PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Cooperativity of myosin molecules through strain–dependent chemistry

Thomas Duke

Phil. Trans. R. Soc. Lond. B 2000 **355**, 529-538
doi: 10.1098/rstb.2000.0594

THE ROYAL SOCIETY

Cooperativity of myosin molecules through strain-dependent chemistry

Thomas Duke†

Institut Curie, Section de Recherche, 26 Rue d'Ulm, 75248 Paris Cedex 05, France

There is mounting evidence that the myosin head domain contains a lever arm which amplifies small structural changes that occur at the nucleotide-binding site. The mechanical work associated with movement of the lever affects the rates at which the products of ATP hydrolysis are released. During muscle contraction, this strain-dependent chemistry leads to cooperativity of the myosin molecules within a thick filament. Two aspects of cooperative action are discussed, in the context of a simple stochastic model. (i) A modest motion of the lever arm on ADP release can serve to regulate the fraction of myosin bound to the thin filament, in order to recruit more heads at higher loads. (ii) If the lever swings through a large angle when phosphate is released, the chemical cycles of the myosin molecules can be synchronized at high loads. This leads to stepwise sliding of the filaments and suggests that the isometric condition is not a steady state.

Keywords: myosin; muscle contraction; mechanochemistry; chemical kinetics; collective properties; theory

1. INTRODUCTION

There are numerous situations in which linear motor proteins work together in vast assemblies. The most striking example—and certainly the best studied—is the contraction of skeletal muscle (Huxley 1974). The shortening of a muscle fibre involves the concerted action of many billions of myosin molecules. Even within each sarcomere, which forms the basic repeating unit of the fibre, about 10 000 molecules act together to drive the sliding of the interdigitated arrays of thick and thin filaments (Huxley & Niedergerke 1954; Huxley & Hanson 1954). Can we understand the properties of such a system, starting from a knowledge of the nature of the individual components? Conversely, can we glean clues about the constituent molecules and their interactions by studying the macroscopic behaviour?

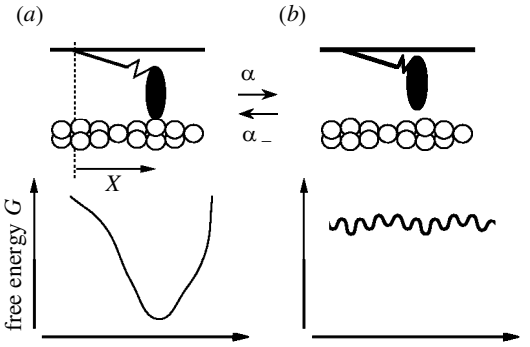
To do so we must construct simplified models of the way in which the individual molecules interact biochemically with each other, and compare the predicted behaviour of large ensembles with experimental observations. The high number of molecules involved is a benefit, since it permits us to describe the overall behaviour by statistical averages. This is analogous to the statistical mechanical treatment of solids, liquids and gases, in which macroscopic thermodynamic properties are related to the microscopic interactions between molecules. One of the fundamental lessons of statistical physics has been that even a very simple system, composed of identical elements with pairwise interactions, can display emergent collective properties. An example is the abrupt condensation of a gas as the temperature falls below a critical value. Such phase transitions, in which one type of collective behaviour becomes unstable and another comportment is favoured, are a consequence of cooperativity in the microscopic interactions. A system in which many motor proteins act collectively is considerably more complex than a gas. The individual entities are not just passive molecules, but active force generators, powered by a chemical reaction which is far from equilibrium. The direct interactions between motor proteins are mechanical but, since the laws of thermodynamics dictate that mechanics and chemistry are closely connected, these induce a more subtle coupling of the kinetics of the molecules. A collection of motor proteins is a dynamic system, but by analogy with a thermodynamic system we should expect that cooperativity in the kinetic coupling can induce dynamic instabilities. For example, a dynamic transition might occur at a critical value of the applied force, where one type of motion is replaced by another. Such instabilities are likely to play important functional roles in motor protein assemblies. Actomyosin is a good system in which to investigate some of the consequences of cooperativity, since there is a large body of experimental data on muscle contraction. The lessons drawn may be relevant to other systems in which large numbers of motor proteins cooperate, such as the axoneme.

2. KINETIC MODELS

(a) *Mechanochemical coupling*

Since the transduction of chemical energy to mechanical work by motor proteins occurs via a series of biochemical reactions, the most natural theoretical description is a kinetic one. Kinetic models of myosin action are based on the supposition that the actomyosin complex can exist in several different biochemical states:

† Present address: Cavendish Laboratory, Madingley Road, Cambridge CB3 0HE, UK.



igure 1. Examples of different biochemical states of the ctomyosin complex. (*a*) When the myosin head is bound to ne thin filament, the dependence of the free energy G on the isplacement x of the thin filament, relative to the thick lament, is principally due to the mechanical deformation of ne myosin molecule. (*b*) When the myosin head is detached, here may be a residual weak interaction which has the same eriodicity as the thin filament. The ratio of forward and everse transition rates, α/α_-, is related to the difference in ree energies of the two states, ΔG.

n some, the myosin is bound tightly to the thin filament; n others it is detached (figure 1). Each state is considered o be internally at equilibrium, and may be characterized y a Gibbs free energy G which, in general, depends on ne position x of the thick filament, relative to the actin-inding site. For a state in which the myosin head is trongly bound to the thin filament, the principal contri-ution to this dependence comes from the mechanical eformation of the myosin molecule; for a detached state, here is a residual, weak interaction between the myosin ead and the actin filament, which reflects the periodicity f the thin filament. In a given state, the actomyosin ndergoes Brownian motion in the potential $G(x)$ and the nstantaneous force exerted on the thin filament by the nyosin head is equal to the gradient of the potential at onstant temperature:

$$(x) = -\left.\frac{\partial G}{\partial x}\right|_T. \qquad (1)$$

Jo net force or movement would be produced if the arious actomyosin states were in equilibrium with each ther. However, the transitions between states are oupled to the hydrolysis of ATP and, in physiological onditions, the concentration of ATP is maintained very nuch higher than it would be at equilibrium. Indeed, the ree energy of ATP hydrolysis

$$\Delta G_{\mathrm{ATP}} = \Delta G^0_{\mathrm{ATP}} + k_{\mathrm{B}}T \ln \left|\frac{[\mathrm{ADP}]\,[\mathrm{Pi}]}{[\mathrm{ATP}]}\right| \qquad (2)$$

where activities should strictly be used in place of oncentrations) is large and negative at physiological oncentrations ($\Delta G_{\mathrm{ATP}} \approx -23 k_{\mathrm{B}}T$), so the transitions are trongly driven in the direction that corresponds to the plitting of ATP. It is this asymmetry which permits the eneration of force and movement. A portion of the nergy of hydrolysis can be used to do mechanical work nd the remainder is dissipated as heat.

Mechanochemical coupling—the interrelationship of nechanical force and chemical kinetics—is the key to understanding motor protein action. The classic paper of Hill (1974), which laid the foundations for the construction of kinetic models that are consistent with the laws of thermodynamics, provided a thorough discussion of this issue. For each transition between a pair of states, the principle of detailed balance dictates that the ratio of forward and reverse rates is related to the difference of the free energies ΔG:

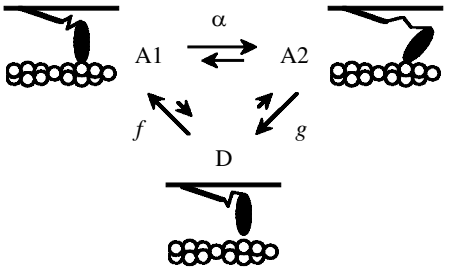
$$\frac{\alpha}{\alpha_-} = \exp\left|\frac{\Delta G}{k_{\mathrm{B}}T}\right|. \qquad (3)$$

In general, ΔG depends on the relative displacement of the thick and thin filaments, so either the forward or the reverse transition rate (or both) must be strain dependent. Put another way, the kinetic rates depend on the force experienced by the myosin molecule. Indeed, comparison of equations (1) and (3) indicates that mechanical force and chemical kinetics are inextricably linked, since both are related to the free energy. Thus thermodynamics provides strong constraints on theoretical models. The relationships between kinetic rates must be associated with the mechanical properties of the molecules. However, there remains considerable freedom to choose the functional form of individual rates. The number of actomyosin states can also be chosen freely, so many different models can fit this general framework. The task is to find a minimal model which is consistent with what is known about myosin structure and chemistry, and which explains the majority of phenomena observed in muscle contraction and in motility assays.

(b) *The swinging cross-bridge model*

The simplest kinetic scheme, proposed by A. F. Huxley soon after the discovery that muscle contracts by the relative sliding of thick and thin filaments, involved transitions between just two states, one attached and one detached (Huxley 1957). Subsequently, microscopic observations of myosin heads (or 'cross-bridges') bound to the thin filament indicated that there were two, structurally distinct, attached states (Reedy *et al.* 1965). This led to the swinging cross-bridge model (figure 2) (Huxley 1969), which is the foundation of nearly all of the kinetic schemes that have followed. The basic assumption is that the myosin head, when bound to actin, can undergo a structural change which alters its orientation. In addition, the dependence of free energy on position is simplified and given a physical interpretation, by supposing that the myosin molecule contains a linear elastic element. Thus two parameters characterize the mechanical properties of a cross-bridge: the distance d that the distal end of the head moves when the myosin makes a transition between the two attached states, A1 and A2; and the spring constant K. The force exerted by the myosin head in each state is Kx and $K(x+d)$, respectively. The transition A1→A2 is sometimes referred to as the 'power stroke' since, by stretching the elastic element, it puts the myosin head into a force-generating state.

The seminal experiments of Huxley & Simmons (1971), which examined the transient response of muscle to sudden changes in length, provided evidence that attached heads can, indeed, undergo a conformational change. They also indicated that the kinetics of this



igure 2. Basic cycle of the swinging cross-bridge model. The nyosin molecule makes stochastic transitions between a etached state D, and two attached states, A1 and A2, which re structurally distinct. In general, the transition rates f, α, g nd the corresponding reverse rates depend on the strain of ne elastic element. Owing to the free-energy change ssociated with ATP hydrolysis, the forward rates are redominately faster than the reverse rates and the molecule driven one way around the cycle: D→A1→A2→D. One TP molecule is split during each cycle.

ransition is much more rapid than the attachment and etachment of the myosin head. This implies that these wo states are, effectively, in equilibrium with each other, lthough neither is in equilibrium with the detached state). The basic cycle involves binding of the myosin head at ate f, which is quickly followed by the power stroke tran- tion at a fast rate α. This puts the head in a force-gener- ting, tightly bound state, from which it dissociates at rate . Detailed balance holds for each of these transitions, but ne corresponding changes of free energy are not known. t is usual, however, to assume that one traversal of the ycle is coupled to the hydrolysis of a single ATP mole- ule, which puts a definite constraint on the product of ne rates for all three transitions:

$$\frac{\times \alpha \times g}{f_- \alpha_- g_-} = \exp\left(\frac{\Delta G_{\mathrm{ATP}}}{k_{\mathrm{B}} T}\right). \tag{4}$$

(c) *Dynamics generated by many myosin molecules*

In muscle, many myosin molecules act together to ause the shortening of a sarcomere. As a simplification, is normal to consider the sliding of a single pair of fila- ents, caused by an ensemble of N molecular motors. What methods can be used to calculate the overall ynamics, given the basic mechanochemical cycle of an ndividual cross-bridge outlined above?

The method that has been employed most extensively ollows the standard, ensemble-averaging approach of tatistical physics. One asks the following question: at a iven time, what is the probability that any myosin head s in a specified biochemical state, with a particular value f the strain? According to the kinetic scheme, general quations can be written for the evolution of these prob- bility distributions (Huxley 1957; Hill 1974). For xample, the equation for the detached state D in figure 3 s

$$\left.\frac{\partial p_{\mathrm{D}}}{\partial t}\right|_x + \left.\frac{\partial p_{\mathrm{D}}}{\partial x}\right|_t \frac{\mathrm{d}x}{\mathrm{d}t} = f_- p_{\mathrm{A1}} + g p_{\mathrm{A2}} - (f + g_-) p_{\mathrm{D}}. \tag{5}$$

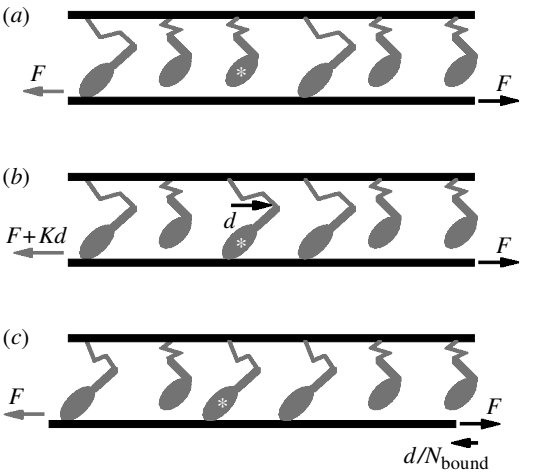


Figure 3. When a thin filament of length L is propelled by N motor proteins, the typical time between chemical events is of the order or $\tau_{\mathrm{chem}} = 1/Nf$. Each event perturbs the system mechanically, and the time that it takes the filaments to adjust their position is of the order of $\tau_{\mathrm{visc}} = \eta L/NK$, where η is the viscosity of the surrounding fluid. For a sarcomere, $\tau_{\mathrm{visc}}/\tau_{\mathrm{chem}} \approx 10^{-2}$, so the filaments are in quasi-mechanical equilibrium. (*a*) For a pair of filaments in mechanical equilibrium, the sum of the forces exerted by the motor proteins (grey arrow) is equal and opposite to the applied load (black arrow). (*b*) If one of the detached heads (marked with an asterisk) binds to the thin filament and undergoes a power stroke transition, the filaments are momentarily out of equilibrium, owing to the additional force Kd exerted by this molecule. (*c*) As a consequence, the thin filament slides leftwards through a small displacement Kd/N_{bound}, reducing the strain of all N_{bound} attached motors. Similar adjustments occur when a head detaches, but the size and sign of the displacement varies according to the strain of the molecule immediately prior to dissociation.

In order to progress further, it is often necessary to make further simplifications. For example, to calculate the relationship between sliding velocity and applied load, it is assumed that a steady state exists, in which the filaments slide at a uniform speed v and the proportion of motors in each state does not change with time. Then equation (5) simplifies to

$$-v \left.\frac{\partial p_{\mathrm{D}}}{\partial x}\right|_t = f_- p_{\mathrm{A1}} + g p_{\mathrm{A2}} - (f + g_-) p_{\mathrm{D}} \tag{6}$$

and the full set of coupled equations can be solved to find the steady-state probability distributions. The force corresponding to this velocity can be obtained by integrating the tension in the elastic elements of all of the motors:

$$F = N \int [p_{\mathrm{A1}}(x) K x + p_{\mathrm{A2}}(x) K (x + d)] \mathrm{d}x. \tag{7}$$

The advantage of this approach is that, once the probability distributions have been obtained, many dynamic and thermodynamic properties can be calculated from them. The drawback is that it may overlook some important physics, for there is no guarantee that a steady state exists in all circumstances, as assumed. Indeed, the force–velocity curve determined in this way really describes the tension that is generated when steady shortening is imposed, rather than the velocity of shortening under a

onstant external load. These two situations are not ecessarily equivalent, since instabilities may arise in one ase, but not the other.

A second method, which can be used to study more eneral situations, including those in which a steady-state esponse is unstable, is stochastic simulation (Duke 1999). With this technique, a record is kept of the biochemical tate and the strain of each motor protein. The time-volution of the system is simulated by modifying the tate of the molecules as reactions occur and by moving he filaments in response to the forces that are generated. ince the Reynolds number is very low at this micro-copic scale, inertial effects can be neglected and the ynamics is described by the Langevin equation, which tates that the sliding velocity is directly proportional to he total shear force acting on the filaments. A further mplification can be made if the dynamic response time f the system is short compared with the typical time etween chemical events. In this case, the filaments are in quasi-mechanical equilibrium' (see figure 3). Each time hat a chemical transition occurs, the total force acting on he filaments is altered by a slight amount; in response hey slide through a small displacement, until mechanical quilibrium is restored. Following the time-evolution of he system is then straightforward. Based on the expres-ions for the rates specified in the model, a random umber generator can be used to determine the instants t which chemical events occur stochastically. Following ach chemical event, the position of mechanical equili-rium is adjusted accordingly. This stochastic simulation ethod is versatile and can be used to determine the verage sliding velocity under constant load, as well as ransient responses to sudden changes in conditions. mportantly, it provides a record of events at the mole-ular level, facilitating the detection of cooperative effects hich are less obvious at the macroscopic level.

(d) *Strain dependence of the kinetics*

In the swinging cross-bridge model, the performance of n individual myosin molecule may be characterized by he unitary force that it can generate, $F_{\mathrm{unit}} = Kd$, and the ypical sliding velocity that it can cause $v_{\mathrm{unit}} = df$. What orce and velocity is produced by many motors? From gure 3 it is clear that when many myosin molecules ork together, they cannot do so independently, since hey are fixed together by the thin and thick filaments, hich are relatively rigid. This linkage communicates the rain induced by one head to all of the others. Thus, the ollective behaviour is highly dependent on the functional orm of the strain dependence of the reaction rates.

As mentioned in § 2a, most of the kinetic rates are likely o vary with strain. However, to understand the role of echanochemical coupling, it is instructive to consider the ypothetical situation in which they are all constant which corresponds to the improbable case of identical x ependence of the free energies for all states). Then the verage fraction of heads bound to the thin filament at any oment is fixed, $r = (1+g/f)^{-1}$, and it is easy to deduce hat the sliding velocity v declines linearly as the external oad F is raised. Of interest, though, is the way in which the orce–velocity relationship depends on the proportion of ound heads (figure 4). If r is small, the sliding velocity at ero load, v_0, is fast because the molecules act like a relay

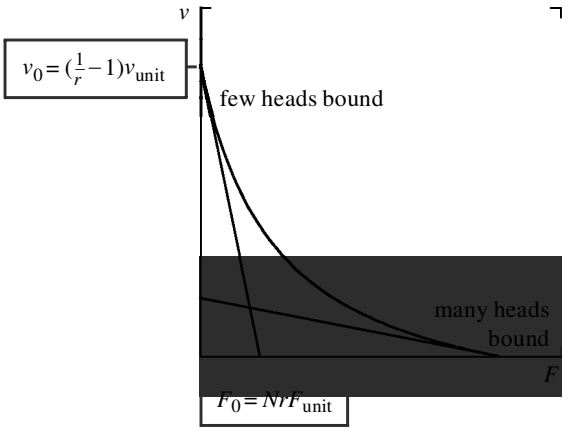


Figure 4. If attachment and detachment rates f and g are strain independent, the force–velocity relationship is linear. If $f \ll g$, few heads are bound, so sliding is rapid, but only a small load can be supported. Conversely, if $f \gg g$, many heads are bound and a high load can be sustained, but sliding is slow. Strain dependence of the detachment rate g permits regulation of the fraction r of bound motors, according to the load. If the fraction of bound heads increases with applied load, the force–velocity relationship is approximately hyperbolic and the combination of rapid sliding and high stall force can be achieved. A. V. Hill's characteristic relationship is well approximated if r increases by a factor of between 3 and 5 as the external load increases from zero to the stall force.

team: a few molecules propel the thin filament, then hand over to another set while they get ready to work again. However, because there are only a few bound cross-bridges at any instant, the filaments cannot support a high load without sliding backwards. On the other hand, if r is close to unity, the stalling force, F_0, is much larger, because nearly all of the myosin molecules share the strain. In this case, though, the unloaded filaments slide only slowly, since the attached heads hinder each other. A motor system that has evolved to work effectively would presumably combine the two desirable features: fast sliding at zero load; and high stall force. Strain dependence of the reaction rates provides a means to do this, because the fraction of bound motors is then regulated according to the load. For example, if the detachment rate g increases monotonically as sliding progresses (Huxley 1957), the average detachment rate is faster if sliding is permitted than if it is not. Consequently, the fraction of bound heads is low at zero load, but extra cross-bridges are recruited as the load is augmented. This leads to a concave force–velocity curve, similar to that observed in muscle.

Many models have elaborated on the basic swinging cross-bridge picture (Julian *et al.* 1974; Eisenberg *et al.* 1980; Pate & Cooke 1989; Chen & Brenner 1993; Pate *et al.* 1993; Piazzesi & Lombardi 1995; Huxley & Tideswell 1996), but all of them conserve the major features outlined above: namely, a fast power stroke transition and a detachment rate that increases as the strain x becomes more negative. Typically, additional states have been incorporated into the cycle, in order to make a more direct correspondence with what is known about the chemistry of actomyosin (Lymn & Taylor 1971). Some authors have supposed that the power stroke is divided into two smaller steps (Piazzesi & Lombardi 1995;

Huxley & Tideswell 1996). Others have considered the possibility that cross-bridge attachment and detachment may not be tightly coupled to ATP hydrolysis (Piazzesi & Lombardi 1995). These models provide an interpretation of a large body of data on the mechanics and thermodynamics of muscle, both during steady sliding and during transient responses to sudden changes in conditions. The price paid for more detailed models, however, is that a greater number of transition rates must be specified. Since the strain dependencies of these rates cannot be ascertained experimentally from solution biochemistry, they can only be guessed. Usually they have been chosen to fit some experimental data, keeping the ratios of forward and reverse rates thermodynamically consistent. There remains a doubt whether similar results might not be obtained under quite different sets of assumptions.

In fact, a rigorous method for constructing the strain dependence of transition rates is available. Kramers' (1940) theory of chemical reactions states that the kinetic rate depends exponentially on the height of an energy barrier required to attain a transition state. The only problem is that structural information is required in order to determine what that energy change might be. For this reason, the recent solution of the structure of the myosin head by Rayment *et al.* (1993) has been an extremely important development for model builders. By connecting kinetics to structural changes, a rational choice of the functional form of the strain dependence can be made (Smith & Geeves 1995; Duke 1999).

3. A PHYSICAL MODEL OF MYOSIN KINETICS

(a) *The swinging lever arm and its implications for kinetics*

The determination of the crystal structure of the myosin head indicated that the neck region contains an exceptionally long α-helix. At one end, the helix connects to the rod domain, which is anchored in the thick filament. At the other end, it extends a short distance into the catalytic domain of the myosin head. This observation led to the proposal that the α-helix acts as a lever arm, rotating about a fulcrum located within the catalytic domain (Rayment *et al.* 1993; Uyeda *et al.* 1996). The swinging lever-arm hypothesis (Holmes 1997) is a refinement of the swinging cross-bridge model; the main difference is that only the neck region of the myosin molecule changes orientation, rather than the entire head. The discovery is very significant, however, because the fulcrum is thought to be located at (or very close to) the nucleotide-binding site. This suggests that changes in the orientation of the lever are directly associated with local conformational changes which occur when ligands bind at, or dissociate from, the nucleotide-binding pocket. Thus, quite generally, we should expect that each biochemical state corresponds to a structural state in which the lever is rotated by a different amount.

On this basis, and using Kramers' (1940) theory of chemical reactions, it is possible to construct a physical argument for the strain dependence of the kinetics. The important observation is that the lever arm amplifies the local conformational changes so that, however small they may be, the distal end of the lever moves through a significant distance whenever a biochemical transition occurs. If this movement is in the direction which stretches the elastic element, mechanical work must be done during the transition, and this increases the free energy of the product, relative to the reactant. As a consequence, the transition rate, which depends exponentially on the free energy difference, is diminished. Thus the physical properties of the molecule—the distance through which the lever arm moves and the compliance of the elastic element—determine the strain dependence of the reaction rates.

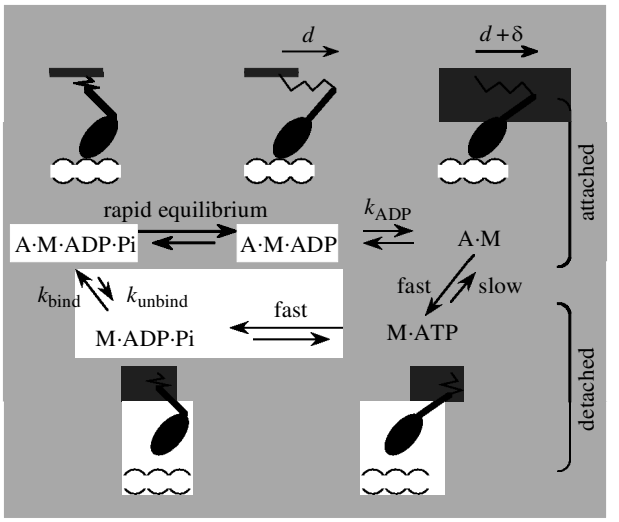


Figure 5. A minimal kinetic scheme of the mechanochemical cycle of the myosin, based on the swinging lever-arm hypothesis. The potentially rate-limiting reactions are the binding of M·ADP·Pi to the thin filament (at rate k_{bind}), and ADP release (at rate k_{ADP}). Since the other forward transitions are fast, only the three states boxed in white are occupied to a significant degree. Thus, the scheme reduces to an effective three-state model (cf. figure 3) in which the effective detachment rate is controlled by ADP release.

The reaction scheme illustrated in figure 5 (Duke 1999) is a minimal model which is consistent with the chemistry of the Lymn–Taylor cycle (Lymn & Taylor 1971), the structure of the swinging lever arm and the mechanochemical coupling suggested by Kramers' theory. Hydrolysis takes place while myosin is detached, and the head then binds to the thin filament as M·ADP·Pi. The products of hydrolysis are released separately. First, inorganic phosphate (Pi) dissociates rapidly. This transition is associated with the main power stroke, in which the lever arm moves through a large displacement d. Subsequently, ADP dissociates more slowly, and this transition causes the lever to move through an additional displacement δ. Following this, ATP binds quickly and destabilizes the actomyosin interaction so that the head rapidly dissociates. This event completes the cycle, during which one molecule of ATP has been hydrolysed.

Because a number of transitions are rapid, this scheme effectively reduces to a three-state model of the type shown in figure 2. The detached head undergoes Brownian motion in the parabolic potential of the elastic element, so the strain dependence of the binding rate f is

$$f = k_{bind}^{0} \exp\left|-\frac{Kx^2}{2k_B T}\right| . \qquad (8)$$

ince both ATP binding and the subsequent detachment f the head are fast, the effective detachment rate g is ontrolled by the rate of the previous transition, i.e. the elease of ADP. According to Kramers' theory, this epends on strain in a simple exponential way:

$$= k^0_{\mathrm{ADP}} \exp\left|-\frac{K\delta(x+d)}{k_{\mathrm{B}}T}\right| . \qquad (9)$$

f δ has the same sign as d (i.e. if the release of Pi and ADP cause the lever to rotate in the same direction) the etachment rate increases as sliding progresses which, as rgued above, makes for an effective motor system. Thus, he structural change on ADP release, in itself, is suffiient to regulate the fraction of bound motors. The strain ependence of ADP release, and hence the degree of egulation, depends on the value of the dimensionless arameter $Kd\delta/k_{\mathrm{B}}T$ (the ratio of the typical work done uring ADP release and the thermal energy). The force–elocity relationship becomes more concave as the value f this parameter is raised.

The power stroke transition is more rapid than both the receding reaction and the following one, so it is effectively quilibrated. Consequently, the relative proportions of ttached heads in the post- and pre-power stroke states is qual to the ratio of forward and reverse transition rates, hich is specified by the condition of detailed balance:

$$\frac{\alpha}{} = \exp\left|-\Delta G_{\mathrm{stroke}} - \frac{Kd(2x+d)}{2k_{\mathrm{B}}T}\right| , \qquad (10)$$

here $\Delta G_{\mathrm{stroke}}$ is the (negative) free energy change ccompanying Pi release. The strain dependence of this eaction is controlled by another dimensionless parameter, $|\frac{1}{2}Kd^2/\Delta G_{\mathrm{stroke}}|$. The two cases in which this parameter is less than, or greater than unity, are qualitatively ifferent. The latter situation, which corresponds to a trong elastic element or a large power stroke, is of particular interest. In this case, the power stroke cannot take lace immediately after the head has bound to the thin lament, because the mechanical work that must be done o stretch the elastic element exceeds the chemical energy hat is available (see figure 6). Consequently, the power troke transition is postponed until the filaments have noved though a short distance. Since this sliding must be aused by the other myosin heads, it is clear that the nolecules act cooperatively in this situation. A single nolecule cannot work on its own and needs the help of he others.

While either of these two situations might apply for keletal muscle myosin, there are reasons to favour the atter. First, when the elastic element is strong, the myosin nolecule is capable of producing a high unitary force unit. Second, although the power stroke transition is postponed, when it does take place it typically does so without an abrupt change in free energy (see figure 6*b*), nd hence with little heat loss. This contrasts with the ituation for a weak spring, where the free energy differnce between A·M·ADP·Pi and A·M·ADP (which is ypically about $-\Delta G_{\mathrm{stroke}} - \frac{1}{2}Kd^2$ when the transition ccurs) is dissipated as heat. Thus a strong spring makes or better efficiency. The conclusion is rather startling: the ollective efficiency with which the ensemble of motors enerates force and motion is optimal when the performance of an individual molecule, acting on its own, is poor.

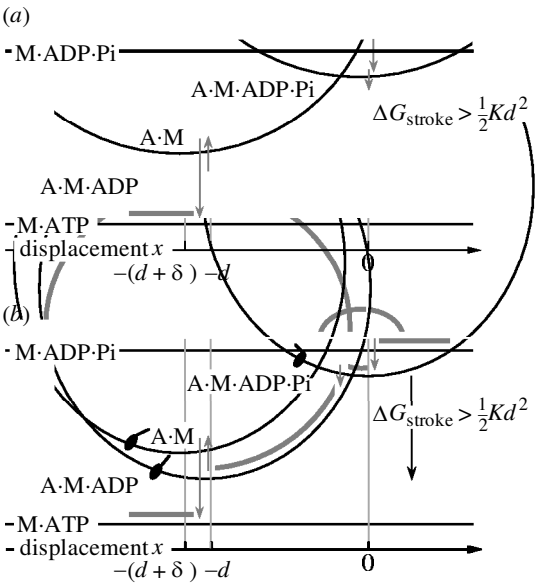
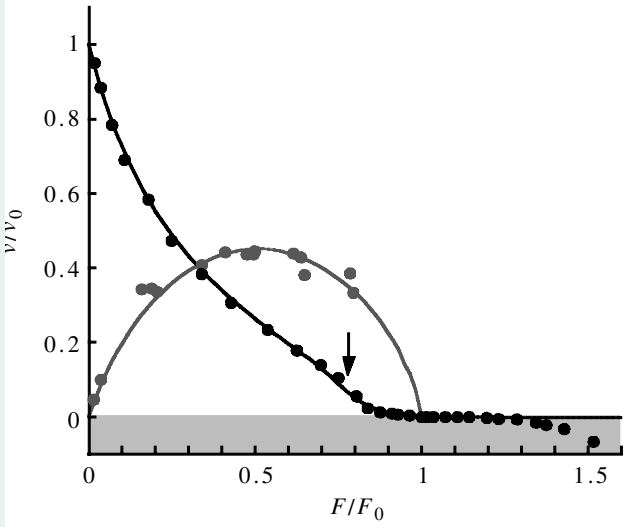


Figure 6. Free energy of the biochemical states shown in figure 5, as a function of the strain x. The thin filament slides leftwards when it is propelled by an ensemble of motors (see figure 3), so the strain of an individual myosin head decreases as time progresses if a moderate load, lower than the stalling force, is applied. The grey trace indicates the reaction pathway of a typical molecule. (*a*) If the elastic element is weak, so that $|\frac{1}{2}Kd^2/\Delta G_{\mathrm{stroke}}| < 1$, Pi can be released immediately after the head binds to the thin filament. (*b*) If the elastic element is strong, the power stroke transition is delayed until the filaments have slid through a small distance, due to the action of other motors.

(b) *Cooperative effects during sliding*

The relative velocity of the filaments, when sliding is opposed by a constant load, can be determined by stochastic simulation. Figure 7 shows the result for the case of a strong spring, in which cooperative effects are expected to be significant. The force–velocity relationship does not descend in a smooth curve, but is split into two regions. At loads less than about 80% of the stalling force, the velocity decreases with increasing load in an approximately hyperbolic way. As the stalling force is approached, however, the average sliding speed declines more rapidly and becomes negative, following a different curve. Data from experiments on single muscle fibres display the same type of behaviour (Edman 1988). In the model, the point of inflection, which separates the two regimes, is the signature of a dynamic transition. At low forces, a steady-state solution exists and the sliding is smooth. But close to the stalling force, an instability arises, owing to the cooperativity of the myosin molecules. The steady-state solution is replaced by a quasi-periodic one, in which the chemical reactions of a large fraction of the myosin molecules are synchronized and the filaments move in a stepwise fashion (figure 8).

The transition is a direct consequence of the fact that myosin heads must cooperate with one another in order to execute their power strokes. At zero load, they are able to do so in an uncorrelated way, with individual molecules binding and detaching at random intervals.



igure 7. Force–velocity relationship for the model of gure 5. The parameter which governs the strain dependence f the power stroke was set at $|\frac{1}{2}Kd^2/\Delta G_{\mathrm{stroke}}| = 1.3$, which is ne value that maximizes the peak mechanical efficiency. The alue of the parameter which governs the strain dependence f ADP release, $Kd\delta/k_{\mathrm{B}}T = 1.6$, was chosen to reproduce the urvature of the experimental force–velocity curve (black ircles; Edman 1988). Note that there is a point of inflexion ist below the stalling force (arrowed). The experimental data or the mechanical efficiency (grey circles; Hill 1964) are uantitatively fit if $Kd^2 = 38\ k_{\mathrm{B}}T$ and $\delta = 0.05d$.

hose heads which have been bound for the longest eriod of time get dragged into a position in which they xert negative force. This balances the positive force xerted by the heads that have just executed their power troke. Because of the strain dependence of ADP release, is the former subset of heads that has the highest instananeous detachment rate. Their continual dissociation auses the thin filament to advance sufficiently rapidly to nsure that any newly bound heads are always able to xecute their power stroke. Consequently, the sliding is mooth and the proportion of heads in each state fluctutes only slightly about a fixed level. As the load is raised nd the average sliding velocity diminishes, this situation reaks down. The power stroke transitions of individual notors begin to fail. Nevertheless, if an individual head oes succeed in accomplishing a power stroke, it causes ne thin filament to advance by a small amount and educes the strain of all the other attached heads, thereby acilitating their own power stroke transitions. Owing to is cooperativity, a large fraction of the bound heads can troke almost simultaneously, like a rowing crew (figure), causing the thin filament to lurch forward through a istance approximately equal to the length of the power troke. Subsequently, the heads detach stochastically and ney must rebind in sufficient numbers to be able to coordinate another cascade of power stroke transitions. hus the dynamics is quasi-periodic, with a period efined by the cycle time of an individual molecule. The robability distributions of the various myosin states are ot fixed, but evolve with time.

As the force is raised further, the likelihood of making power stroke transition declines sharply, and consequently so does the sliding velocity. Even so, stepwise

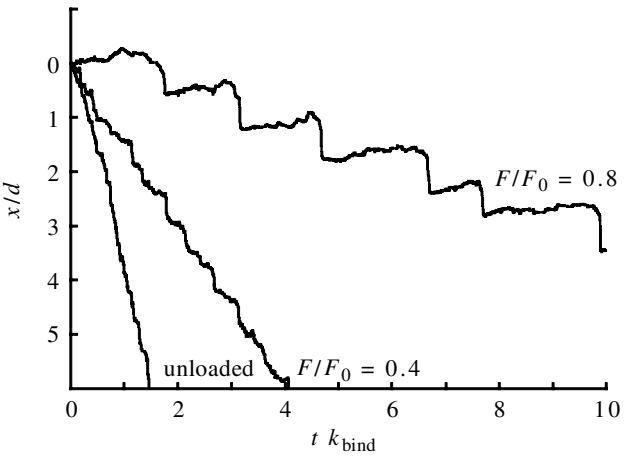


Figure 8. Trace of the position of the thin filament, x, with time. At low loads, the filament slides smoothly but, as the load approaches the stalling force, there is a transition to stepwise motion.

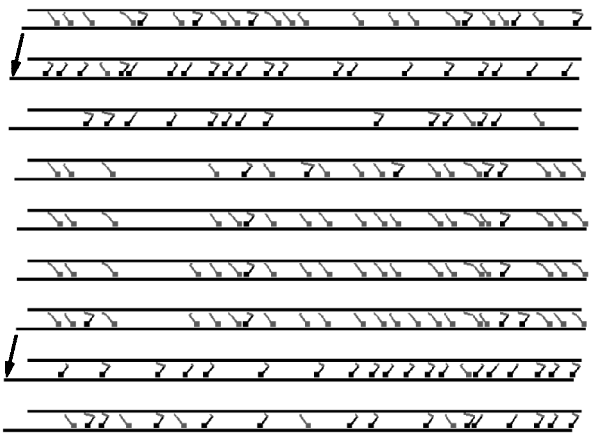


Figure 9. A sequence of snapshots showing the dynamics at a load slightly less than the stalling force, $F = 0.8F_0$. Only bound myosin heads are shown: pre-power stroke A·M·ADP·P_i (grey) and post-power stroke A·M·ADP (black). Instants at which synchronized power stroke transitions occur, and the thin filament moves sharply to the left, are indicated by the arrows.

motion can still occur when the average speed is zero. This suggests that the isometric condition of muscle may not be a steady state, but rather one in which some sarcomeres are lengthening and others are shortening at any instant of time. Since the stall force is determined by inhibition of the power stroke transition, it is ultimately specified by the chemical energy available to power the stroke, $\Delta G_{\mathrm{stroke}}$. From equation (2) it can be seen that this interpretation is consistent with the logarithmic dependence of the isometric load of muscle fibres on phosphate concentration, observed experimentally (Pate & Cooke 1989). At still higher loads, almost no power stroke transitions can occur and the myosin heads simply bind and detach reversibly, without releasing the products of hydrolysis. In doing so, they create a high friction, so the filaments slide backwards only very slowly. This suggests a further reason why a strong spring may be favourable. It provides a large range of forces, either side of the stalling force, for which the average velocity is very close to zero and the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

ATP rate is low. This permits muscle to support a variety of loads while remaining at an almost constant length and without expending much energy.

(c) *Instabilities, transients and oscillations*

The shape of the force–velocity curve provides a clue that a dynamic transition may exist in muscle fibres. Is there any more direct experimental evidence for instabilities in muscle? In practice, the range over which the chemistry of myosin molecules could be synchronized is limited by the compliance of the thin and thick filaments. The strain generated by one head is not communicated over an indefinite distance, but diminishes exponentially with the number of myosin heads passed. Given that filament compliance in muscle fibres is found to be approximately equal to the combined compliance of the myosin cross-bridges (Huxley *et al.* 1994; Wakabayashi *et al.* 1994; Higuchi *et al.* 1995), one can estimate that the range of coordination extends over a few sarcomeres, at most. Thus, the detection of stepwise motion or biochemical synchronization during shortening under a constant load would require a very local probe. It is questionable, then, whether the apparent steps detected in laser diffraction experiments (Pollack *et al.* 1977) are a signature of this instability: more likely, they are an artefact of the experimental technique (Rüdel & Zite-Ferenczy 1979; Altringham *et al.* 1984).

There is, however, one experiment in which the absence of a steady state in near-isometric conditions would have a clear, observable consequence: The isotonic transient response, in which an isometric muscle fibre is subjected to a sudden decrease in load (Podolsky 1960). The external mechanical stimulus would synchronize the power stroke transitions of the bound myosin heads throughout the filament. Subsequently, individual sarcomeres would start to shorten in a stepwise fashion but, owing to filament compliance, the coordination of different sarcomeres would gradually be lost. The consequence would be an apparent, damped oscillatory motion of the fibre. This is precisely what is observed in muscle (Podolsky 1960; Huxley 1974)

More generally, the ability to synchronize biochemical reactions through strain-dependent kinetics suggests a mechanism to generate sustained oscillations. Oscillations are characteristic of many motor protein systems, including insect flight muscle and the axoneme of cilia, and are most probably caused by an instability in the collective dynamics of the motor proteins. The type of dynamic transition discussed above, in which a steady state becomes unstable, could naturally provoke a self-sustained vibration with an amplitude approximately equal to the length of the power stroke and a period similar to the cycle time. It is not the only one in which the collective dynamics of motor proteins can generate an oscillation, however. Another type of instability can arise if the detachment rate g, instead of increasing steadily with sliding, reaches a sharp maximum at the point where the elastic element is unstretched. Then, the force–velocity relationship can be non-monotonic so that, over a particular range of loads, two different steady sliding velocities are possible. This type of instability was first discussed in the context of a Brownian ratchet model (Jülicher & Prost 1995), but has a direct counterpart in kinetic models (Vilfan *et al.* 1999). If the two stable velocities have opposite sign, the instability can be used to generate oscillations by coupling the system to an external spring (Jülicher & Prost 1997). In this case, the frequency and amplitude can be tuned, according to the value of the elastic constant.

(d) *Implications for single-molecule experiments*

Could unstable dynamics, such as stepwise motion, be observed in a motility assay? Since biochemical synchronization depends sensitively on the mechanical coupling, it would be essential to control the compliance and the orientation of the molecules very carefully. Thus, to investigate cooperative effects, micromanipulation experiments using myofilaments would probably be more suitable than motility assays in which actin filaments glide on a bed of myosin molecules. Even so, a gliding assay in which an actin filament was loaded by an electrophoretic force did provide evidence for an instability close to the stalling force (Riveline *et al.* 1998).

The strong strain dependence of the kinetics means that great caution must be exercised in interpreting the results of micromanipulation experiments on single molecules. The behaviour can be extremely dependent on the compliance of the linkages used to hold the molecules in position, on the orientation of the molecules, and on the concentrations of nucleotides used. For example, if an individual myosin molecule could be pinned down as firmly as it is in a myofibril, it might be possible to witness inhibition of the power stroke transition. On the other hand, if a myosin head is attached extremely loosely, it could potentially interact with the thin filament over long distances via the weak binding potential (figure 1), before binding more strongly. Such biased diffusion along the actin helix might explain the successive 5 nm steps (the actin monomer size) that have been observed when a myosin head attached to a very flexible microneedle interacts with an actin bundle (Kitamura *et al.* 1999). Most micromanipulation experiments on single myosin molecules fall between these two extremes, since they have been performed with moderate compliance in the links (Finer *et al.* 1994; Molloy *et al.* 1995; Guilford *et al.* 1997; Mehta *et al.* 1997). In this case, the measurements are likely to underestimate the true value of the power stroke displacement and unitary force of a myosin molecule. Experiments in which the orientation of the myosin molecules was carefully controlled, by binding them in sparse myosin–rod co-filaments (Tanaka *et al.* 1998), may provide the most reliable figure; they indicate that the step size is about 10 nm.

The fit of force production and energy consumption in figure 7 suggests values of the combinations Kd^2 and δ/d, but does not provide individual figures for the three physical parameters, K, d and δ. However, a large power stroke of size $d = 10–12$ nm is most consistent with data from a variety of other sources, in addition to the micromanipulation experiments. An instantaneous step of about this size, per half-sarcomere, is observed when the load on an isometrically contracting muscle is suddenly reduced to zero. Also, the crystal structure of the one M·ADP·Pi analogue that has been solved (Dominguez *et al.* 1998) indicates that the catalytic domain might rotate through as much as 70° on Pi release, which translates to

10 nm displacement of the lever arm. The additional conformational change that occurs when ADP dissociates from the head is predicted to be much smaller than the power stroke, $\delta = 0.5$ nm. Thus, a very modest movement of the lever arm on ADP release is sufficient to regulate the fraction of bound heads. This is consistent with the fact that no conformational change has been detected for this transition in skeletal muscle myosin (Gollub *et al.* 1996). Interestingly, there is structural evidence for a significant shift in the lever-arm position on ADP release in other types of myosin: smooth muscle myosin (Whittaker *et al.* 1995) and brush border myosin (Jontes *et al.* 1995). This is complemented by the recent observation that the head domains of these motors generate displacement in two distinct strokes (Veigel *et al.* 1999). According to the model, it might be anticipated that such structural modifications are functional adaptations, designed to regulate the chemistry according to the load in a different way.

4. SUMMARY

Physical systems in which many identical elements are coupled together generally display complex emergent behaviour. Thus, we should not be surprised to find that collective effects occur when many motor proteins act together—indeed, it would be more surprising if dynamic transitions and instabilities were absent. For molecular motors, cooperativity arises through strain dependence of the chemistry, which ensures that molecules which are mechanically connected are also kinetically coupled. The structure of the myosin head, which contains a movable lever arm, suggests a physical basis for the strain dependence of the kinetics; the transition rates are altered according to the work done in deforming an elastic element within the molecule when the arm shifts position. An important implication is that structural variations of the molecule can be used to adapt the motor to specific tasks. For skeletal muscle myosin, we have argued that a small movement of the lever on ADP release permits regulation of the fraction of bound motors according to the load, providing a combination of rapid, unloaded shortening and a high isometric tension. A strong elastic element, which causes the power stroke to fail at high load, would equip muscle to support a wide range of loads at nearly constant length and, at the same time, would ensure high collective efficiency. It would also lead to a dynamic instability at high load, in which the chemical reactions of the individual myosin molecules become synchronized. Such instabilities might form the basis of oscillations in this, and other motor protein systems.

REFERENCES

Altringham, J. D., Bottinelli, R. & Lacktis, J. W. 1984 Is stepwise sarcomere shortening an artefact? *Nature* **307**, 653–655.

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

Dominguez, R., Freyzon, Y., Trybus, K. M. & Cohen, C. 1998 Crystal structure of a vertebrate smooth muscle myosin motor domain and its complex with the essential light chain: visualization of the pre-power stroke state. *Cell* **94**, 559–571.

Duke, T. A. J. 1999 Molecular model of muscle contraction. *Proc. Natl Acad. Sci. USA* **96**, 2770–2775.

Edman, K. A. P. 1988 Double-hyperbolic force–velocity relation in frog muscle fibres. *J. Physiol.* **404**, 301–321.

Eisenberg, E., Hill, T. L. & Chen, Y. D. 1980 Cross-bridge model of muscle contraction. *Biophys. J.* **29**, 195–227.

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

Gollub, J., Cremo, C. R. & Cooke, R. 1996 ADP release produces a rotation of the neck region of smooth myosin but not skeletal myosin. *Nat. Struct. Biol.* **3**, 796–802.

Guilford, W. H., Dupuis, D. E., Kennedy, G., Wu, J., Patlack, J. B. & Warshaw, D. M. 1997 Smooth muscle and skeletal muscle myosins produce similar unitary forces and displacements in the laser trap. *Biophys. J.* **72**, 1006–1021.

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

Hill, A. V. 1964 The efficiency of mechanical power development during muscular shortening and its relation to load. *Proc. R. Soc. Lond.* B **159**, 319–324.

Hill, T. L. 1974 Theoretical formalism for the sliding filament model of contraction of striated muscle: part I. *Prog. Biophys. Mol. Biol.* **28**, 267–340.

Holmes, K. C. 1997 The swinging lever-arm hypothesis of muscle contraction. *Curr. Biol.* **7**, R112–R118.

Huxley, A. F. 1957 Muscle structure and theories of contraction. *Prog. Biophys. Biophys. Chem.* **7**, 225–318.

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

Huxley, A. F. & Niedergerke, R. 1954 Interference microscopy of living muscle. *Nature* **173**, 971–973.

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

Huxley, A. F. & Tideswell, S. 1996 Filament compliance and tension transients in muscle. *J. Muscle Res. Cell Motil.* **17**, 507–511.

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

Huxley, H. E. & Hanson, J. 1954 Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature* **173**, 973–976.

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

Jontes, J. D., Wilson-Kubalek, E. M. & Milligan, R. A. 1995 A 32° tail swing in brush border myosin-I on ADP release. *Nature* **378**, 751–753.

Julian, F. J., Sollins, K. R. & Sollins, M. R. 1974 A model for the transient and steady-state behavior of contracting muscle. *Biophys. J.* **14**, 546–562.

Jülicher, F. & Prost, J. 1995 Cooperative molecular motors. *Phys. Rev. Lett.* **75**, 2618–2621.

Jülicher, F. & Prost, J. 1997 Spontaneous oscillations of collective molecular motors. *Phys. Rev. Lett.* **78**, 4510–4513.

Kitamura, K., Tokunaga, M., Iwane, A. H. & Yanagida, T. 1999 A single myosin head moves along an actin filament with regular steps of 5.3 nm. *Nature* **397**, 129–134.

Kramers, H. A. 1940 Brownian motion in a field of force. *Physica Utrecht* **7**, 284–304.

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

Mehta, A. D., Finer, J. T. & Spudich, J. A. 1997 Detection of single-molecule interactions using correlated thermal diffusion. *Proc. Natl Acad. Sci. USA* **94**, 7927–7931.

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

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

ate, E., White, H. & Cooke, R. 1993 Determination of the myosin step size from mechanical and kinetic data. *Proc. Natl Acad. Sci. USA* **90**, 2451–2455.

iazzesi, 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.

odolsky, R. J. 1960 The kinetics of muscle contraction: the approach to the steady state. *Nature* **188**, 666–668.

ollack, G. H., Iwazumi, T., Ter Keurs, H. E. D. J. & Shibata, E. F. 1977 Sarcomere shortening in striated muscle occurs in stepwise fashion. *Nature* **268**, 757–759.

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

.eedy, M. K., Holmes, K. C. & Tregear, R. T. 1965 Induced changes in orientation of the cross-bridges in glycerinated insect flight muscle. *Nature* **207**, 1276–1280.

.iveline, D., Ott, A., Julicher, F., Winkelmann, D. A., Cardoso, O., Lacapere, J. J., Magnusdottir, S., Viovy, J. L., Gorre-Talini, L. & Prost, J. 1998 Acting on actin: the electric motility assay. *Eur. Biophys. J.* **27**, 403–408.

.üdel, R. & Zite-Ferenczy, F. 1979 Do laser diffraction studies on striated muscle indicate stepwise sarcomere shortening. *Nature* **278**, 573–575.

Smith, D. A. & Geeves, M. A. 1995 Strain-dependent cross-bridge cycle for myosin. *Biophys. J.* **69**, 524–537.

Tanaka, H., Ishijima, A., Honda, M., Saito, K. & Tanagida, T. 1998 Orientation dependence of displacements by a single one-headed myosin relative to the actin filament. *Biophys. J.* **75**, 1886–1894.

Uyeda, T. Q. P., Abramson, P. D. & Spudich, J. A. 1996 The neck region of the myosin domain acts as a lever arm to generate movement. *Proc. Natl Acad. Sci. USA* **93**, 4459–4464.

Veigel, C., Coluccio, L. M., Jontes, J. D., Sparrow, J. C., Milligan, R. A. & Molloy, J. E. 1999 The motor protein myosin-I produces its working stroke in two steps. *Nature* **398**, 530–533.

Vilfan, A., Frey, E. & Schwabl, F. 1999 Force–velocity relations of two-state crossbridge model for molecular motors. *Europhys. Lett.* **45**, 283–289.

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

Whittaker, M., Wilson-Kubalek, E. M., Smith, J. E., Faust, L., Milligan, R. A. & Sweeney, H. L. 1995 A 35 Å movement of smooth muscle myosin on ADP release. *Nature* **378**, 748–751.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES