Mechanical Components of Motor Enzyme Function

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ABSTRACT Motor enzymes use energy from ATP dephosphorylation to generate movement by a mechanical cycle, moving and pushing in one direction while attached to their cytoskeletal substrate, and recovering by moving relative to their substrate to a new attachment site. Mainstream models assert that movement while attached to the substrate results from preexisting strain in the attached motor. The additional underlying ideas can be described in terms of three components for strain amplification: a rotating lever arm, multiple attached states, and elastic compliance. These components determine how energy is recovered during the mechanical cycle and stored in a strained motor. They may coexist in a real motor; the challenge is to determine the contributions of each component. Because these components can generate similar relationships between strain energy and strain, standard measurements of motor function do not discriminate easily between these components. However, important information could be is provided by observations that suggest weak coupling between chemical and mechanical cycles, observations of negative force and movement events in single motor experiments, and the discovery that two motors that move in opposite directions have very similar structures. In models incorporating changes in conformation between attached states, these observations are only explained easily if the conformational changes are tightly coupled to changes in the strength of motor-substrate binding.

INTRODUCTION

Motor enzymes—myosins, dyneins, and kinesins—power the movements of muscle and cilia, and many microscopic movements of and within cells. For 40 years, our understanding of the generation of movement by these enzymes has been guided by the ideas in the seminal paper by Andrew Huxley (1957). The fundamental idea is that the motor enzyme forms a strained, force-producing "cross-bridge" between two components, such as the actin and myosin filaments of muscle, and that the release of this strain causes movement between the two components. Beginning with the Huxley (1957) paper, numerous mathematically detailed models have been presented for the operation of these enzymes. These models have been elaborated to fit some of the large body of mechanical and biochemical observations on motor enzyme function, especially observations on properties of skeletal muscle. In the past decade, information from measurements of movement and force produced by individual motor enzyme molecules and from high-resolution determinations of molecular structure has become available. This article attempts to build upon 40 years of cross-bridge modeling studies and identify the mechanical components of motor enzyme function—strain amplification, strain energy storage, and strain recovery—that need to be defined by the structural and functional studies that are now possible at the molecular level. Original papers must be consulted for details of the mathematical modeling that demonstrates that particular components can work in a realistic model with thermodynamic detailed balancing.

Throughout this article, the word "motor" is used as a synonym for "motor enzyme molecule." In most cases, a motor does not move independently, but is attached to a "cargo" that moves with it, usually with the help of additional motors. The portion of the molecule that interacts with a cytoskeletal substrate is often referred to as the "head" of the motor. Most motors have more than one head, but single-headed motors have been shown to function effectively under some conditions. In kinesins and dyneins, interactions between heads may be important for normal motor function, but the focus of this article is on the operation of individual motor heads. The next two sections will review the basic assumptions of models that are descendents of the Huxley (1957) model, as a preliminary to discussion of strain amplification and strain recovery.

FOUR BASIC OBSERVATIONS

A polarized substrate polymer

Motors operate on a specific cytoskeletal substrate: myosins on actin filaments, and kinesins and dyneins on microtubules. The cytoskeletal substrate is a polymer with a well-defined polarity that is recognized and utilized by the motor to determine the direction of translocation along the substrate. The substrate is not permanently altered by the passage of the motor.

Energy from ATP dephosphorylation cycles

The energy needed to operate the motor and perform external work is obtained from repeated chemical cycles involv-

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ing the dephosphorylation of ATP as the motor progresses along its cytoskeletal substrate.

A strong binding state

At least one state in the chemical cycle involves strong interaction of the motor with a specific site on the substrate, similar to the strong binding that is seen in the absence of ATP and described as the "rigor" state. These substrate sites form a repeating series along the cytoskeletal polymer, with spacing equal to the cytoskeletal subunit spacing: 8 nm in the case of microtubules and 5.5 nm in the case of actin filaments.

The existence of a rigor state in the absence of ATP is well documented. Individual myosin molecules in the absence of ATP require an average force of 9.2 pN to mechanically detach the myosin from an actin filament (Nishizaka et al., 1995). Proving the existence of this state within a chemical cycle that is coupled to movement of a motor is more challenging. Oplatka (1996) summarizes arguments against the existence of this state and discusses a model that does not require it. However, the mechanical definition of the attached state is simply an interaction that can generate a sustained force if the motor is not at its equilibrium attachment position. This has now been demonstrated by measurements on single myosin molecules in the presence of ATP (Finer et al., 1994). These measurements appear to exclude models in which the chemical cycle of the motor transfers an impulse to the substrate (e.g., Oplatka, 1996).

An exceptional case is provided by the β/IC$_1$ fragment of axonemal outer-arm dynein. These single-headed motors translocate microtubules at high velocity in an in vitro motility assay (Sale and Fox, 1988), but do not bind stably to microtubules, in either the absence of ATP or the presence of β,γ-imidoadenosine-5′-triphosphate (AMPPNP) (Moss et al., 1992). A short-lived, strong binding state may be sufficient to explain this motor's function, but this state has not been demonstrated. More commonly, the strongly bound state is a stable state that has an extremely low rate of spontaneous detachment.

The term "cross-bridge" is used to refer to a motor that is attached to its substrate.

Dissociation by ATP

Binding and hydrolysis of ATP destabilize the strongly bound state and allow the chemical cycle to proceed through a series of detached or weakly interacting states. For simplicity, these states will be referred to here as detached. The operational definition is that movement of the motor head while "detached" does not drag the substrate along with it to any significant extent. In the case of myosin, binding of ATP is sufficient for destabilization, but with kinesin, destabilization does not occur until after the ATP is hydrolyzed (Crevel et al., 1996; Hackney, 1996).

THE FUNDAMENTAL ASSERTION: CROSS-BRIDGE STRAIN PRECEDES MOVEMENT

When an attached motor is strained, by pulling it away from its equilibrium position, it generates a force and possesses strain energy. An opposing force is required to maintain the strain and prevent the motor from moving toward its equilibrium, unstrained, position. A strained motor can convert its strain energy to external work when it moves toward its unstrained position. The assertion that strain energy exists in the cross-bridge before movement distinguishes motor enzyme function from other possible mechanisms for biological motion, such as rectification of the thermal motion of a motor protein and its attached cargo (e.g., Peskin et al., 1993).

The role of strain energy can be portrayed by a free energy diagram, such as the example in Fig. 1. The slope of the free energy curve represents force. As shown here, the motor can exert a force during either positive or negative strains; the symmetry is not necessary and is abandoned in some models (e.g., Cooke and Bialek, 1979). The presence of force in the negative strain region was incorporated into the Huxley (1957) model as a major part of the explanation for the decrease in force at high shortening velocities, when cross-bridges are pulled into the negative strain region before they have time to detach. Equilibrium between detached and attached states has been examined in detail by Schoenberg (1985). If force is a linear function of strain, corresponding to the example in Fig. 1, the strain dependency of the equilibrium can be described by a Gaussian

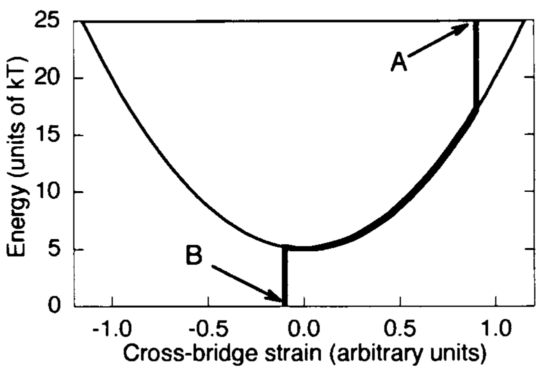

FIGURE 1 An energy level diagram for a conventional cross-bridge working stroke, described in terms of an oversimplified two-state model. The parabolic curve represents the strain-dependent energy, $A(x)$, in an attached motor, with x representing cross-bridge strain. If the motor has a linear force-versus-strain relationship with a force constant, K_F, then $A(x) = A(0) + 1/2\ K_F x^2$ (cf. Hill, 1974). The relative energy levels of detached motors are represented by the horizontal lines at 0 and $25kT$, and the force constant has been given an arbitrary value of $30kT$ per strain unit^2. The energy unit, kT, is Boltzmann's constant multiplied by the absolute temperature. As an example, the heavy line represents energy changes for a motor that starts in the detached state at A, attaches at a strain of 0.9 units, moves to a strain of -0.1 units, and is detached by ATP binding into the deattached state at B, with a total energy loss of $25kT$. The strain energy loss (in this case, $12kT$) that occurs while the motor is attached can be used to perform external work as it moves.

function (Fig. 2). The solid curve in Fig. 2 describes the strain dependency of the equilibrium constant, resulting from the difference in energy between a strained, attached state and a detached state in equilibrium with it. The equilibrium probability of being in the attached state is illustrated by the dashed curves in Fig. 2, for several cases with differing stabilities of the attached state.

The existence of a strongly bound state requires that long-distance movement of a motor along a substrate must result from repeated mechanical cycles of attachment and detachment. To generate movement in a consistent direction, the entry and exit of motors into strongly bound states (or, "the attachment and detachment of cross-bridges") must be biased, so that, on average, motors attach with high positive strain and detach when they have low strain, as illustrated by the heavy line in Fig. 1. The mechanical cycle of a working motor is therefore a sequence involving attachment, strain release, detachment, and strain recovery, with biases that determine the direction of movement. The motor moves in one direction during strain release while attached to the substrate, and then recovers while detached from the substrate. When formulated by Huxley (1957), it was a bold assertion to say that familiar macroscopic movement cycles used for animal locomotion, such as walking and rowing, could be extended to the molecular level. Subsequently, it was given a solid thermodynamic basis by the work of Hill (1974, 1975). Forty years later, this idea has survived many challenges, and is now being confirmed by observations and measurements at the single-motor level.

The chemical and mechanical cycles must be coupled so that the energy of the ATP dephosphorylation cycle is used to bias the attachment and detachment of cross-bridges. This coupling is not considered in detail here, but has been examined thoroughly by Eisenberg and Hill (1985), Smith and Geeves (1995a), Krupka (1996), and many other authors. Most models have assumed that the coupling allows the energy available from hydrolysis of one ATP molecule (up to ~60 kJ/mol, equivalent to 25*kT* or 100 pN nm per ATP molecule) to be used efficiently by one attachment-detachment cycle. In this case, the difference in strain energy between the points of attachment and detachment needs to be as much as 10–15*kT*, and should be at least as large as measured values of work output per ATP molecule (8–12*kT*; e.g., Curtin and Wooledge, 1993; Bagshaw, 1993). By specifying free energy curves, such as that in Fig. 1, and a thermodynamically consistent set of rate functions for attachments and detachments with and without ATP binding, many mathematical models have been constructed that successfully predict the basic features of motor performance. This can be done independently of any molecular explanations for strain energy storage, strain release, strain recovery, or chemomechanical coupling, but there have been many attempts to derive model specifications from molecular mechanisms. These attempts differ primarily by emphasizing distinct mechanical components for strain amplification, discussed in the following section.

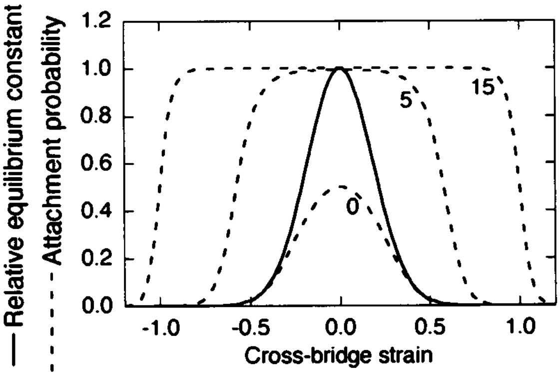

FIGURE 2 The effect of cross-bridge strain on a simple equilibrium between attached and detached motors, governed by $K_{EQ} = \exp(A_1 - A_2(x)/kT)$, where A_1 represents the free energy of the detached state and $A_2(x)$ represents the free energy of the attached state. The solid curve can be interpreted as the effect of strain on the attachment rate, if the detachment rate is constant, or the equilibrium constant for the case where there is no free energy difference between attached and detached states at 0 strain ($A_1 = A_2(0)$). The dashed curves show the equilibrium probability that a motor will be in the attached state, for cases where attachment of the unstrained motor is stabilized by 0, 5, or 15*kT* of free energy difference. This probability equals $K_{EQ}/(1 + K_{EQ})$. For this example, as in Fig. 1, one unit of strain corresponds to 15*kT* of strain energy.

STRAIN AMPLIFICATION

The strain of an attached motor (a cross-bridge) is measured by the displacement between the cargo end of the motor and the substrate, in a direction parallel to the substrate, that is required for complete strain release. Strain release is complete when the cross-bridge is no longer exerting any force in this direction.

The amount of strain involved in motor enzyme function is difficult to measure precisely, because it is difficult to eliminate all of the compliances in series with a cross-bridge. Experimental observations of striated muscle were originally interpreted as evidence of cross-bridge strain of 10 nm or more. However, recent recognition of significant actin filament compliance has led to a reevaluation of these observations (Huxley et al., 1994; Higuchi et al., 1995), suggesting that myosin motors may operate with cross-bridge strains of 5 nm or less. The current consensus is that cross-bridges operate with strains of approximately the same magnitude as the binding site repeat distances on the cytoskeletal substrate. It is difficult to see how single motors could produce movement (Howard et al., 1989; Vale et al., 1996) if their operating strains were much less than the binding-site repeat distances. If a myosin exerts the force of 9.2 pN measured by Nishizaka et al. (1995) and does 10*kT* of work during strain release, an initial strain of at least 4.3 nm is required.

The intermolecular forces responsible for the strong binding interaction between a motor and its substrate are a potential source of force that can resist externally imposed strain. New structural information has allowed molecular dynamics calculations that confirm that the local binding energies between myosin and actin are comparable to the energy converted to work by the mechanical cycle of a motor (Díaz Baños et al., 1996). The energy of interaction between a motor and a binding site on the cytoskeletal substrate will probably be described by a sigmoidal curve (e.g., Pauling, 1940; Moy et al., 1994), such as the ones shown by the dashed curves in Fig. 3 *B*. Regardless of the exact shape of the curve, the necessary feature is a region around 0 strain where the slope of the curve (the force) increases with distance (strain). In this region, a stable equilibrium between an external force and the binding force can be obtained at some nonzero value of strain. The binding of a ligand to a site on a protein is likely to involve forces acting over distances of less than 1 nm (Cordova et al., 1992; Eisenberg and Hill, 1978; Moy et al., 1994). Only limited amounts of strain can be accommodated at the binding site before detachment occurs. To explain strains on the order of 5 nm or more, the strain allowed by the binding site itself must be amplified by other components of the motor. Because a major component of binding force might be expected to be normal to the substrate polymer, whereas strain and movement are parallel to the substrate polymer, a change in force direction by the amplification mechanism is also required.

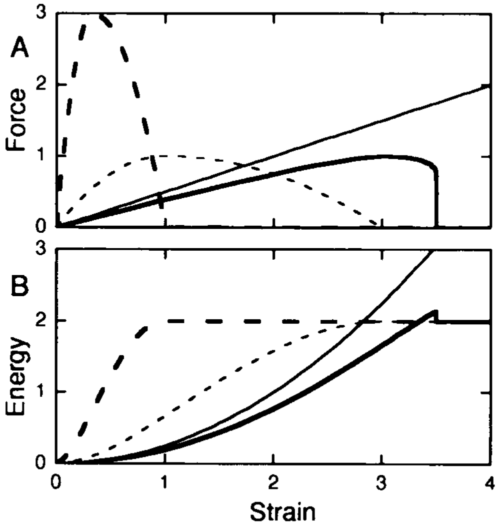

FIGURE 3 Amplification of binding-site strain. The heavy dashed curves represent the binding force and energy for motor-substrate interaction, measured along the reaction coordinate between attached and detached states. The lighter dashed curves show the binding force and energy after amplification by a lever arm with a 1:3 amplification factor. All values are relative, with unit force corresponding to the maximum force that is obtained after amplification, at 1 unit of strain. The thin solid line represents a linear elastic compliance, with a force constant that gives 1 unit of force at 2 units of strain. The thick solid line represents the series combination of the amplified binding-site interaction and the elastic compliance. Addition of this elastic compliance gives a further 1:3 factor of strain amplification. These 1:3 amplification factors have been chosen simply for clarity of presentation; values for a real motor could be very different. At the level of a single motor molecule, these curves only describe time averages. Instantaneous values will reflect substantial variations resulting from thermal energy fluctuations. The curves should be used as a basis for determining probabilities of stable attachment, rather than deterministically.

Three mechanical components have been proposed as strain amplifiers. These components increase the compliance of the cross-bridge; this is equivalent to a reduction in "force constant" of the cross-bridge, or an appropriate expression for a nonlinear relationship between force and strain. The distinction between these three components is significant, because they correspond to different forms of energy storage in a strained motor. Strain amplification could occur either within the substrate or within the motor, or both, but most models consider strain amplification to be a property of the motor. Models involving strain amplification within the substrate (e.g., Schutt and Lindberg, 1992) will not be discussed here, because the purpose is to illuminate general mechanical ideas.

Strain amplification by a lever arm

With appropriate geometry, a lever arm provides a natural mechanism for changing the direction of force and movement and amplifying the intrinsic compliance of a binding site. H. Huxley (1969) proposed that small strains or changes in conformation near the myosin-actin interface could be amplified by action of the rest of the elongated head, or subfragment-1 (S1) segment, of the myosin molecule as a rigid lever arm, so that rotation of this lever arm could produce a relatively large translation between the actin and myosin filaments. This idea has recently been supported by information about the structure of the myosin S1 segment (Rayment et al., 1996) and by experiments in which the length of the lever arm portion of the myosin motor has been experimentally manipulated (reviewed by Block, 1996; Holmes, 1996; Ruppel and Spudich, 1996).

Fig. 4 illustrates these ideas in a cartoon form that indicates the structural requirement for a fulcrum as well as a lever arm. The effect of lever amplification on the force and energy functions of strain is illustrated by the dashed curves in Fig. 3. For the binding interaction itself, the heavier pair of dashed curves should be interpreted as functions of distance along the reaction coordinate, which might be approximately perpendicular to the cytoskeletal filament. Amplification by a simple lever with a 1:3 ratio gives the lighter pair of dashed curves, now plotted as functions of strain along the direction of movement, parallel to the cytoskeletal filament. This extends the range of strains over which force is produced by a factor of 3, as well as changing the direction of strain and force. For illustration, parabolic force curves have been drawn to obtain a continuous slope at the force maximum; the appropriate shapes for an actual motor-substrate interaction are not known. All of the strain energy is still stored in the binding interaction between the motor and the substrate site. With the particular curves drawn here, only 33% of the binding energy is stored and available for performance of work at the strain that gener-

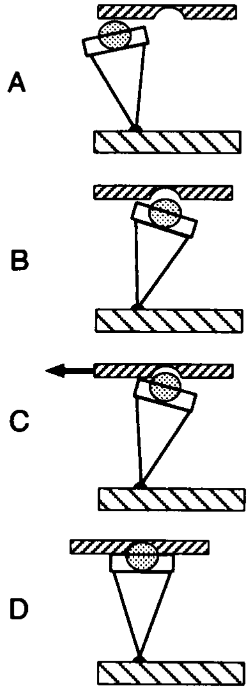

FIGURE 4 Cartoon representation, derived from Brokaw (1995), of a motor in which a lever arm amplifies the strain in the binding interaction between the motor and its substrate. The upper bar represents the substrate, with one of the substrate binding sites represented by the open semicircle. The lower bar represents the cargo or a filament such as a myosin filament. *A* and *B* represent just two of the many configurations that are possible in the detached state, with the motor pivoting diffusively around its attachment point on the lower bar. When it is in the position shown in *B*, it can equilibrate with the strongly attached state shown in *C*, where the binding force that is trying to pull the motor head into the binding site generates sliding force, indicated by the heavy arrow. If movement is possible, the strain in the binding site, amplified by the lever arm, will cause the motor to move to the unstrained position shown in *D*.

ates maximum force. However, in a real motor, evolutionary adaptation might produce very different curves to give optimal motor function.

Strain amplification by an elastic compliance within the motor

An elastic compliance, located somewhere between the attachment of the motor to its cargo and the site of attachment to a cytoskeletal substrate, can amplify strain and modify the shape of the force-strain relationship of the motor. It provides an additional site for storage of strain energy that can be utilized to do work during strain release. In muscle, elastic compliance in the actin and myosin filaments may contribute to the total elastic compliance between an individual motor and the external load (Mijailovich et al., 1996). The traditional location proposed for an elastic compliance within the motor has been as a link between the head domain and the rest of the motor molecule, as discussed, for example, by Duke and Leibler (1996). It is not known whether such a component could swivel around to amplify both positive and negative strain by extension; other difficulties with this location are mentioned later. An elastic compliance in the motor could arise from bending or torsion, as well as by extension or compression. However, for the purpose of communicating ideas by cartoons, representing the elastic element by something that extends like a familiar coil spring between the motor head and its support (Fig. 5) may be particularly effective. It is conventional to assume a spring with a linear force-strain relationship, but it is easy to think of molecular situations that would give a nonlinear relationship (e.g., Hill, 1974). The effects of combining an elastic compliance in series with the compliance of a binding site interaction are illustrated in Fig. 3. In this particular combination, the magnitude of the elastic compliance is matched to the properties of the binding interaction, with the result that the force increases with strain over most of the range of strains that can be sustained by the motor before detachment. In all three cases, application of an external force greater than the maximum force shown in Fig. 3 *A* causes strain to increase rapidly until complete detachment occurs. In the cases indicated by the dashed lines, the external force continues to do work on the system while the strain increases to the value where the interaction force is 0. However, with an elastic element as illustrated by the solid line, the energy stored in the elastic element performs this work, and there is an

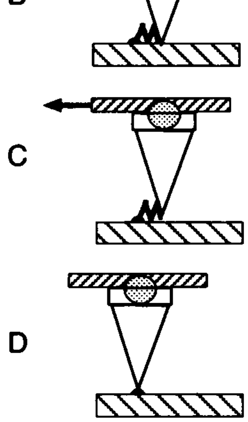

FIGURE 5 Cartoon representation of a motor in which strain energy is stored in an elastic compliance. In this case, the movement of the detached motor (*A*, *B*) involves extension of an elastic element, represented by the heavy jagged lines, that tethers it to the lower bar, and the motor does not rotate. When it is in the position shown in *B*, it can equilibrate with the strongly attached state in *C*, and the strain in the elastic element generates force, which may lead to movement to the unstrained position shown in *D*.

abrupt drop in the externally detectable force. As a result, almost all of the energy in the elastic compliance is recovered in the higher energy level of the detached motor.

Some models, such as the usual explanation of the Huxley (1957) model and the pure thermal ratchet model of Cordova et al.(1992), have been based on the idea that an elastic compliance is the only site of strain energy storage in the motor (Fig. 5). In this case, the strain coordinate must be perpendicular to and completely independent of the reaction coordinate for the binding-site interaction. Describing the role of the elastic element as strain amplification is no longer accurate, because strain parallel to the substrate does not cause any strain in the binding-site interaction. In this case, the probability of detachment of the motor from the binding site would be independent of strain, and it would be difficult to understand experiments (Nishizaka et al., 1995) where strain causes detachment. If the elastic compliance is located as indicated in the cartoon representation in Fig. 5, this imposes a very stringent structural requirement for an "orientationally rigid" (Smith and Geeves, 1995a) connection between the head of the motor and the elastic element, so that no rotation is possible. This is an idealized situation that is unlikely to be realized in any real motor. Any real motor is likely to combine elastic compliance and binding-site compliance, but they will not necessarily be matched as in the example shown in Fig. 3.

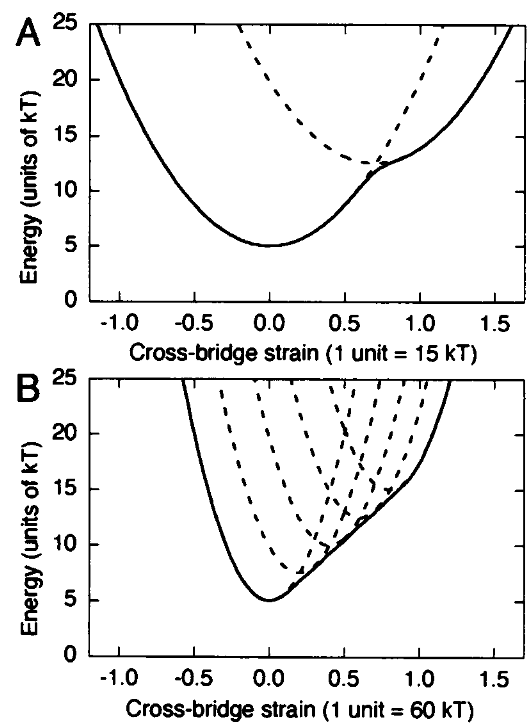

FIGURE 6 Energy levels for an attached motor with multiple states. Energy levels for individual states are shown by the dashed lines. The composite energy for the system when there is rapid equilibration between the states is shown by the solid lines, calculated using equation 70 of Hill (1974).

Strain amplification by a wedge, created by multiple strongly bound states

Motor compliance can be increased by the presence of multiple attached states, with the unstrained position for each state corresponding to attachment at sites that give different values for the overall strain of the motor. The simplest version of this idea, with two strongly bound states, is illustrated by Fig. 6 *A*. The dashed curves in Fig. 6 *A* show energy levels for the individual states, with each attached state having a parabolic dependence of strain energy upon strain. Under conditions where there is very rapid and unimpeded equilibration between these states, the composite effect of these two states is illustrated by the solid line in Fig. 6 *A*. Another example, with five states, is illustrated in Fig. 6 *B*. In this manner, a cross-bridge force-strain relationship extending over 5 nm or more could be constructed from a series of binding-site interactions, each with a realistically narrow range of binding-site compliance. As illustrated in Fig. 6 *B*, the force, derived from the slope of the composite energy curve (*solid line*), can be nearly constant, independent of strain, over a large range (Huxley and Simmons, 1972; Hill, 1974; Astumian and Bier, 1996). In a real situation, where there are finite rates of equilibration between states, effective compliance will depend on the rate of change of strain. Very fast changes will measure the force-strain relationship of individual states, whereas slow changes will measure the composite force-strain relationship. Starting with Huxley and Simmons (1971), fast force transients and other dynamic experiments have been recognized as an important source of information about multiple attached states.

Multiple states of motor-substrate binding, with additional strain amplification by a rotating lever, appeared in the models of Huxley and Simmons (1971, 1972) that were designed to explain force recovery after rapid stretch or release of skeletal muscle. A structural basis for this type of multiple-state binding is now available (Díaz Baños et al., 1996). A more extended interaction region, equivalent to multiple states derived from a series of substrate sites as in Fig. 6 *B*, without lever rotation, appeared in the model of Astumian and Bier (1996) to produce an asymmetrical force-strain relationship that could function as a molecular ratchet, without internal motions of the motor. A ratchet uses a wedge, or inclined plane, instead of a lever to produce strain amplification and change the direction of force. In addition, a ratchet has asymmetry that biases the direction of strain recovery, as described later. The model of Astumian and Bier (1996) is dependent upon the creation of a wedge by a region of interaction covering most of the subunit repeat distance, within which the motor is drawn toward positions of gradually more stable interaction, to obtain a working stroke of 5 nm or more. This requirement is unsupported by structural studies. It requires more than just the extended region of protein-protein interaction on the surfaces of the substrate and the motor that is suggested by structural studies (Milligan, 1996). It offers no easy explanation for the results of experimental changes in the length of the lever arm and experimental observations interpreted as rotation of the lever arm (e.g., Irving et al., 1995).

Composite models

A lever arm can amplify strain that has already been amplified by multiple states or by elastic compliance. The more common use of multiple states has been for conformational changes near the motor-substrate interface, in combination with rotation of a lever arm, to produce a working stroke of 5 nm or more. The lever arm can simply expand the strain scale for the energy curves shown in Fig. 6 *A*. However, many models with two or more attached conformations have postulated much narrower ranges of intrinsic strain within each conformational state, giving energy diagrams more like the heavy curve in Fig. 7 (Huxley and Simmons, 1971, 1972; Smith and Geeves, 1995a). In this case, intermediate degrees of strain move the motor through a large range of very unstable intermediate conformations. In these models, the addition of elastic compliance is assumed to convert an energy diagram with very narrow potential wells to the type shown by the thinner curves in Fig. 7. The underlying rationale for these diagrams is that the rotating lever arm amplifies the difference between the two states, which lies along the strain coordinate, but does not amplify strain along the reaction coordinate for binding to the substrate. It is even more difficult to imagine a structure that could achieve this isolation when the motor has two conformations with very different orientations of a lever arm.

There is evidence for strain amplification by a lever arm, and there are strong reasons to favor models in which strain amplification is provided by multiple mechanically distinct states of motor attachment. However, all of these components can generate similar energy diagrams, so little information is available to assess the relative contributions of the three forms of strain amplification in real motors. In particular, the location of an elastic compliance and its role in strain amplification and strain energy storage have not been established.

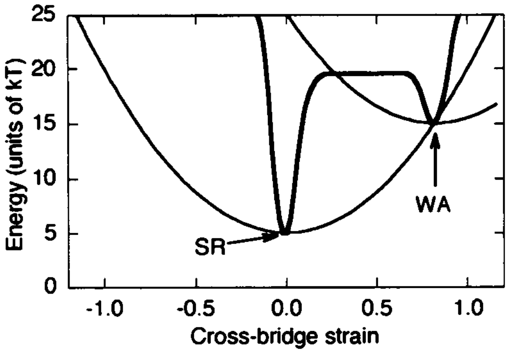

FIGURE 7 Energy levels for weakly attached (WA) and strongly attached (SR) states of a motor, which have different unstrained positions, indicated by the arrows. If the binding energies of the attached states are very steep functions of strain, even after strain amplification by a lever arm, they might be represented by the heavy curve. In this case, a large amount of strain amplification by elastic compliance is required to give the curves shown by the thin curves, which represent the total strain dependence for the attached states of a composite model. The solid curve is intended to represent only the ideas in papers by Smith and Geeves (1995a) and others, and is not intended to be an exact representation of any model.

RECTIFICATION OF CROSS-BRIDGE DISTRIBUTIONS BY STRAIN-DEPENDENT DETACHMENT

Regardless of the mechanism for strain amplification, an equilibrium distribution of strained cross-bridges, such as those shown in Fig. 2, can be "rectified" by a strain-dependent detachment rate that uses energy from the ATP dephosphorylation cycle to selectively detach cross-bridges that have negative strains. Smith and Geeves (1995a) rationalized this in molecular terms as blockage of ADP release and ATP binding at positive strains. The result will be an asymmetrical distribution of cross-bridges with mostly positive strains that can produce a nonzero average force and translocation toward negative values of strain. Direct evidence for different detachment rates at positive and negative strains, in the presence of ATP, has now been reported from single-motor experiments with myosin (Molloy et al., 1995b). By itself, this mechanism is not very efficient (Hill, 1974), because many motors attach at negative or weakly positive strains, and are detached by ATP binding, without doing significant work. To obtain reasonable efficiency, the mechanism for strain recovery also must be biased to selectively favor attachment at positive strains. The simplest possible models, with only one strain-dependent rate function, incorporate strain dependency during strain recovery, and abandon strain-dependent detachment (Duke and Liebler, 1996).

STRAIN RECOVERY

After ATP-induced detachment, the mechanical cycle must continue with strain recovery, involving the movement of the motor head to a favorable position for reattachment and the reacquisition of strain energy by the motor. A detached motor state has a free energy that is independent of strain. The overall energetics of strain recovery are summarized by the simple free energy diagram in Fig. 1, where the horizontal line at $25kT$ represents the free energy of the detached motor state, and the parabola represents the free energy of a strongly attached state. The vertical line from point *A* to the parabola represents a difference in free energy that stabilizes the attached state if the strain is fixed at this value. The total free energy difference of $20kT$ between the detached state and the unstrained attached state is necessary to maintain very stable attachment when the attached motor is at a value of strain where the strain energy is up to $\sim 15kT$ (Fig. 1). This free energy difference explains the direction of the chemical reaction—from the detached state to the strained, attached state, and ultimately to the unstrained, attached state—but does not define the rates of the reaction or their dependence upon strain (Hill, 1974).

Each of the three components of strain amplification has distinct requirements for strain recovery. Hypothetical models using only one type of strain amplification will be discussed first.

Strain recovery with lever-arm strain amplification of motor-substrate binding

When all of the strain amplification is provided by a rotating lever arm, as cartooned in Fig. 4, strain energy is obtained from the energy of formation of the strongly attached state, after a motor head diffuses to an appropriate position for reattachment. The movement of the detached motor head, relative to the substrate site on the upper bar, results from rotational diffusion around the joint at the lower end of the motor and the elastic element, as well as relative movement of the substrate and cargo. When the detached motor head is positioned relative to an attachment site as shown in Fig. 4 *B*, it is allowed to undergo the transition to the strongly attached state shown in Fig. 4 *C*. This is clearly a two-step process, with attachment following movement to the configuration shown in Fig. 4 *B*, but it does not require that the detached configuration in Fig. 4 *B* be defined as a distinct state. This type of model has structural requirements for a lever arm and a fulcrum, to amplify the strain inherent in the binding-site interaction. It also requires a strain-dependent transition rate, to ensure that the transition to the strongly bound state only occurs when the motor is appropriately positioned. In particular, transitions between the detached and attached states must be blocked when the motor is positioned so that it would not be strained after attachment (Fig. 4 *D*).

An improved version of this model, cartooned in Fig. 8, considers the detached state to be a state that allows free rotation, within a range including the configurations drawn in Fig. 8, *A* and *B*, of the lever-arm portion of the motor relative to the portion of the motor head that is in contact with the substrate. This allows the detached state to be a weakly interacting state that keeps the head of the motor close to a binding site. Because free rotation within the motor is allowed, translation does not strain the weak binding interaction, until the range of free rotation is exceeded, at which time the head releases and diffuses to an adjacent binding site.

When the motor is in the configuration shown in Fig. 8 *B* and positioned for attachment to a substrate site, as indicated, it can equilibrate with the attached state shown in Fig. 8 *C*, just as already suggested for equilibration between the states shown in Fig. 4, *B* and *C*. Because this is not the equilibrium conformation for the attached state, it is strained, and generates sliding force, represented by the heavy arrow. This improved version of the model allows the operation of the motor to be described in terms of two distinct conformational changes within the motor. One conformational change occurs in the substrate-binding region of the motor, changing it from a W conformation that binds the substrate site very weakly to a S conformation that has a more favorable conformation for binding to the substrate site, and forms a stable attached state. This W→S change is depicted as the difference between the relative positions of the rectangle and stipled circle in the motor head (Fig. 8, *C* and *D*). The second conformational change (A→R) eliminates the freedom of rotation between the lever arm and the

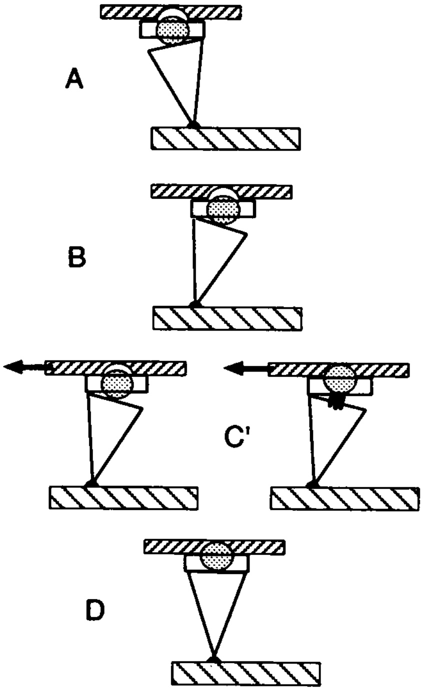

FIGURE 8 Cartoon representation of a composite motor. *A* and *B* can be interpreted as the detached state of a two-state model, which allows free rotation of the lever arm, relative to the motor head, so that the motor head can move freely through a range including the configurations shown in *A* and *B*. There could be a weak interaction between the motor head and substrate sites, with the motor head spending most of its time in positions such as these, where it is weakly bound to a site. Because of the free rotation of the lever arm, the motor in this state does not have strain energy, and is effectively a detached state. Alternatively, *A* and *B* can be interpreted as stable alternative conformations for a weakly attached (WA) state in a three-state model. In this case, the lever arm does not rotate with complete freedom, and the motor can be strained. With either interpretation, when the motor is the configuration shown in *B*, it can equilibrate with the strained, strongly bound state shown in *C*. The force of the binding interaction can generate force, as indicated by the heavy arrow, as it tries to pull the motor head into a stronger interaction with the binding site (the W→S transition) that is shown by the movement of the stippled circle to the positions shown in *C'* or *D*. This W→S transition is coupled to the A→R transition, which eliminates the freedom of rotation of the lever arm, rotating it forcefully to the position shown in *D*. If the motor is provided with an elastic compliance that is large compared to the compliance of the binding site, the position shown in *D* could be reached through the intermediate step shown in *C'*, where a strained elastic compliance in the linkage between the W→S transition and the A→R transition is indicated by the triplet of heavy lines.

motor head, and causes the lever arm to extend perpendicularly and rigidly from the motor head (Fig. 8 *D*). For the motor to perform efficiently, the W→S conformational change is tightly coupled within the motor molecule to the A→R conformational change. In this way, attachment of the motor to the substrate site will generate force (Fig. 8 *C*) or movement as the WA conformation of the motor changes to the unstrained SR conformation (Fig. 8 *D*). However, strain energy recovery results from the overall energy difference between the WA state and the SR state, regardless of the internal details. Eisenberg and Hill (1978) and Krupka (1996) discuss in more detail the direct coupling of binding energy to a conformational change that generates external work, without an elastic compliance. This model is dependent upon a portion of the motor functioning as a lever arm, but the internal conformational changes are substituted for the fulcrum required in Fig. 4. A biased distribution of strained cross-bridges is obtained by specifying that the rate for the WA→SR transition is determined within the motor, by the orientation of the lever arm relative to the substrate-binding domain. In the detached (WA) state, acquiring this orientation does not require the acquisition of significant strain energy.

Strain recovery with multiple-state strain amplification

Multiple-state strain amplification can easily be asymmetrical, as in Fig. 6, creating a molecular ratchet. In this case, with states that are actually different attachment sites along the substrate, Astumian and Bier (1996) have pointed out that even without a state-dependent attachment rate, strain recovery will be biased if ATP-induced detachment always occurs from the most stable state. After ATP-induced detachment, the diffusion distance to the adjacent polymer subunit in the negative direction will be shorter than the diffusion distance to the adjacent polymer subunit in the positive direction. There will therefore be a higher probability that the next reattachment will move the motor in the negative direction (the same direction that is produced by the transition from higher energy sites to the most stable binding site). For this bias to be significant, an extended series of sites on each polymer subunit, similar those in Fig. 6 *B*, is necessary. Details of this type of model are provided by Astumian and Bier (1996).

More commonly, multiple attached states have been described as different conformations, with the transitions between the states amplified by a rotating lever arm. This combination has been commonly used in models for myosin, beginning with H. Huxley (1969) and A. Huxley and Simmons (1971). Although there is evidence supporting at least three states (Huxley and Simmons, 1971; Díaz Baños et al., 1996), simpler two-state models are useful for illustrating the features of multiple-state models. A detailed modern presentation of a model with two attached states, referred to as the A and R states, is provided by Smith and Geeves (1995a,b). The cartoons in Fig. 8 can be reinterpreted for a model with two attached states by specifying that the WA conformation in Fig. 8 *B* is the stable conformation for a weakly attached state, rather than one of many equally stable detached configurations allowed by free rotation of the lever arm. The WA state is then an attached state that has a strain-dependent free energy, and energy levels for the WA and SR states are described by diagrams such as Fig. 7. Strain release involves both movement and a transition from the higher energy attached WA state to the more stable attached SR state that has a more negative unstrained position. However, Fig. 7 should be used only qualitatively; a quantitative picture requires much more detailed consideration of the contribution of multiple chemical states to each of the mechanically distinct states, as presented by Smith and Geeves (1995a).

This combination not only provides significant strain amplification, but also provides a natural explanation for the strain-dependent formation of strongly attached cross-bridges that is required for an efficient motor. Formation of strained cross-bridges during strain recovery is explained by a specification that the rates of equilibration with the detached state are high when the motor is in the WA state, and low when it is in the SR state, without requiring an explicit dependence upon strain. The result is that in a working motor, as in solution (Smith and Geeves, 1995a), the WA state is a near-obligatory intermediate in the formation of the SR state. An ATP-detached motor diffuses rapidly and establishes an equilibrium with the WA state at various values of strain, similar to the equilibrium described in Fig. 2, but shifted to a higher strain value. This symmetrical equilibrium distribution is then rectified by transitions between the WA state and the SR state, to produce a population of motors in the SR state with predominantly positive strains (Smith and Geeves, 1995a). An example is illustrated in Fig. 9. The details of the resulting distribution depend upon the strain dependency for the transition between the WA and SR states; Smith and Geeves (1995a) argue for a form different from the minimal form used for this example and provide a complete model that gives a more realistic demonstration of the role of the WA state in producing a biased distribution of attached motors. This two-step attachment process represents another way in which a biased distribution of attached motors, with positive strain energy, can be said to be created by rectification of an equilibrium distribution. The source of strain energy is still the energy of formation of the attached states. However, because the initial distribution of motors in the WA state is the result of random thermal diffusion, or Brownian movement, this situation has also been described as rectification of thermal energy (Cordova et al., 1992; Smith and Geeves, 1995a).

A mechanically distinct detached state in addition to the WA state is still necessary, although it may be a very transient state that exists only while a motor is moving from one binding site to another. If detachment from the SR state after ATP binding required a direct return to a short-lived

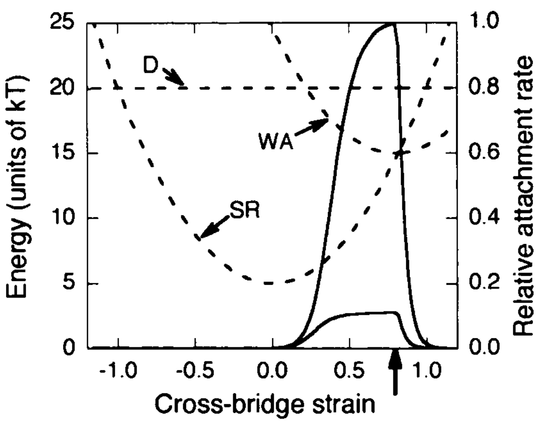

FIGURE 9 Illustration of the effect of an intermediate weakly attached state (WA) on the rate of formation of strongly attached motors. The dashed lines represent energy levels for the two attached states shown in Fig. 7, and a detached state (D) with a strain-independent energy level of 20*kT*. The free energy minimum for the WA state is at a strain value of $x_W = 0.8$ strain units (*heavy arrow*). The model system involves the following scheme:

$$D \underset{k_{1-}}{\overset{k_{1+}(x)}{\longleftrightarrow}} WA \xrightarrow{k_{2+}(x)} SR \xrightarrow{k_{3+}} D$$

The solid lines show the forward, strain-dependent rate of transitions from the detached state to the strongly attached (SR) state, for two values of $k_{2+}(x_W)$, equal to 0.1 $k_{1+}(x_W)$ (*upper curve*) and 0.01 $k_{1+}(x_W)$ (*lower curve*). To enhance the comparison with Fig. 2, k_{1-} is held constant and $k_{1+}(x)$ is determined by the equilibrium constant equation in the legend for Fig. 2, with $x - x_W$ substituted for x. As an illustration, a simple strain dependence is chosen for $k_{2+}(x)$, such that $k_{2+}(x) = k_{2+}(x_W)$ for $x \leq x_W$, and for $x > x_W$ it is decreased by a factor $\exp(-A)$, where A is the additional energy required for the transition from the WA state to the SR state when $x > x_W$. To simplify this illustration, k_{3+} is assumed to be very fast and strain-independent, so that no significant fraction of the motors accumulates in the SR state.

WA state before detachment (Eisenberg and Hill, 1985), the energy barrier resulting from the high strain of the WA state would be too high to allow a realistic detachment rate at high shortening velocities (Smith and Geeves, 1995a). A direct pathway for detachment from the SR state circumvents this problem. Most models with two distinct internal conformations for the attached motor have therefore included two corresponding conformations for the detached motor, with ATP-induced detachment into an R state, followed by transition to a detached A state before reattachment to form the WA state (e.g., Krupka, 1996). However, the otherwise very complete model of Smith and Geeves (1995a,b) did not need to discriminate between these two detached states, and in the structural model of Díaz Baños et al. (1996), the coupling between the W→S and S→R transitions is probably so strong that distinct A and R conformations of the detached motor are not expected.

Some conformational change models correspond to a different interpretation of the coupling between W→S and A→R transitions. In these models, the A→R transition is considered to be driven by energy stored in the motor during the ATP dephosphorylation cycle, when the detached R state changes to the detached A state. Upon attachment, the W→S transition "triggers" the A→R transition, without providing energy for it (e.g., Cordova et al., 1992). Krupka (1996) discusses difficulties with this type of model, in which the A→R transition is not tightly coupled to binding of the motor to its substrate. Although the underlying molecular mechanism is different, the performance during a normal work-producing cycle of attachment and detachment may not reflect this difference.

The "kinetic model" shown in Figs. 6 and 7 of Astumian and Bier (1996) provides further details about how the use of some of the energy of ATP dephosphorylation for strain recovery is a mechanism for increasing efficiency by ensuring that the chemical cycle is coupled to mechanical steps in one direction. In effect, a pair of detached states and a pair of attached states each act as ratchets to cause unidirectional mechanical cycling, by rectifying the ATP-driven cycle of attachments and detachments that are state dependent but not strain dependent. The pair of detached states need not be mechanically distinct A and R states; the significant requirement is a transition to a detached state that cannot directly equilibrate with the SR state. In this view, the difference between a Brownian ratchet model (Vale and Oosawa, 1990) and a conformational change model is not fundamental, but merely a difference in the details of strain amplification and recovery.

Strain recovery with strain energy storage in an elastic compliance

In models where all of the strain energy is stored in an elastic compliance, strain energy must be acquired simultaneously with movement of the motor head to an appropriate position for attachment. The molecular mechanism for acquisition of strain energy is described as rectification of thermal fluctuations. These models utilize only two mechanically distinct states—attached and detached—as represented in Figs. 1 and 5. Beginning with Huxley (1957), two-state models have been shown to reproduce major features of the performance of real motors, if the attachment rate is strain dependent, to increase the probability that attachment will occur when it produces a cross-bridge with a high level of positive strain.

Molecular bombardment of a detached motor will produce fluctuations in energy, averaging *kT*/2 in the strain coordinate. Direct observation of the effects of thermal energy fluctuations, as fluctuations in force or position ("Brownian movement") of a motor, have now been made at the single-molecule level (Molloy et al., 1995a, etc.). Occasionally these fluctuations will give a detached motor an increment of thermal energy comparable to the energies involved in state transformations and work production during a cross-bridge cycle. If the fluctuations in position of a motor head that bring it close to an attachment site require an amount of thermal energy equal to the strain energy in a strained attached motor, attachment does not require any additional source of strain energy. Whether these fluctuations occur frequently enough to explain the properties of skeletal muscle has been questioned (e.g., Huxley and Simmons, 1971, 1972), and combining the two components of strain recovery in this manner has stringent structural requirements. The diffusion of the motor head must be tightly coupled to strain in the elastic compliance of the motor. If the elastic compliance is in the position shown in the representation in Fig. 5, all other connections between the head of the motor and its cargo must be orientationally rigid. Evidence for restricted flexibility at the cargo end of a motor head domain is provided by structural evidence for differences in preferred position of the detached head between kinesin and ncd homodimers that are tethered to a microtubule by one head (Hirose et al., 1996; Arnal et al., 1996). However, it is not easy to see how this requirement could be satisfied when motor heads are randomly attached to surfaces for in vitro motility assays.

To favor attachment at large positive strains, a molecular mechanism is required that rectifies the thermal fluctuations, by strongly suppressing equilibration between attached and detached states when the strain of an attached motor is less than values that are optimal for cross-bridge formation. In molecular terms, information from the strained elastic compliance must be communicated to the substrate-binding domain of the motor to modulate its attachment rate. Structural studies on motors have not yet revealed how this could occur.

The addition of an elastic compliance to a composite model containing conformational changes and a rotating lever arm introduces an additional site for energy storage. Energy diagrams drawn for such models, with curves such as the thinner curves in Fig. 7, are considered to represent strain energy that is temporarily stored in elastic compliance. This will be approximately true if the elastic compliance is much greater than the lever-arm-amplified compliance of the binding sites or conformational states indicated by the heavy curve in Fig. 7 or by figure 2 of Smith and Geeves (1995a). In these models, recovery of strain energy involves the formation of a weakly attached (WA) state, followed by a transition to the strongly attached (SR) state that stores energy in the elastic compliance, as cartooned in Fig. 8 *C′*. With these ideas, locating the elastic compliance in the linkage between the W→S transition and the A→R transition (Fig. 8 *C′*), before amplification by the lever arm, might be more reasonable. This nonvectorial (Brokaw, 1995) location would more easily explain the ability of the elastic compliance to amplify both positive and negative strains. It does not require the rapid back-and-forth rotation of the lever arm during equilibration between the WA and SR states that would be required by an elastic element at the cargo end of the motor. However, unlike the usual placement of an elastic compliance between the motor head and its cargo (e.g., Fig. 5), it only provides elastic strain amplification for the SR state, and does not provide the compliance for the WA state that is indicated by the strain energy curve in Fig. 7. Elastic compliance could be even more closely integrated with the binding interaction, perhaps as part of the flexible P and M loops examined by Díaz Baños et al. (1996), making it even more difficult to distinguish contributions to the strain energy curve from binding interaction and elastic compliance (Hill, 1974). There has been little detailed examination of composite models in which the reaction coordinate for the motor-substrate binding interaction is not completely isolated from the strain coordinate, giving composite strain relationships of the type illustrated in Fig. 3.

STRONG OR WEAK COUPLING?

Strong coupling encapsulates the idea that there is exactly one mechanical cycle of the motor for each chemical cycle. This has been the common interpretation of the original Huxley (1957) model, with all motor detachments resulting from ATP binding and followed by ATP hydrolysis. Some experimental observations with muscle at maximum sliding velocities have been difficult to explain with strongly coupled models. It has been argued that the rate of actin-myosin detachment measured by solution biochemistry of actomyosin is too low to explain the rapid detachment at high shortening velocities that is required by a strongly coupled model (Cooke et al., 1994). It is difficult to obtain both a high translocation efficiency (nanometers of movement per ATP hydrolyzed by one motor enzyme molecule) and a high fraction of attached cross-bridges (as measured by stiffness measurements on rapidly contracting muscle) with strongly coupled models. These problems can be circumvented by weakly coupled models in which a large fraction of cross-bridge attachments are terminated, without ATP binding, by mechanical detachment at large negative strains (Cooke et al., 1994; Brokaw, 1995). This allows multiple cycles of cross-bridge attachment and mechanical detachment to occur before an ATP-induced detachment occurs. The average work output per mechanical cycle is a small fraction of the free energy of the chemical cycle, but this average results from a combination of several uncoupled cycles that absorb work for every coupled work-producing cycle (Cooke et al., 1994).

Weakly coupled models require a mechanism for strain recovery after mechanical detachment, without requiring ATP binding and dephosphorylation. This means that mechanical detachment must restore the energy level of the motor to a level sufficient for attachment in a strained state. The mechanical work required for this detachment must not be much greater than the strain energy that is recovered after reattachment, to minimize the "protein friction" (Tawada and Sekimoto, 1991) that ultimately limits the efficiency of a weakly coupled model. In simple two-state models, there is no distinction between detached states formed by mechanical detachment or by ATP binding, and no need for a special mechanism for strain recovery after mechanical detachment. For example, models in which all of the strain energy is obtained by rectification of thermal energy fluctuations in an elastic compliance have no difficulty explaining the reacquisition of strain energy after mechanical detachment. This is also the case for conformational change models in which an A→R transition is tightly coupled to the W→S binding transition. When external work is used to break this stable attachment of the SR state, the resulting detached conformation of the motor is not an exceptionally stable state. Strain energy can be recovered when the motor rebinds. On the other hand, if the R conformation is intrinsically much more stable than the A conformation, irrespective of attachment, there is no obvious way that negative strain and detachment can reverse the A→R conformational change or otherwise conserve the strain energy and use it to generate another working stroke (Brokaw, 1995). Experimental evidence for a weakly coupled model therefore will require a model with tight coupling between motor-substrate binding and conformational changes that cause changes in strain. If there is a significant elastic compliance, it must be matched to the properties of the binding interaction, as illustrated in Fig. 3, to prevent major energy loss by mechanical detachment.

Mechanical detachment must also restore mechanisms that ensure that a motor reattaches to an appropriate site. In a weakly coupled model, during normal forward translocation, the configuration of the motor immediately after mechanical detachment will be like that in Fig. 8 *A*, rather than the normal preattachment WA configuration shown in Fig. 8 *B*. If these figures represent a detached state that permits free rotation of the lever arm between the configurations shown in Fig. 8, *A* and *B*, both of these configurations must allow equilibration with the strongly attached state, by a strain-dependent specification of equilibration rates that does not allow equilibration at intermediate values of strains. A variant of this type of model was studied quantitatively by Brokaw (1995). If the configuration shown in Fig. 8 *B* is an attached state with this preferred conformation, a new attached state with the conformation of Fig. 8 *A* must be defined that can equilibrate rapidly with the state with the conformation of Fig. 8 *B*. This version of a weakly coupled model has not yet been examined thoroughly.

THE DIRECTION OF MOVEMENT

The discovery of kinesin as a plus-end-directed microtubule motor, in contrast to the minus-end-directed microtubule motor, dynein, first raised the question of how the direction of movement is determined. Initial indications of unique features of the chemical cycle of kinesin, especially the observation that ATP binding was not sufficient for detachment, suggested that major differences in coupling between chemical and mechanical cycles might explain movement in different directions along a microtubule. If the directions of the working and recovery strokes are defined for one type of motor, the other type of motor might bind strongly during movement in the recovery direction and be detached during movement in the working direction. This idea has largely been abandoned, with more understanding of the kinesin biochemical cycle (Hackney, 1996) and discovery of ncd, a minus-end-directed microtubule motor that shares the major biochemical features of kinesin (Lockhart and Cross, 1994; Crevel et al., 1996). The most straightforward mechanism for changing the direction of translocation might be to change the polarity of the microtubule-binding site on the motor (Lockhart and Cross, 1994). This possibility has been eliminated by information that ncd and kinesin have very similar motor domain structures (Sablin et al., 1996) and bind to microtubules with similar polarities (Hoenger and Milligan, 1997).

Weakly coupled models provide insight into this question. In any reasonable weakly coupled model, the energy loss in each uncoupled mechanical cycle must be low. Strain recovery after mechanical detachment, involving transitions between configurations such as those in Fig. 8, *A* and *B*, must therefore be reversible. Motors that are in a state that can attach at positive strains (i.e., the conformation shown in Fig. 8 *B*) should be able to reverse these transitions, reaching the conformation shown in Fig. 8 *A*, and attach at negative strains. This possibility has now been reinforced by the observation of negative force and movement events in single-motor experiments with myosin (Finer et al., 1994; Molloy et al., 1995a,b; Ishijima et al., 1996; Guilford et al., 1997). The frequency of negative attachments is small, as expected if strain recovery is normally biased to produce efficient movement in one direction.

For a motor to reverse its direction of operation, the execution of a working stroke in the direction opposite that of the normal direction is straightforward, if the motor attaches at large negative strain instead of at positive strain. There are one or two additional requirements. If the strain dependency of the ATP-induced detachment rate is asymmetrical, this asymmetry must be reversed. However, in a weakly coupled model, it may be sufficient for this detachment rate to be symmetrical, with a narrow peak near the position of 0 strain (Brokaw, 1995). The more important requirement is a change in the direction of strain recovery, so that attachment at negative strains becomes more frequent than attachment at positive strains (Lockhart and Cross, 1994). This is equivalent to changing the direction of the strain recovery step. There are several possibilities for determining the direction of strain recovery by biasing the transitions between configurations such as those in Fig. 8, *A* and *B*, and these will probably all be easy to reverse without major structural changes in the motor head. These possibilities generally introduce differences that will decrease the efficiency of a weakly coupled model (Brokaw, 1995). Further analysis of weakly coupled models may provide additional insight into the question of determining the direction of motion. However, the symmetry in these proposed models is not supported by current information about the rigor state of myosin-actin interaction, which shows S1 heads attached at a ~45° angle to the actin filament axis (Milligan, 1996). It is not clear that modification of a reversible model to accommodate this observation is possible or worthwhile. The possibility of changing the direction of motion without major structural change and the ability to work in a weakly coupled mode may not both be required to be present in one type of motor. The former is at present only relevant to motors in the kinesin family, and weak coupling is an idea that has arisen to explain observations of skeletal muscle myosin. Structural studies of the kinesin-microtubule and ncd-microtubule complexes will determine whether mechanisms based on reversibility of the strain recovery step will have to be considered further.

CONCLUSIONS

Starting with the assumption of a mechanical cycle with a working stroke that is driven by the release of preexisting strain energy and terminated by ATP-induced detachment of the motor from its substrate, a complete model requires mechanisms for strain amplification and strain recovery. Three components of strain amplification have been identified, involving different forms of energy storage in a strained cross-bridge. There is strong evidence for strain amplification by a lever arm. There are compelling reasons to favor models in which strain amplification is provided by multiple mechanically distinct states of motor attachment, corresponding to conformational changes within the motor head, rather than attachment at different sites along the substrate. Multiple states provide mechanisms for strain amplification, an explanation for strain-dependent attachment in terms of state-dependent transitions, ratchet effects that can influence the directions of the working stroke and/or the strain recovery step in the mechanical cycle, and rectification of the distributions of attached states that are determined by thermal fluctuations. However, very little information is available to assess the relative contributions of the three forms of strain amplification, and in particular, the role of elastic compliance in strain amplification and strain energy storage. The mechanisms of strain amplification and recovery cannot be easily distinguished by measurements of motor performance. They presume major differences in motor structure. Increasing information about motor structure and the functions of subdomains of the motor enzyme molecule, such as recent experimental alterations of myosin lever arm length, should help us to identify the contributions from each mechanism. An additional source of information that has not yet been fully explored by modeling studies is provided by dimeric motors such as kinesin, in which operation of the two heads in a cooperative manner appears to be an important feature of normal function.

Considerations of coupling and reversibility argue against one subclass of models involving strain amplification by two (or more) mechanically distinct attached motor states. Models in which strain energy results from a transition between mechanically distinct attached states that is not tightly coupled to the binding of a motor to its substrate appear unrealistic. They do not allow the weak coupling between mechanical and chemical cycles that has been suggested to fit some experimental data, do not predict negative forces and backward steps in single-motor assays, and appear to require major structural differences between motors such as kinesin and ncd that move in opposite directions.

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