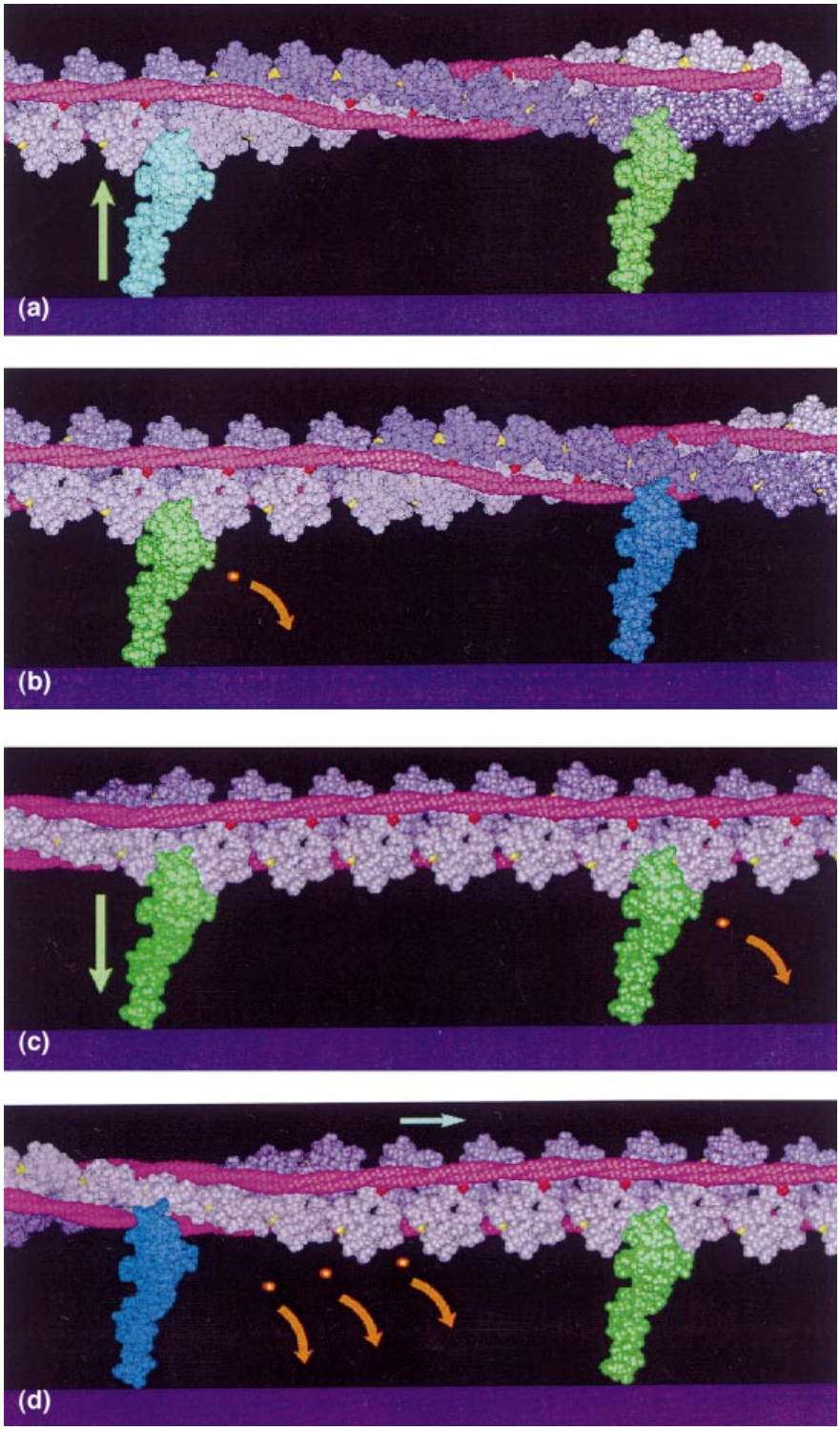
$$f = -\frac{\mathrm{d}G(x)}{\mathrm{d}x}, \qquad (1)$$

where, f is the force, $G(x)$ the Gibbs free energy, and x the length of the segment, measured in the direction of the length change.

The source of the free energy for the mechanical work performed by the actin segment is the hydrolysis of ATP. As the conversion of each actin monomer from the h-state to the r-state involves the binding and hydrolysis of a single ATP molecule, the mean force as the segment contracts is:

$$f_{\mathrm{rh}} = -\epsilon\left[\frac{\Delta G_{\mathrm{ATP}}}{\Delta x_{\mathrm{rh}}}\right], \qquad (2)$$

where ΔG_{ATP} is the free energy available from ATP hydrolysis on one actin monomer, Δx_{rh} the length change associated with the r $\rightarrow$ h transition (0.83 nm), and ϵ the efficiency of the energy conversion. The instantaneous force fluctuates around a mean value, f_{rh},



independent of segment length, as long as monomers contract one at a time in succession. A constant force–extension curve (the T2 curve) for the actomyosin contractile component for length changes less than about 40 Å per half sarcomere is an established feature of independent force generators acting in parallel (Huxley & Simmons 1971).

THE HILL EQUATION AND THE FENN EFFECT

A muscle in an 'isometrically contracting state' does no work but generates heat at a steady rate. If allowed to shorten by decreasing the load by an amount ΔP, it performs mechanical work and is observed to generate

Figure 1 The myosin-activated actin power stroke. An actin filament is shown as it reptates through the actomyosin lattice towards the right of the figure. In this model (Schutt & Lindberg 1992, Schutt & Lindberg 1993, Schutt *et al.* 1995a), both myosin and actin molecules are ATPases, each activated by the other. Myosin heads are represented as space-filling molecules based on the S1 crystal structure (Rayment *et al.* 1993). An ADP-P_i state of myosin engaging the ribbon–helix boundary is represented in aquamarine. The actin-bound myosin-ADP state is in green. The detached myosin-ATP or -ADP-P_i state is in blue. The myosin thick filament shaft is represented as a structureless support (purple). Inorganic phosphate molecules are small spheres (orange). Actin subunits are represented as space-filling molecules (grey, ivory) based on the crystal structure of the ribbon form (Schutt *et al.* 1993). Two states of actin are present in the filaments: h-actin, the classical ADP-containing helical form, modelled as will be described elsewhere (Page *et al.*, in press), and r-actin, the ATP-bound form observed in profilin–actin crystals (Schutt *et al.* 1993). The two forms are related by a ribbon-to-helix transition comprising of a twist of 13° and a shortening of 0.83 nm per actin monomer. Tropomyosin (purple) is modelled as a space-filling helical coiled coil based on the crystal structure (Phillips *et al.* 1986). It is strongly bound when associated with actin in the h-state and unbound when adjacent to r-actin. Only equivalent myosin heads separated by 429 Å along the thick filament are shown. Other 'crowns' of myosin heads spaced at 143 Å intervals bind actin filaments when waves of ribbonization, out of phase with the one shown, pass through the lattice. (a) *Ground state*. Myosin-ADP-P_i on the left (aquamarine) binds to a targetted actin subunit. The actin subunits to the left of this head are in the r-actin state, and those on the right of are in the h-actin state. The myosin head on the right (green) is tightly bound to r-actin. (b) *Energization primed by myosin*. As myosin opens up the first few actin subunits (h → r transition) for nucleotide exchange, it releases an inorganic phosphate, becoming strongly bound to actin in the process. Tropomyosin is released from the actin subunits as they successively take on the r-actin form. Ribbonization proceeds toward the centre of the sarcomere (to the right) driven by the cooperative uptake and hydrolysis of ATP on the actin filament. (c) *Metastable intermediate*. The energization of the ribbon is complete when the 13th r-actin subunit is delivered to the next myosin head to the right along the myosin rod. It binds to that head, initiating formation of the next ribbon segment to the right. The tightly bound myosin head on the left that had activated the ribbon detaches upon binding ATP and in response to the forces developed at the helix–ribbon boundary to its left. (d) *Force generation by actin*. As each subunit returns to the ground state (helicalization of the actin monomers that lie to the left) inorganic phosphate (P_i) is released. At this point tropomyosin is rebound by the helicalizing actin filament. Force generation is initiated when the bound head (green) on the left is forcibly detached by the helicalization from arriving from the left. Force is maintained between the 'grip site' of the helical actin monomers to the tropomyosin on the left and the bound myosin head to the right (green). Force development persists as long as there are r-actin monomers remaining in the segment between the helicalization front and the bound head to the right. Force generation in this segment ends when the last r-actin monomer returns to the ground state forcing (as it rotates) the detachment of the bound head that had been bearing the tension. The detached head (blue), binds ATP, hydrolyzes it and retains the products until another targetted monomer arrives from the left.

an additional increment of heat. Thus the chemical reactions that drive work production in shortening muscle fibres provide an increment of free energy above that in the initial isometric conditions (Fenn 1924, Fenn & Marsh 1935). This is known as the Fenn effect (Hill 1938). The extra energy released (which appears as heat + work) is nearly linear in ΔP, the reduction in load. Hill's force–velocity curve (Hill 1938), originally derived from these observations, contains two empirical constants which must be related in some fundamental manner to the molecular mechanism that converts chemical energy into mechanical work by the actomyosin ATPase(s).

The Hill equation is usually written:

$$(P + a_o)(V + b(T)) = K, \tag{3}$$

where P is the load, V the velocity of shortening, a_o, $b(T)$ and K are constants at a given temperature T. If P_o is the maximal isometric load (where $V = 0$) and V_{max} the velocity of shortening under no load ($P = 0$), then the constant K can be written as:

$$K = (P_o + a_o)b(T) = a_o(V_{max} + b(T)). \tag{3a}$$

Hill found that to a good approximation $a_o = P_o/4$, and $b(T) = V_{max}/4$. These constants are found to be applicable to a wide range of muscle types from different species. Substituting Hill's approximate values for a_o and $b(T)$ into Eqns (3) and (3a) gives a reduced form for the force–velocity curve:

$$\left[4\left(\frac{P}{P_o}\right) + 1\right]\left[4\left(\frac{V}{V_{max}}\right) + 1\right] = 5. \tag{3b}$$

This is a convenient expression for interpreting ATP-dependent length changes in actin as a means of transforming chemical energy to mechanical energy.

In this Markovian model, one molecule of ATP is hydrolyzed *by each myosin-head* involved in the initiation of a ribbon segment. The myosin head will remain attached to the actin monomer until the stepwise contraction of the ribbon segment lying on its I-band side is complete (on the left in Fig. 1), with the exception of the myosin heads which initiate the process at the entrance to the overlap zone. In the latter instance, the impulses transmitted through the I-band to these 'phase boundary' heads stimulate the dissociation of myosin, thereby allowing the first segment to begin to shorten. A postulate of this model is that the actomyosin links internal to the overlap zone, bearing the tension produced by adjacent ribbons segment on the I-band side, *are not expected to dissociate even in the presence of physiological concentrations of ATP*. The actomyosin link is only broken when the actin monomer to which the myosin head is bound twists off at the end of the stepwise contraction of the adjacent ribbon segment (to the left in Fig. 1). The breakage of these internal actomyosin links requires the free energy of hydrolysis of one ATP molecule *by an actin monomer* undergoing the r → h transition for each link broken. This is in contrast to the

situation in solution, where acto-S1, not mechanically constrained by the lattice, can be dissociated as a result of thermal agitation in the presence of ATP. As the free energy of one molecule of ATP is consumed during the r → h transition, an actin monomer exerts a force equal to f_{rh} as it twists free. Contracting muscle fibres moving small loads ($P \sim 0$ in Eqn 3) must still form and break actomyosin links even though very little or no work is being done against an external load. Therefore, we propose that the empirical constant a_o appearing in the Hill equation represents the force required to break actomyosin links during thin filament reptation through the lattice. This term is myosin dependent and is likely to be different for different myosin isoforms. Thus, a_o is equal to f_{rh}, assuming that ϵ, the efficiency of the transduction, is about the same for all actin monomers in a contracting ribbon segment.

The length of the overlap zone is enough to accommodate the number of ribbon segments required to produce the observed values for maximal isometric tension. For instance, a typical value for P_o is 200 pN per actin filament for frog sartorius muscle at 0 °C (Higuchi & Goldman 1991, 1995). From Eqn (2), with $\Delta G_{ATP} = 8.0 \times 10^{-20}$ J per molecule of ATP (Sellers & Homsher 1991) and $\Delta x_{rh} = 0.83 \times 10^{-9}$ m shortening per actin subunit (Schutt & Lindberg 1992), we find that $f_{rh} = 100$ pN. As $a_o = P_o/4$, it follows that the required 50 pN of force (i.e. $P_o/4$) can be produced if an actin subunit converts the energy of ATP hydrolysis to mechanical work at an efficiency $\epsilon = 50\%$. Therefore, the efficient generation of full isometric tension appears to require an average of only four ribbon segments per actin filament. A ribbon segment plus the adjacent helical region occupies a minimum length of 858 Å (2 × 429) (Fig. 1, Schutt & Lindberg 1992), and the maximum length of actin filament overlapping the myosin head lattice is 0.65 μm (= 6500 Å). Thus, the maximum number of active segments possible at full overlap is 7 (~6500/858) per half sarcomere.

As four ribbon segments appear to be sufficient to generate full isometric tension P_o and at least one must be present even at zero load for movement, the average number, $\overline{N}(P/P_o)$, of ribbon segments active per filament as a function of load P relative to maximal isometric tension P_o is given by:

$$\overline{N}\left(\frac{P}{P_o}\right) = 1 + \frac{4P}{P_o}. \quad (4)$$

This allows us to rewrite the Hill equation (Eqn 3b) as

$$\overline{N}\left(\frac{P}{P_o}\right)\left(\frac{4V}{V_{max}} + 1\right) = 5. \quad (5)$$

Therefore, the overall velocity of a filament depends inversely on the number of ribbon segments active along its length and *vice versa*, a kind of 'gearing' formula. Two factors contribute to this dependence, one geometric and the other mechanical. The first can be understood by recognizing that there is no movement of an actin filament in the I-band into the overlap zone while a ribbon segment is being initiated at the boundary of the overlap zone (see Fig. 2 of Schutt & Lindberg 1992). The greater the number of such 'pauses', the slower the contraction, purely as a consequence of the geometry of lattice-activated thin filament reptation. The second factor reflects the fact that the rate of the ribbon-to-helix transition itself depends on the load, as the rebinding of tropomyosin to actin at the helicalization front is influenced by tropomyosin tension.

The Fenn effect implies an element of reciprocity in the control of the mechanics of muscle contraction and the chemical reactions that drive it (Hill 1938). In this model, new ribbon segments must be continually initiated along moving actin filaments as actomyosin links are repeatedly being made and broken. The myosin ATPase cycle, beginning with the binding of an ATP molecule during the forced dissociation of the actomyosin link by the twisting actin filament, and ending with product release upon rebinding another actin monomer, is thus regulated by the propagation of r → h transitions along actin filaments, and cannot be described simply in terms of mass action (dissociation constants). Without loss of generality, an element of statistical uncertainty can be introduced into the process in the form of probability expressions for the binding of myosin heads at the entrance to the overlap zone (Fig. 2) and for the effect of Brownian motion of the heads (Ostap *et al.* 1995) on transitions in the overlap zone (Klebanov 1996).

DISTANCE MOVED PER ATP MOLECULE HYDROLYZED

For the purpose of discussion, we define a 'power stroke' (Fig. 1) as the stepwise contraction of 12 actin monomers in one segment in the ribbon form to the shorter helical form. While tension is being maintained, segments appear to move continuously toward the centre of the sarcomere from the I-band/A–band interface. This apparent translation of a segment (Fig. 2) arises because for every r-actin to h-actin transition at the helicalization front there occurs the reverse transition at the other end. Interior r-actin subunits remain stationary until reached by a helicalization front. At any instant, only a maximum of five actin subunits are actually shortening and pulling on tropomyosin in parallel per actin filament (Eqn 5).

The length of the actin power stroke is 10 nm, i.e. 12 × 0.83 nm. Thus, 12 ATP molecules are consumed by the actin ATPase during one power stroke.

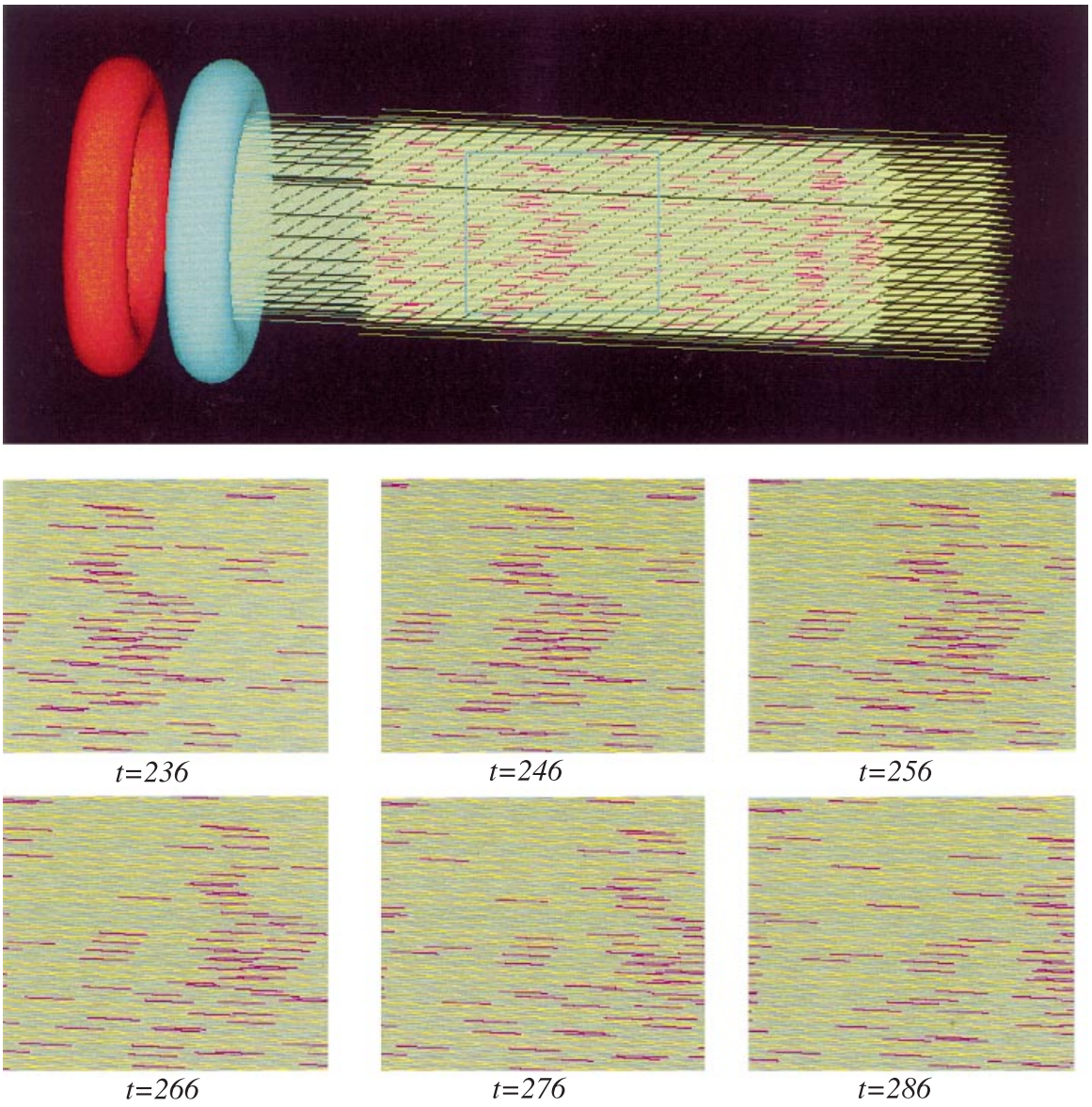


Figure 2 Muscle contraction as a process. Shown are the results of a computer simulation (Klebanov 1996) based on the actin power-stroke model of muscle contraction (Schutt & Lindberg 1992, Schutt *et al.* 1995a). The position of the Z-disk is marked by the red and turquoise toruses on the left. Thin filaments are in green and thick filaments are yellow. The simulation included 300 actin filaments reptating through the lattice according to the mechanism described in Fig. 1. The boundaries of the box over the overlap zone include ribbon 'segments' (purple) that 'move' towards the right. At the front of a segment, actin subunits undergo the helix-to-ribbon transition, while at the rear (on the left) ribbon-to-helix transitions occur. The net result of allowing the simulation to run for 50 ms is shown as temporal 'snapshots' in the six panels at the bottom. Notice that not all actin monomers have ribbons at exactly the same distance from the Z-disk. This is because the initiation of a ribbon at the entrance to the overlap zone is determined by a probability distribution (Klebanov 1996) that is a function of load (velocity). The Markovian nature of the process allows the ready summation of the total number of ATP molecules consumed for any given length change, given by Eqn (6). For any imposed load, the work done can be calculated and compared with published values (Kushmerick & Davies 1969), and force–velocity curves generated. A full description of the programme (Klebanov 1996) includes a detailed consideration of the role of twist in the actin filament.

Assuming (the argument does not depend on the exact number) that the energy expended on the actin filament by myosin heads to initiate ribbon formation requires an additional four ATP molecules (e.g. two S1 domains per myosin molecule acting on the actin filament), a total of 16 ATP molecules are consumed per power stroke. Therefore, N_{ATP}, the total number of ATP molecules hydrolyzed as a function of load and distance travelled by an actin filament is:

$$N_{ATP} = n_o \overline{N}\left(\frac{P}{P_o}\right) D, \qquad (6)$$

where, $\overline{N}(P/P_o)$ is the average number of actin ribbon segments along an actin filament moving a load P, given

by Eqn (4), D is the distance moved by the Z-line end of the actin filament in the non-overlap region, and n_o is the number of ATP molecules consumed per angstrom of movement by one segment of actin ($n_o = (16/100) = 0.16$ ATP molecules $Å^{-1}$) undergoing a power stroke.

In the isometric state, actin filaments hydrolyze ATP at a rate which is about 20% of that observed when muscle fibres are shortening and producing maximal work (Sellers & Homsher 1991). This implies that, even in the isometric state, where D in Eqn (6) might be thought to be zero, relative movement of actin and myosin filaments must be occurring to some extent. We interpret this as actin filaments 'churning' in the myosin lattice. When actin filaments are developing maximal force in the 'clamped state', fluctuations allowed by the elasticity of the Z-disk and length inhomogeneities in sarcomeres enable them to complete a certain number of power strokes, detach completely from myosin, and retract to new starting positions from which they can resume tension development. This would appear to explain why it is necessary to attribute the high variance observed in length–tension curves to length variations in actin filaments (Edman & Reggiani 1987, Schutt & Lindberg 1992), an otherwise perplexing observation in terms of the classical sliding filament mechanism.

THE RATE OF ATP HYDROLYSIS

The total rate of ATP hydrolysis by the force generators, the actomyosin turnover rate, can be calculated using Eqn (6). It is well known that maximal isometric tension increases with filament overlap and that the Hill equation governs the dependence of the velocity of shortening on load. Experimentally, free energy changes are measured for fibres contracting at a constant velocity, so that tension is the dependent variable. In general, for actin filaments moving through the myosin lattice by our proposed mechanism, the rate of ATP hydrolysis in moles per second, η_{ATP}, is given by:

$$\eta_{ATP} = \frac{1}{N_A}\frac{dN_{ATP}}{dt} = \frac{n_o}{N_A}\left(D\frac{dN(P)}{dt} + N(P)\frac{dD}{dt}\right), \tag{7a}$$

where N_{ATP}, n_o, D, and $N(P) = N(P/P_o)$ are defined by Eqn (6) and N_A is Avogadro's number. Now, for an *isovelocity* contraction $dN(P)/dt = 0$ and $N(P)$ can be replaced by $N(V)$ using Hill's equation (Eqn 5). As $V_f = dD/dt$ is the average velocity of an actin filament.

$$\eta_{ATP} = \frac{n_o}{N_A}N(V)V_f \text{ moles s}^{-1} \text{ per actin filament.} \tag{7b}$$

The speed of translocation depends on the facility with which actomyosin links are broken as the attached actin monomers undergo the ribbon-to-helix transition, rebinding tropomyosin in the process (see Fig. 1). As tropomyosin is under tension, 'twisting free' of the myosin links by an attached actin monomer is more difficult at higher loads, and filament translocation is slowed. The dependence of the velocity of propagation (not the same as the velocity of shortening) on load can be expressed as:

$$V_f = V_{max}\, e^{-\gamma p} = V_{max}\, e^{-p/p_0}, \tag{7c}$$

where γ is a parameter describing the degree to which a given myosin subtype resists the 'twisting off' of attached actin monomers undergoing ribbon-to-helix transitions. Computer simulations (Klebanov 1996, exemplified here in Fig. 2) suggest that a good approximation for skeletal muscle is $\gamma = 1/p_0$.

The unloaded velocity of shortening can be expressed in terms of parameters describing the ribbon → helix transition:

$$V_{max} = k_{rh}\Delta x_{rh}, \tag{7d}$$

where k_{rh} is the intrinsic rate of the ribbon → helix transition and Δx_{rh} the length change per monomer. These expressions are useful for interpreting results from *in vitro* motility assays when modified or mutated actin molecules are used, especially those affecting the hinge-like motions in actin and/or the release of phosphate from actin.

Experimentally, the rate of ATP hydrolysis during shortening of frog sartorius muscle at 0 °C at $V_{max}/2$ is 1.60 ± 0.23 μmol g^{-1} s^{-1}, expressed in terms of grams of muscle mass. The maximal velocity of shortening per *half-sarcomere* is 1.42 μm s^{-1}. As there are 10^{15} actin filaments per gram of muscle tissue (N_f = [4 actin filaments/thick filament] ÷ [600 S1 heads/thick filament] × [0.28 μmol S1 heads g^{-1}]), the observed rate of ATP hydrolysis at $V_{max}/2$ is 1.6×10^{-21} mol s^{-1} per actin filament.

Comparing this experimental observation with our model, it appears that maximum efficiency is achieved when each actin filament in the overlap zone has about one active ribbon segment 'moving' along its length, as according to Eqn (7b) the calculated ATPase rate is:

$$\begin{aligned}\eta_{ATP} &= \left(\left[0.16 \times 10^{10}\,\text{m}^{-1}\right] \div \left[6 \times 10^{23}\right]\right) \\ &\quad \times (1.67)\left(0.71 \times 10^{-6}\,\text{m s}^{-1}\right)(0.61) \\ &= 1.8 \times 10^{-21} \text{ moles s}^{-1} \text{ per actin filament.}\end{aligned} \tag{7e}$$

This suggests that the assumed efficiency of 50% for the ribbon-to-helix transition (Eqn 5) is too conservative, as only half as many ribbon segments (1.67/2) are needed under these conditions. Thus, an actin-based model can, in principle, account for the 100% thermodynamical efficiency at $V_{max}/2$ reported by Kushmerick & Davies (1969). The ATPase rate for

isometrically contracting muscle fibres depends on the rate of actin filament 'churning' in the lattice (i.e. as actin filaments shorten locally in this churning mode, $V \neq 0$. The extent of the churning action can be estimated from Eqns 7 (b–d), which accounts for the fact that the measured ATPase rate is about one-fifth that for fibres contracting at a velocity of $V_{max}/2$: that is, the isometric state represents the situation where the ribbon-to-helix transition is maximally restrained by the load.

UNEXPLAINED ENTHALPY OF SHORTENING

Energy balance studies have shown that a significant fraction (50%) of the total enthalpy released during shortening at V_{max} cannot be accounted for by the chemical reactions which occur simultaneously with shortening (Kushmerick & Davies 1969, Irving & Woledge 1981, Homsher *et al.* 1981, Homsher 1987). On returning to an isometric state following the shortening, it has been shown that an extra actomyosin ATP hydrolysis activity makes up the enthalpy deficit. The explanation given by the actin power-stroke model for this phenomenon is that there are more ribbon segments active in the overlap zone prior to shortening than needed to move the lightly loaded filament, even though they may be churning rather slowly in the isometric state. Therefore, when the load on the fibre is dropped, the free energy of the unnecessary ribbon segments is converted to heat in futile power strokes as the fibre readjusts to the lower load requirements during shortening. There is no unexplained enthalpy for shortening velocities less than $V_{max}/2$, because apparently muscle fibres can adjust efficiently to the reduced load when the reduction in the number of ribbon segments is not too great. However, if the fibre is slackened too quickly with no load to brace against, heat is generated because the motions do not result in useful work.

The unexplained enthalpy is equal to 6.5 mJ g^{-1} of muscle tissue. The concentration of actin monomers in the A-band at full overlap is 4.2×10^{-7} mol g^{-1} tissue (this is based on an estimate of 0.28 μmol of myosin S1 heads g^{-1} tissue, and the ratio of 1.5 actin monomers per myosin head in the overlap zone for this model). The unexplained enthalpy, when expressed in terms of actin, thus amounts to 2.6×10^{-20} J per actin monomer in the A-band. As 30% of the actin subunits are in the r-state at full overlap during an isometric contraction, the unexplained enthalpy is 8.6×10^{-20} J per r-actin subunit. This is close to the amount of energy expected on the basis of the actin power-stroke model, as each r-actin monomer hydrolyzes one molecule of ATP (8×10^{-20} J per molecule).

This calculation suggests that an excess of metastable r-actin, present in the isometric state prior to shortening, is converted to the ground state during the ensuing contraction. It is consistent with the observation that the amount of unexplained enthalpy is proportional to the degree of overlap in the isometric state established before the shortening. The unexplained enthalpy of shortening limits the range of plausible theories of muscle contraction (Homsher 1987), because it implies that energy can be stored in one phase of the contraction to be released later in another. The concept of the metastable ribbon state of actin allows a straightforward molecular explanation how this can be achieved.

COMPARISON WITH RESULTS FROM *IN VITRO* MOTILITY ASSAYS

Results from *in vitro* 'motility assays' (Yanagida *et al.* 1985, Toyoshima *et al.* 1990, Uyeda *et al.* 1991), in which actin filaments move on lawns of immobilized myosin heads, can be used to estimate the ratio of the distance travelled by an unloaded actin filament to the number of ATP molecules hydrolyzed. It has been reported (Toyoshima *et al.* 1990) that translocation of a total length of 86 m of filamentous actin on a myosin-coated surface is accompanied by the hydrolysis of 3.4×10^{11} molecules of ATP per second. This amounts to 11 ATP molecules hydrolyzed per actin monomer per second. Using this value and solving for D using Eqn (6) with $P = 0$ (the filaments are unloaded in the motility assay) gives a velocity of 6.9 nm s^{-1} per monomer. As there are 370 actin monomers per micron of filament, this calculation yields a velocity of shortening of 2.6 μm s^{-1} (i.e. 370×6.9 nm s^{-1}), which compares favourably with the measured value of 3.6 μm s^{-1} reported by Toyoshima *et al.* (1990), where the average filament length was slightly longer than 1 μm. To avoid invoking unreasonably long myosin power strokes to explain their data, Toyoshima *et al.* introduced the idea of short myosin 'active duty cycles' during which attached myosin heads impart 'quantal velocities' to actin filaments. However, our analysis suggests that these results can be explained by a simple relationship involving the velocity of shortening, where ATP hydrolysis is coupled to repetitive local changes of length within actin filaments.

An apparent contradiction to our model is the observation that simply changing the source of the myosin used can lead to a 3-fold increase in the force on a glass microneedle attached to the end of an actin filament moving on a myosin lawn (Van Buren *et al.* 1994). In our model, myosin heads serve not only to activate the actin force generators, but also as traction points for the crawling filaments. Changing the source of the

myosin from skeletal to smooth muscle changes the traction properties of these links (see Eqn 7c). The tighter they hold, the greater the torque needed to 'twist free'. The greater the resistance to this 'twisting free' of myosin, or forced dissociation of the actomyosin bond, the slower the velocity of shortening, as observed. Actin force generators working in series (not in parallel because there is no tropomyosin) easily produce the force required initially to bend the needle because it offers little resistance. Individual actin monomers continually bind and 'twist free' of the myosin attachment points as the filament crawls forward, further bending the needle. If the myosin heads cannot hold, or there are too few of them, the filament will stall. The maximum tension produced by an actin filament pulling on the microneedle depends, therefore, not just on the maximum force produced by an actin monomer, but on the total traction afforded by the myosin heads. This explains how tension proportional to overlap can be obtained, even in the absence of tropomyosin, with translocating actin filaments in these assays.

Our analysis suggests that inconsistencies with the cross-bridge theory appear when attempts are made to extrapolate properties (such as actomyosin dissociation constants) observed with isolated myosin molecules to the ordered environment found in sarcomeres. For example, overly long step lengths are inferred when the sarcomere-like organization is more or less preserved in experiments, as in skinned fibres (Higuchi & Goldman 1991) or with reconstituted thick filaments (Saito *et al.* 1994). The use of laser-trapped microspheres attached to the ends of actin filaments to measure forces generated by individual myosin motor molecules (Finer *et al.* 1994, Molloy *et al.* 1995) raises new questions concerning the actomyosin interaction (Block 1995, 1996). Measurements on individual molecules under unloaded conditions appear to be in general agreement with the expectations of the cross-bridge theory for force and displacement, but our model also predicts, under the conditions of these experiments, that impulses would be imparted to actin during the encounter of a myosin molecule with an actin filament to activate the actin nucleotide exchange reaction. The 50–100 pN forces generated by actin, however, should not be observable (and may not even be present) because the compliances are too high in these experiments.

COMPARISON WITH RESULTS USING SKINNED MUSCLE FIBRES

Our contention is that orderly and efficient generation of force, without undue molecular crowding or motion, is only possible when chemomechanical transduction by actin thin filaments and myosin thick filaments is coordinated in a natural way by tropomyosin molecules under tension in a symmetric lattice. It is essential, if phenomena such as the Fenn effect are to be explained, that 'motor properties' be examined under load-bearing conditions, as has been achieved by Higuchi & Goldman (1991, 1995). The distance travelled by an actin filament per ATP molecule can be determined from experiments carried out on skinned muscle fibres into which a controlled amount of ATP is released from caged-ATP. These experiments yield an estimate for the length of the myosin power stroke of 60.0 nm, leading the authors to suggest that multiple power strokes would be needed to account for the high thermodynamical efficiency of contracting muscle.

These measurements can be compared with the expectations of the actin power-stroke model using Eqn (6). The slopes calculated from this formula giving the distance *an actin filament* moved per ATP hydrolyzed at the three loads used in the Higuchi & Goldman (1991, 1995) experiments are 5.0, 3.1, and 2.3 Å per ATP, for loads of $0.07P_o$, $0.25P_o$ and $0.42P_o$, respectively. The result of this calculation agrees well with the relative values of the slopes (0.84, 0.50, and 0.30) measured graphically from Fig. 1B of their paper (15) where the abscissa is expressed in terms of the concentration of caged ATP hydrolyzed. Converting the abscissa values to absolute numbers of ATP molecules consumed in the overlap zone, yields 1.3, 0.8, and 0.6 Å per ATP molecule for the three conditions. This conversion is based on the average volume occupied by a moving actin filament in the hexagonal lattice of myosin thick filaments. The volume is equal to the product of the area of the equilateral triangle having side 450 Å and the average length of the overlap of thick and thin filaments during the shortening process ($V = [1/2]\,[0.866]\,[450\ \text{Å}]^2 \times O_L = 10^{-15}\ \text{m}^2 \times O_L$; the overlap O_L is 0.57, 0.52, and 0.47 μm for the three loading conditions, 1 $\mu\text{m} = 10^{-6}$ m).

Although our model successfully predicts the trend in the data, the observed values for shortening distance per ATP molecule appear to be consistently low by a factor of about 4 compared with the predictions of Eqn (6) (based on 50% thermodynamical efficiency), which is not surprising as the efficiency of chemomechanical transduction by actomyosin in skinned fibres is unlikely to be as high as the values obtained under physiological conditions, owing to a variety of mechanical and chemical causes. For example, under normal conditions, the concentration of ATP is maintained at a steady level by the phosphocreatine buffering system. The experiments analysed here necessarily involved a constant decrease in the available pool of ATP molecules as the movement gradually subsided. Thus, an efficiency of 10–15% (compared with 2–3% deducible

from these data under the assumptions of tightly coupled cross-bridge theories) is reasonable considering the deviations from optimal conditions.

It might seem paradoxical that data apparently suggesting myosin power strokes of hundreds of Angstroms per ATP molecule (Higuchi & Goldman 1991, 1995) can be explained with an actin conformational change of only 0.83 nm per ATP molecule. The source of the apparent confusion is that, when an actin filament slides some distance D_m in the cross-bridge model, all attached myosin heads move with the actin filament. As hundreds of attached heads are assumed to be generating a total force $P_o = 200$ pN, each contributes $f_m = 1$–2 pN. This inevitably gives rise to long strokes per ATP molecule (Sellers & Homsher 1991), as $\Delta G = 8.0 \times 10^{-20}$ J $= f_m \times D_m$, if the process of energy conversion is to be at all efficient. In the actin-based theory, actin molecules pull only a few at a time, but each exerts a much greater force $f_{rh} = 50$–100 pN and over a much shorter distance.

MECHANICAL WORK PERFORMED DURING RAPID REGENERATION OF TENSION

The mechanism of force development by actomyosin in isometrically contracting muscle fibres can be probed by examining the effect of a rapid series of quick releases at various times during tension recovery following an initial longer conditioning release. Recently, it has been shown that the force generators in muscle fibres are able to rebend the elastic myosin bridges in less than 20 ms. This is too short a time for an attached myosin cross-bridge to complete its biochemical cycle (Irving *et al.* 1992). These experiments have generated considerable speculation on the nature of cross-bridge force generation (Huxley 1992, Lombardi *et al.* 1992, Chen & Brenner 1993, Piazzesi & Lombardi 1995, Huxley & Tideswell 1997, Linari *et al.* 1997).

We believe that the paradox of too-rapid tension regeneration arises because the X-ray diffraction measurements used to monitor the time course of the elementary force-generating events have been misinterpreted. Irving *et al.* (1992) and Lombardi *et al.* (1992) assumed that the intensity drop in the 14.3 nm meridional reflection indicated synchronized movements of the myosin heads, the usual interpretation based on the cross-bridge theory (Huxley & Kress 1985). However, it has been pointed out that actin filaments in the ribbon conformation are commensurate with the myosin thick filament 14.3 nm longitudinal spacings and that conformational changes in actin could account for a number of perplexing X-ray results (Schutt & Lindberg 1992). Thus, the drop in intensity of the 14.3 nm reflection could be indicative of ribbon-to-helix transitions in actin filaments. Actin force generators themselves do not need repriming after a rapid drop in tension and recovery can be very rapid. In a quick release, a myosin head attached to a segment can return to its elastic minimum without detaching. The free energy available for performing mechanical work, present in the form of r-actin monomers, is not dissipated during a tension drop, only the elastic component in the myosin head. Actin ribbons present at the time of the release can redevelop force between tropomyosin and an attached myosin head by releasing an inorganic phosphate molecule at the ribbon–helix boundary. Tension rises in the muscle fibre in the time required to rebend the bridges, namely the distance of release divided by the velocity of the helicalization front given by Eqn (5), assuming that the rate of tension recovery directly relates to the velocity of shortening.

In the quick release experiments of Irving *et al.* (1992) on intact frog skeletal muscle fibres, an average force of 0.63 $P_o/2$ is exerted per actin filament on rebending the myosin bridges a distance of 44.3 Å to reestablish the tension during recovery. As P_o is reported to be 206 pN per actin filament in these experiments, the work performed in bending the myosin bridges is 2.9×10^{-19} J. Using Eqn (6), modified to leave out the myosin ATPase as no bridges are broken and no new ribbon segments are initiated, the number of ATP molecules consumed in delivering a force equal to $0.315P_o$ over a distance of 44.3 Å is 11.9. Therefore, the efficiency of the actin power stroke in tension recovery, equal to the work performed divided by the input energy, is 30% (2.9×10^{-19} J/($11.9 \times 8.0 \times 10^{-20}$ J)).

In terms of the cross-bridge model it was concluded that myosin bridges either worked at 4% efficiency or executed multiple power strokes per ATP molecule consumed (Irving *et al.* 1992, Lombardi *et al.* 1992). It is difficult to reconcile multiple myosin power strokes per ATP molecule with the explanation for the Fenn effect given by Eisenberg & Hill (1985) which requires that the strain energy stored in a myosin head be slowly relieved as the actin filament to which it is attached slides by. The heads only detach at the end of the myosin power stroke. Multiple power-stroke theories seem to require that a *detached* myosin head not relax to a state of lower strain energy even though it is subjected to considerable thermal buffeting. In terms of an actin-based Markov process, these questions are simply resolved without the need to neither abandon the principle of tight chemomechanical coupling (Eisenberg & Hill 1985) nor to tolerate large inefficiencies during free energy transduction.

ACTOMYOSIN ATP*ase* IN SOLUTION

The central question posed by our model is whether there are two ATPases operating in the filament lattice of muscle fibres: the well-established actin-activated myosin ATPase and the more elusive myosin-activated actin ATPase suggested by our analysis. It has long been known that ATP bound to monomeric G-actin exchanges rapidly with ATP in solution, but that ADP bound to f-actin exchanges slowly. Asakura *et al.* (1963) reported that sonic vibration of f-actin results in exchange and hydrolysis of nucleotide with characteristics indicating that the major part of the ATP is split into fragments of activated f-actin rather than on reannealing the fragments into long filaments. This led to the suggestion that external forces, in this case arising from sonication, applied to actin filaments could result in the formation of stretched filaments with partially interrupted longitudinal bonds. These stretched regions of the filament could more easily exchange nucleotide and hydrolyze ATP upon the return to the original filament structure. Observations that the actomyosin interaction can be coupled to the exchange of the nucleotide on f-actin have been reported repeatedly (Barany *et al.* 1966, Szent-Györgyi & Prior 1966, Szent-Györgyi 1968, Strzelecka-Golaszewska *et al.* 1975), but the results and conclusions drawn have varied widely between laboratories (Martonosi *et al.* 1960, Moos & Eisenberg 1970, Appenheimer *et al.* 1972, Ward 1979). Furthermore, Yanagida & Oosawa (1978) showed that actin monomers rotated by 10° when heavy meromyosin was introduced into myosin-free single fibres, monitored through changes in polarized fluorescence from epsilon-ADP, which appeared to be exchanging as the experiment proceeded.

The discovery by Lymn & Taylor (1971) that actin accelerates product release from myosin, resulting in a greatly increased ATPase activity, reinforced the view that the rate of ATP hydrolysis on actin-activated myosin alone, when measurements were extrapolated to infinite actin concentrations, was of a magnitude sufficient to account for the steady-state turnover of ATP in muscle (Eisenberg & Moos 1970). The observation that the reassociation rate of actin and myosin equals the rate of the actin-activated myosin ATPase for subfragment-1 and HMM (White & Taylor 1976) apparently eliminated the need to investigate further any possible participation of the actin ATPase in muscle contraction. However, the possibility that these experiments may have missed an actin ATPase is indicated by the fact that higher levels of actin nucleotide exchange are observed in cases where a high degree of sarcomeric organization is maintained. For example, a significant exchange of bound nucleotide, showing dependence on calcium in the presence of tropomyosin–troponin, occurs during superprecipitation of reconstituted myofibrils (Szent-Györgyi & Prior 1966, Szent-Györgyi 1968, Strzelecka-Golaszewska *et al.* 1975). Also, when frogs are injected with tubocurarine and ^{14}C-glucose to label the ATP pool, and subsequently subjected to a brief electrical impulse, a large increase in the specific activity of the actin-bound ADP is observed, suggesting a change in the physical state of actin during muscle contraction (Cheesman & Priston 1972).

It is recognized that actomyosin ATPase kinetic experiments in solution are difficult to interpret, and extrapolation to physiological conditions is problematical (Sleep & Hutton 1980, Hibberd & Trentham 1986). In particular, steps in the reaction which are influenced by load and speed of shortening, as implied by Eqns (5) and (6), cannot in principle be reproduced with dispersed molecules in solution. Solution studies of the acto-S1 ATPase cannot be expected to reveal the full extent of an actin ATPase whose mechanism is part of a highly coordinated process occurring within structured muscle fibres performing work. Evidence from kinetic experiments indicating the existence of two pathways of ATP hydrolysis in the actomyosin system has never been interpreted in terms of an actin ATPase. Instead, in the case of measurements of ^{18}O isotope exchange between phosphate and water (Hibberd *et al.* 1985), or ATP and water (Bowater *et al.* 1989), with contracting skinned fibres, the existence of a second ATPase was attributed to the effects of myosin heads being strained in the lattice. However, as strain energy would be relieved upon detachment (Eisenberg & Hill 1985), this explanation appears to be inconsistent with cross-bridge models of muscle contraction that necessarily invoke multiple attachment/detachment cycles to account for the very long myosin step length data (Higuchi & Goldman 1991, 1995)

In the actin power-stroke model of muscle contraction, tropomyosin plays a key role in the biochemistry of the actin and myosin ATPases by controlling P_i-release from the actin filaments (see Fig. 2). When AMPPNP is substituted for ADP in F-actin, the Ca^{2+}-regulated myosin-S1 ATPase is strongly enhanced, indicating that occupation of the terminal phosphate binding site of the actin-bound ATP is important for regulating myosin (Miki 1990). Rosenfeld & Taylor (1987) reported what appeared to be a *second* myosin ATPase that could be unmasked in the presence of tropomyosin under low salt conditions, consistent with the proposed role of tropomyosin in the theory presented here. Low-salt conditions are known to strengthen the bond between actin and myosin in the presence of ATP, which should lead to an increased rate of ATP hydrolysis by actin filaments according to our model. Recently, larger than expected 'P_i-bursts' were observed with dispersed myofibrils as compared

with acto-S1 even under near-physiological ionic strength (Herrmann *et al.* 1992). So, it should no longer be considered certain that the myosin ATPase accounts for the sum total of the ATPase activity in contracting fibres.

The fact that isometric tension declines with an increase in P_i in the millimolar range of concentrations (Brandt *et al.* 1982, Hibberd *et al.* 1985, Kawai & Halvorson 1991, Dantzig *et al.* 1992) is consistent with the actin power-stroke model. Our studies on the sensitivity of P_i release from freshly polymerized actin filaments to weak shear forces (Pinaev *et al.* 1995), and on the deformability of actin ribbons in crystals in response to osmotic pressure (Chik *et al.* 1996), suggests that bound P_i acts as a negative switch in the ribbon-to-helix transition. The activation barrier for P_i release appears to be about 1 kT. Ribbon-bound P_i is released as the helicalization front passes through, accompanied by the rebinding of tropomyosin, consistent with the biochemical studies discussed in the preceding paragraph. A ribbon segment, bound between a myosin molecule on one end and a tropomyosin on the other, would be metastably maintained by weak cooperative interactions with P_i, just as alpha-helices or other 'zippered' systems, such as the TMV assembly intermediate (Caspar & Namba 1990), are stabilized by protons or Ca^{2+} ions. Raising the P_i concentration 3-fold would inhibit P_i release at the helicalization front, leading to the observed decline in force development.

As actomyosin in solution quickly dissociates in the presence of ATP, many studies of the actomyosin ATPase rely on the use of zero-length cross-linking of myosin subfragment S1 to actin by EDC (1-ethyl-3-[3-dimethylaminopropyl]) carbodiimide. Using this approach, Kasprzak (1994) has shown that myosin cross-linked to actin monomers greatly stimulates the actin ATPase. Differences in actin filament structure induced by an ATPase activity in neighbouring cross-linked myosin heads have been observed by electron microscopy (Craig *et al.* 1985, Applegate & Flicker 1987). Evidence that these changes are vectorially propagated along actin filaments has been obtained by Ando (1989), who analysed the biphasic behaviour of the actomyosin ATPase as a function of the ratio of cross-linked myosin to actin in the presence of varying amounts of soluble myosin subfragment S1.

COOPERATIVITY IN THE ACTIN FILAMENT

The allosteric transmission of conformational changes along actin filaments, linked to ATP exchange and hydrolysis on both actin and myosin, is the motivating idea behind viewing of muscle contraction as a Markov process. Although it is intrinsically difficult to infer from the properties of the isolated parts of a self-coordinating piece of machinery how it works, there does exist for the case of muscle several lines of evidence for the kind of cooperativity required for such a model.

Bremel & Weber (1972) discovered particular conditions that favoured a 3- to 4-fold increase in the ATPase activity of tropomyosin-regulated acto-S1 in solution. This 'potentiation' phenomenon has been taken as evidence for thin filament-mediated cooperativity in the binding of myosin heads during the transition from the relaxed to the fully activated state (Guth & Potter 1987, Zot & Potter 1989, Swartz & Moss 1992, Schaertl *et al.* 1995). There is a considerable body of biophysical data suggesting that the binding of myosin initiates cooperative changes in actin filament structure (Yanagida *et al.* 1974, Loscalzo *et al.* 1975, Yanagida & Oosawa 1978, Prochniewicz-Nakayama & Yanagida 1982, Borovikov & Gusev 1983, Prochniewicz-Nakayama *et al.* 1983, Kasprzak *et al.* 1988, Orlova & Egelman 1997). The degree of cooperativity between functional groups, defined as seven contiguous actin subunits spanned by one tropomyosin molecule and bound to one troponin molecule (Murray & Weber 1980), is quite high as evidenced by Hill coefficients of between 4 and 8 derived from tension–pCa curves (Brandt *et al.* 1984, Metzger & Moss 1991). Interfering with the continuous polymer of head-to-tail linked tropomyosin chains by truncation of chain lengths significantly decreases, but does not abolish, cooperative S-1 binding (Pan *et al.* 1989), consistent with the view that the transmittal of conformational changes along actin filaments within a functional group may be important in the cooperative interactions between adjacent functional groups (Hill *et al.* 1980, Butters *et al.* 1993). Recently, gelsolin-induced cooperative changes in unregulated F-actin have been observed, although they are smaller in magnitude than those predicted here (for a review, see Egelman & Orlova 1995, see also Orlova *et al.* 1995, Prochniewicz *et al.* 1996).

The physiological importance of thin filament cooperativity is underscored by the fact that the steepness of the tension–pCa curve is sharply reduced after partial extraction of troponin C from thin filaments (Brandt *et al.* 1984, Moss *et al.* 1985, Metzger 1995). The role of Ca^{2+} regulated tropomyosin may not be merely to expose myosin binding sites on actin thin filaments (Chalovich *et al.* 1981, Schaertl *et al.* 1995). The mechanical parameters affecting the kinetics of force redevelopment are strongly influenced by the level of thin filament activation either by Ca^{2+} (Brenner 1988) or with activating factors (Martyn *et al.* 1994). Furthermore, the Ca^{2+} dependence of the rates of tension recovery during phase 2 suggests sensitivity of the elementary force generating transitions to the number of attached heads and/or the degree of thin filament

activation (Martyn & Chase 1995). In principle, the actin power-stroke model can account for these observations, as well as the observation that the ratio of stiffness to force is elevated at submaximal isometric levels compared with maximal activation (Martyn & Gordon 1992), as passive stiffness resides in the myosin heads, while forces generated within actin filaments create tension in tropomyosin. Moreover, the striking fact that the product Kh appearing in the equation describing the rate of tension recovery following a quick release (Huxley & Simmons 1971) is independent of Ca^{2+} activation (Martyn & Chase 1995) is simply explained by the actin power-stroke model, because all force generators independently produce constant forces, according to Eqn (1). These characteristics of muscle suggest that there is feedback between tension on the thin filaments and the rates of the biochemical reactions producing it, again pointing to the fundamental difficulty of extrapolating results from solution studies, where loads cannot be applied, to events in the sarcomere, and to the need for theories incorporating these basic observations.

CONCLUSIONS

In a Markovian description of muscle contraction, all biochemical processes are governed by a single rate: the velocity of helicalization fronts moving towards the centre of the sarcomere. This rate depends on the external load, which is transmitted to the helicalization fronts by tropomyosin. Several paradoxes, associated with energy production and mechanical behaviour of the actomyosin system, such as overly long myosin power strokes and the unexpectedly rapid rate of tension regeneration following a quick release, can be resolved with the actin power-stroke model. Our analysis of ATP usage in loaded skinned muscle fibres is consistent with the concept that ATP exchange and hydrolysis on actin filaments increases with the load. The success of this analysis in resolving these paradoxes, without incurring unreasonable losses in thermodynamical efficiency, and the fact that a single process determines the rates of all force-generating biochemical reactions, lend credence to the idea that hydrolysis of ATP on actin is one of the principal sources of free energy driving chemomechanical transduction in muscle. We propose this as the essence of the Fenn effect.

This paper is dedicated to D. L. D. Caspar to mark the occasion of his 70th birthday. We are deeply grateful to Walter Kauzmann for his patient effort in scrutinizing our arguments. We also thank Jim Myslik, Mike Rozycki, Stephen Miller, Daniel Klebanov, Rebecca Page, Constantine Kreatsoulas, Roger Karlsson and George Pinaev for fruitful discussions. This work was supported by a grant to C. E. S. from the N. I. H. (GM44038) and by grants to U. L. from the Swedish National Research Council and the Swedish Cancer Fund. Special grants from the Swedish National Research Council, the Swedish Cancer Fund, and Granholms Foundation enabled C. E. S. to spend a significant portion of a sabbatical semester at Stockholm University as a Visiting Scientist.

REFERENCES

Ando, T. 1989. Propagation of acto-S1 ATPase reaction-coupled conformational change in actin along the filament. *J Biochem* **105**, 818–822.

Appenheimer, M., von Chak, D. & Weber, H. 1972. Der Nucleotidaustausch des F-actin in kontrahierten, erschlafften und totenstarren Fibrillen in seiner Bedeutung für den molekularen Mechanismus der Muskelkontration. *Biochim Biophys Acta* **256**, 681–694.

Applegate, D. & Flicker, P. 1987. New states of acto-myosin. *J Biol Chem* **262**, 6856–6863.

Asakura, S., Taniguchi, M. & Oosawa, F. 1963. Mechano-chemical behaviour of F-actin. *J Mol Biol* **7**, 55–69.

Barany, M., Tucci, A.F. & Conover, T.E. 1966. The removal of bound ADP of F-actin. *J Mol Biol* **19**, 483–502.

Block, S.M. 1995. One small step for myosin. *Nature* **378**, 132–133.

Block, S.M. 1996. Fifty ways to love your lever: myosin motors. *Cell* **87**, 151–157.

Borovikov, Y.A. & Gusev, N.B. 1983. Effect of tropon-tropomyosin complex and Ca^{++} on conformational changes in F-actin induced by myosin subfragment-1. *Eur J Biochem* **136**, 363–369.

Bowater, R., Webb, M.R. & Ferenczi, M.A. 1989. Measurement of the reversibility of ATP binding to myosin in calcium-activated skinned fibers from rabbit skeletal muscle. *J Biol Chem* **264**, 7193–7201.

Brandt, P.W., Cox, R.N., Kawai, M. & Robinson, T. 1982. Regulation of tension in skinned muscle fibres. Effect of cross-bridge kinetics on apparent Ca^{2+} sensitivity. *J Gen Physiol* **79**, 997–1016.

Brandt, P.W., Diamond, M.S. & Schachat, F.H. 1984. The thin filament of vertebrate skeletal muscle co-operatively activates as a unit. *J Mol Biol* **180**, 379–384.

Bremel, R.D. & Weber, A. 1972. Cooperation within actin filament in vertebrate skeletal muscle. *Nature* (*London*) *New Biol* **238**, 97–101.

Brenner, B. 1988. Effect of Ca^{2+} on cross-bridge turnover kinetics in skinned single rabbit psoas fibers: implications for regulation of muscle contraction. *Proc Natl Acad Sci USA* **85**, 3265–3269.

Butters, C.A., Willadsen, K.A. & Tobacman, L.S. 1993. Cooperative interactions between adjacent troponin-tropomyosin complexes may be transmitted through the actin filament. *J Biol Chem* **268**, 15565–15570.

Caspar, D.L. & Namba, K. 1990. Switching in the self-assembly of tobacco mosaic virus. *Adv Biophys* **26**, 157–185.

Chalovich, J.M., Chock, P.B. & Eisenberg, E. 1981. Mechanism of action of troponin-tropomyosin: inhibition of actomyosin ATPase activity without inhibition of myosin binding to actin. *J Biol Chem* **256**, 575–578.

Cheesman, D.F. & Priston, A. 1972. Exchange of actin-bound nucleotide in brief electrical stimulation of muscle. *Biochem Biophys Res Comm* **48**, 552–558.

Chen, Y. & Brenner, B. 1993. On the regeneration of the actin-myosin power stroke in contracting muscle. *Proc Natl Acad Sci USA* **90**, 5148–5152.

Chik, J., Schutt, C.E. & Lindberg, U. 1996. The structure of an open state of β-actin at 2.65 Ångstroms resolution. *J Mol Biol* **263**, 607–623.

Cooke, R., White, H. & Pate, E. 1994. A model for the release of myosin heads from actin in rapidly contracting muscle fibers. *Biophys J* **66**, 778–788.

Cordova, N.J., Ermentrout, B. & Oster, G.F. 1992. Dynamics of single-motor molecules: the thermal ratchet model. *Proc Natl Acad Sci USA* **89**, 339–343.

Cox, D.R. & Miller, H.D. 1965. The Theory of Stochastic Processes. London: Methuen & Co. Ltd.

Craig, R., Greene, L.E. & Eisenberg, E. 1985. Structure of the actin-myosin complex in the presence of ATP. *Proc Natl Acad Sci USA* **82**, 3247–3251.

Dantzig, J.A., Goldman, Y.E., Millar, N.C., Lacktis, J. & Homsher, E. 1992. Reversal of the crossbridge force-generating transition by photoregeneration of phosphate in rabbit psoas muscle fibres. *J Physiol* **451**, 247–278.

Edman, K.A.P. & Reggiani, C. 1987. The sarcomere length-tension relation determined in short segments of intact muscle fibres of the frog. *J Physiol* **385**, 709–732.

Egelman, E.H. & Orlova, A. 1995. New insights into actin filament dynamics. *Curr Opin Struct Biol* **5**, 172–180.

Eisenberg, E. & Hill, T.L. 1985. Muscle contraction and free energy transduction in biological systems. *Science* **227**, 999–1006.

Eisenberg, E. & Moos, C. 1970. Actin activation of heavy meromyosin adenosine triphosphatase. Dependence on adenosine triphosphate and actin concentration. *J Biol Chem* **245**, 2451–2456.

Fenn, W.O. 1924. The relation between the work performed and the energy liberated in muscular contraction. *J Physiol* **58**, 373–395.

Fenn, W.O. & Marsh, B.S. 1935. Muscular force at different speeds of shortening. *J Physiol* **85**, 277–297.

Finer, J.T., Simmons, R.M. & Spudich, J.A. 1994. Single myosin molecule mechanics: piconewton forces and nanometre steps. *Nature* **368**, 113–119.

Ford, L.E., Huxley, A.F. & Simmons, R.M. 1981. The relation between stiffness and filament overlap in stimulated frog muscle fibres. *J Physiol* **311**, 219–249.

Guth, K. & Potter, J.D. 1987. Effect of rigor and cycling cross-bridges on the structure of troponin C and Ca^{++} affinity of the Ca^{++}-specific regulatory sites in skinned rabbit psoas fibers. *J Biol Chem* **262**, 13627–13635.

Harrington, W.F. 1971. A mechanochemical mechanism for muscle contraction. *Proc Natl Acad Sci USA* **68**, 685–689.

Herrmann, C., Houadjeto, M., Travers, F. & Barman, T. 1992. Early steps of the Mg^{++}-ATPase of relaxed myofibrils. A comparison with Ca^{++}-activated myofibrils and myosin subfragment 1. *Biochem* **31**, 8036–8042.

Hibberd, M.G. & Trentham, D.R. 1986. Relationship between chemical and mechanical events during muscular contraction. *Ann Rev Biophys Chem* **15**, 119–161.

Hibberd, M.G., Webb, M.R., Goldman, Y.E. & Trentham, D.R. 1985. Oxygen exchange between phosphate and water accompanies calcium-regulated ATPase activity of skinned fibers from rabbit skeletal muscle. *J Biol Chem* **260**, 3496–3500.

Higuchi, H. & Goldman, Y.E. 1991. Sliding distance between actin and myosin filaments per ATP molecule hydrolysed in skinned muscle fibres. *Nature* **352**, 352–354.

Higuchi, H. & Goldman, Y.E. 1995. Sliding distance per ATP molecule hydrolyzed by myosin heads during isotonic shortening of skinned muscle fibers. *Biophys J* **69**, 1491–1507.

Higuchi, H., Yanagida, T. & Goldman, Y.E. 1995. Compliance of thin filaments in skinned fibers or rabbit skeletal muscle. *Biophys J* **69**, 1000–1010.

Hill, A.V. 1938. The heat of shortening and the dynamic constants of muscle. *Proc R Soc Lond* **B126**, 136–195.

Hill, T.L., Eisenberg, E. & Greene, L. 1980. Alternate model for cooperative equilibrium binding of myosin subfragment-1-nucleotide complex to actin troponin-tropomyosin. *Proc Natl Acad Sci USA* **77**, 3186–3190.

Homsher, E. 1987. Muscle enthalpy production and its relationship to actomyosin ATPase. *Ann Rev Physiol* **49**, 673–690.

Homsher, E., Irving, M. & Wallner, A. 1981. High-energy phosphate metabolism and energy liberation associated with rapid shortening in frog skeletal muscle. *J Physiol* **321**, 423–436.

Huxley, A.F. 1957. Muscle structure and theories of contraction. *Progr Biophys Chem* **7**, 255–318.

Huxley, H.E. 1969. The mechanism of muscular contraction. *Science* **164**, 1356–1366.

Huxley, A.F. 1974. Muscular contraction. *J Physiol.* (*London*) **243**, 1–43.

Huxley, A.F. 1992. A fine time for contractural alterations. *Nature* **357**, 110.

Huxley, H.E. & Kress, M. 1985. Crossbridge behaviour during muscle contraction. *J Muscle Res Cell Motil* **6**, 153–161.

Huxley, A.F. & Simmons, R.M. 1971. Proposed mechanism of force generation in striated Muscle. *Nature* (*London*) **233**, 533–538.

Huxley, H.E., Stewart, A., Sosa, H. & Irving, T. 1994. X-ray diffraction measurements of the extensibility of actin and myosin filaments in contracting muscle. *Biophys J* **67**, 2411–2421.

Huxley, A.F. & Tideswell, S. 1997. Rapid regeneration of power stroke in contracting muscle by attachment of second myosin head. *J Muscle Res Cell Motil* **18**, 111–114.

Irving, M., Allen, T.St.C., Sabido-David, C. *et al.* 1995. Tilting of the light-chain region of myosin during step length changes and active force generation in skeletal muscle. *Nature* **375**, 688–691.

Irving, M., Lombardi, V., Piazzesi, G. & Ferenczi, M. 1992. Myosin head movements are synchronous with the elementary force-generating process in muscle. *Nature* **357**, 156–158.

Irving, M. & Woledge, R.C. 1981. The energy liberation of frog skeletal muscle in tetanic contractions containing two periods of shortening. *J Physiol* **321**, 401–410.

Kasprzak, A.A. 1994. Myosin subfragment-1 activates ATP hydrolysis on Mg^{++} G-actin. *Biochem* **33**, 12456–12462.

Kasprzak, A.A., Takashi, R. & Morales, M.F. 1988. Orientation of actin monomer in the F-actin filament: radial coordinate of glutamine-41 and effect of myosin subfragment-1 boinding on the monomer orientation. *Biochem* **27**, 4512–4522.

Kawai, M. & Halvorson, H.R. 1991. Two step mechanism of phosphate release and the mechanism of force generation in chemically skinned fibers of rabbit psoas muscle. *Biophys J* **59**, 329–342.

Klebanov, D. 1996. Analysis of muscle contraction through computer modelling. Senior Thesis. Dept. of Chemistry, Princeton University.

Kojima, H., Ishijima, A. & Yanagida, T. 1994. Direct measurement of stiffness of single actin filaments with and without tropomyosin using in vitro nano-manipulation. *Proc Natl Acad Sci USA* **91**, 12962–12966.

Kushmerick, M.J. & Davies, R.E. 1969. The chemical energetics of muscle contraction. II. The chemistry, efficiency and power of maximally working sartorius muscle. *Proc Roy Soc Lond* **B**, 1969, 315–353.

Leibler, S. & Huse, D.A. 1993. Porters versus rowers: a unified stochastic model of motor proteins. *J Cell Biol* **121**, 1357–1368.

Linari, M., Lombardi, V. & Piazzesi, G. 1997. Cross-bridge kinetics studied with staircase shortening in single fibres from frog skeletal muscle. *J Muscle Res Cell Motil* **18**, 91–101.

Lombardi, V., Piazzesi, G. & Linari, M. 1992. Rapid regeneration of the actin-myosin power stroke in contracting muscle. *Nature* **355**, 638–641.

Lorenz, M., Popp, D., Holmes, K.C. 1993. Refinement of the F-actin model against X-ray fiber diffraction data by the use of a directed mutation algorithm. *J Mol Biol* **234**, 826–836.

Loscalzo, G., Reed, G.H. & Weber, A. 1975. Conformational change and cooperativity in actin filaments free of tropomyosin. *Proc Natl Acad Sci USA* **72**, 3412–3415.

Lymn, R.W. & Taylor, E.W. 1971. Mechanism of adenosine triphosphate hydrolysis by actomyosin. *Biochem* **10**, 4617–4624.

Martonosi, A., Goueva, M.A. & Gergeley, J. 1960. Studies on actin. III G-F transformation of actin and muscular contraction (experiments in vivo). *J Biol Chem* **235**, 1707–1710.

Martyn, D.A. & Chase, B. 1995. Faster force transients kinetics at submaximal Ca^{++} activation of skinned psoas fibers from rabbit. *Biophys J* **68**, 235–242.

Martyn, D.A., Chase, P.B., Hannon, J.D., Huntsman, L.L., Kushmerick, M.J. & Gordon, A.M. 1994. Unloaded shortening of skinned muscle fibers from rabbit activated with and without Ca^{++}. *Biophys J* **67**, 1984–1993.

Martyn, D.A. & Gordon, A.M. 1992. Force and stiffness in glycerinated rabbit psoas fibers. Effects of calcium and elevated phosphare. *J Gen Physiol* **99**, 795–816.

Metzger, J.M. 1995. Myosin binding-induced cooperative activation of the thin filament in cardiac myocytes and skeletal muscle fibers. *Biophys J* **68**, 1430–1442.

Metzger, J., M. Moss, R. L. 1991. Kinetics of a Ca^{2+} -sensitive crossbridge state transition in skeletal muscle fibers: effects due to variations in thin filament activation by extraction of troponin C *J Gen Physiol* **98**, 233–248.

Mijailovich, S.M., Fredberg, J.F. & Butler, J.P. 1996. On the theory of muscle contraction: filament extensibility and the development of isometric force and stiffness. *Biophys J* **71**, 1475–1484.

Miki, M. 1990. Interaction of F-actin-AMPPNP with myosin subfragment-1 and troponin-tropomyosin: influence of an extra phosphate at the nucleotide binding site in F-actin and its function. *J Biochem* **108**, 457–461.

Mitsui, T. & Oshima, H. 1988. A self-induced translation model of myosin head motion in contracting muscle I. Force-velocity relation and energy liberation. *J Muscle Res Cell Motil* **9**, 248–260.

Molloy, J.E., Burns, J.E., Kendrick-Jones, J., Tregear, R.T. & White, D.C.S. 1995. Movement and force produced by a single myosin head. *Nature* **378**, 209–212.

Moos, C. & Eisenberg, E. 1970. Effect of myosin on actin-bound nucleotide exchange in the presence and absence of ATP. *Biochim Biophys Acta* **223**, 221–229.

Moss, R.L., Allen, J.D. & Greaser, M.L. 1985. The effects of partial extraction of TnC upon tension-pCa relationship in rabbit skinned skeletal muscle fibers. *J Gen Physiol* **86**, 585–600.

Murray, J.M. & Weber, A. 1980. Cooperativity of the calcium switch of regulated rabbit actomyosin system. *Mol Cell Biochem* **35**, 11–15.

Orlova, A. & Egelman, E.H. 1997. Cooperative rigor binding of myosin to actin is a function of F-actin structure. *J Mol Biol* **265**, 469–474.

Orlova, A., Prochniewicz, E. & Egelman, E.H. 1995. Structural dynamics of F-actin: II. Cooperativity in structural transitions. *J Mol Biol* **245**, 598–607.

Ostap, E.M., Barnett, V.A. & Thomas, D.O. 1995. Resolution of three structural states of spin-labelled myosin in contracting muscle. *Biophys J* **69**, 177–188.

Page, R., Lindberg, U. & Schutt, C. 1988. Domain motions in Actin. *J Mol Biol* (in press).

Pan, B.S., Gordon, A.M. & Luo, Z.X. 1989. Removal of tropomyosin overlap modifies cooperative binding of myosin S-1 to reconstituted thin filaments of rabbit strioated muscle. *J Biol Chem* **264**, 8495–8498.

Pate, E. & Cooke, R. 1989. A model of crossbridge action: the effects of ATP, ADP and P_i. *J Muscle Res Cell Motility* **10**, 181–196.

Phillips, G.N., Fillers, J.P. & Cohen, C. 1986. Tropomyosin crystal structure and muscle regulation. *J Mol Biol* **192**, 111–131.

Piazzesi, G. & Lombardi, V. 1995. A cross-bridge model that is able to explain mechanical and energetic properties of shortening muscle. *Biophys J* **68**, 1966–1979.

Piazzesi, G. & Lombardi, V. 1996. Simulation of the rapid regeneration of the actin-myosin working stroke with a tight coupling model of muscle contraction. *J Muscle Res Cell Motil* **17**, 45–53.

Pinaev, G., Schutt, C.E. & Lindberg, U. 1995. The effect on actin ATPase of phalloidin and tetramethylrhodamine phalloidin. *FEBS Lett* **369**, 144–148.

Prochniewicz, E., Zhang, Q., Janmey, P.A. & Thomas, D.O. 1996. Cooperativity in F-actin: binding of gelsolin at the barbed end affects structure and dynamics of the whole filament. *J Mol Biol* **260**, 756–766.

Prochniewicz-Nakayama, E. & Yanagida, T. 1982. The effect of crosslinking of thin filament with glutaraldehyde on the contractility of muscle fiber. *J Biochem* **92**, 1269–1277.

Prochniewicz-Nakayama, E., Yanagida, T. & Oosawa, F. 1983. Studies on the conformation of F-actin in muscle fibers in the relaxed state, rigor, and during contraction using fluorescent phalloidin. *J Cell Biol* **97**, 1663–1667.

Rayment, I., Holden, H.M., Whittaker, M., Yohn, C.B., Lorenz, M., Holmes, K.C. & Milligan, R.A. 1993. Structure of the actin-myosin complex and its implications for muscle contraction. *Science* **261**, 58–65.

Rosenfeld, S.S. & Taylor, E.W. 1987. The mechanism of regulation of actomyosin subfragment 1 ATPase. *J Biol Chem* **262**, 9984–9993.

Saito, K., Aoki, T., Aoki, T. & Yanagida, T. 1994. Movement of single myosin filament and myosin step size of an actin filament suspended in solution by a laser trap. *Biophys J* **66**, 769–777.

Schaertl, S., Lehrer, S.S. & Geeves, M.A. 1995. Separation and characterization of the two functional regions of troponin involved in muscle thin filament regulation. *Biochem* **34**, 15890–15894.

Schutt, C.E., Kreatsoulas, C., Page, R. & Lindberg, U. 1997. Plugging into actin's architectonic socket. *Nat Struct Biol* **4**, 169–172.

Schutt, C.E. & Lindberg, U. 1992. Actin as generator tension during muscle contraction. *Proc Natl Acad Sci USA* **89**, 319–323.

Schutt, C.E. & Lindberg, U. 1993. A new perspective on muscle contraction. *FEBS Lett* **325**, 59–62.

Schutt, C.E., Lindberg, U., Myslik, J. & Strauss, N. 1989. Molecular packing in profilin-actin crystals and its implications. *J Mol Biol* **209**, 735–746.

Schutt, C.E., Myslik, J., Rozycki, M., Goonesekere, N. & Lindberg, U. 1993. The structure of crystalline profilin-β-actin. *Nature* **365**, 810–816.

Schutt, C.E., Rozycki, M., Chik, J. & Lindberg, U. 1995a. Structural studies on the ribbon-to-helix transition in profilin: actin crystals. *Biophys J* **68**, 12s–18s.

Schutt, C.E., Rozycki, M.D., Myslik, J.C. & Lindberg, U. 1995b. A discourse on modelling F-actin. *J Struct Biol* **115**, 186–198.

Sellers, J.R. & Homsher, E. 1991. A giant step for myosin. *Curr Biol* **7**, 347–349.

Sleep, J.A. & Hutton, R.L. 1980. Exchange between inorganic phosphate and adenosine 5′-triphosphate in the medium by actomyosin subfragment 1. *Biochem* **19**, 1276–1283.

Spudich, J.A. 1994. How molecular motors work. *Nature* **372**, 515–518.

Strzelecka-Golaszewska, H., Jukubiak, M. & Drabikowski, W. 1975. Changes in the state of actin during superprecipitation of actomyosin. *Eur J Biochem* **55**, 221–230.

Swartz, D.R. & Moss, R.L. 1992. Influence of a strong-binding myosin analogue on calcium-sensitive mechanical properties of skinned skeletal muscle fibers. *J Biol Chem* **267**, 20497–20506.

Szent-Györgyi, A.G. 1968. The role of actin-myosin interaction in contraction. *Symp Exptl Biol Woods Hole Mar Biol Laboratory* **22**, 17–42.

Szent-Györgyi, A.G. & Prior, G. 1966. Exchange of adenosine diphosphate bound to actin in superprecipitated actomyosin and contracted myofibrils. *J Mol Biol* **15**, 515–538.

Thomas, D., Ramachandran, R., Roopnarine, O., Hayden, D.W. & Ostap, E.M. 1995. The mechanism of force generation in myosin: a disorder-to-order transition, coupled to internal structural changes. *Biophys J* **68**, 135s–141s.

Toyoshima, Y.Y., Kron, S.J. & Spudich, J.A. 1990. The myosin step size: measurement of the unit displacement per ATP hydrolyzed in an in vitro assay. *Proc Natl Acad Sci USA* **87**, 7130–7134.

Uyeda, T., Kron, S.J. & Spudich, J.A. 1990. Myosin step size: estimation from slow sliding moving of actin over low densities of heavy meromyosin. *J Mol Biol* **214**, 699–710.

Uyeda, T.G.P., Warrick, J.M., Kron, S.J. & Spudich, J.A. 1991. Quantized velocities at low myosin densities in an *in vitro* motility assay. *Nature* **352**, 307–311.

Vale, R.D. & Oosawa, F. 1990. Protein motors and Maxwell's demons: does mechanochemical transduction involve a thermal ratchet? *Adv Biophys* **26**, 97–134.

Van Buren, P., Work, S.S. & Warshaw, D.M. 1994. Enhanced force generation by smooth muscle myosin *in vitro*. *Proc Natl Acad Sci USA* **91**, 202–205.

Wakabayashi, K., Sugimoto, Y., Tanaka, H., Ueno, Y., Takezawa, Y. & Anemiya, Y. 1994. X-ray diffraction evidence for the extensibility of actin and myosin filaments during muscle contraction. *Biophys J* **67**, 2422–2435.

Ward, L.C. 1979. The turnover of F-actin-bound ADP in vivo. *Experientia* **35**, 1145–1146.

White, H.D. & Taylor, E.W. 1976. Energetics and mechanism of actomyosin adenosine triphosphatase. *Biochem* **15**, 5818–5826.

Yanagida, T., Arata, T. & Oosawa, F. 1985. Sliding distance of actin filament induced by a myosin crossbridge during one ATP hydrolysis cycle. *Nature* **316**, 366–369.

Yanagida, T. & Oosawa, F. 1978. Polarized fluorescence from e-ADP incorporated into F-actin in a myosin-free single fiber: conformation of F-actin and changes induced in it by heavy meromyosin. *J Mol Biol* **126**, 507–524.

Yanagida, T., Taniguchi, M. & Oosawa, F. 1974. Conformational changes of F-actin in the thin filaments of muscle induced *in vivo* and *in vitro* by calcium ions. *J Mol Biol* **90**, 509–522.

Zot, A.S. & Potter, J.D. 1989. Reciprocal coupling between troponin C and myosin cross-bridge attachment. *Biochem* **28**, 6751–6756.