and Chalk River, Canada—will ensure that this interest is matched by an ever-increasing capability to synthesize new isotopes and to endow the nuclear laboratory with unprecedented flexibility.

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Muscle Contraction and Free Energy Transduction in Biological Systems

Evan Eisenberg and Terrell L. Hill

One of the most ubiquitous properties of living systems is their ability to transform chemical free energy into motion. Muscle is highly specialized to perform this type of energy transduction, but motility also occurs in many important processes in cells. Ciliary motion, cytoplasmic streaming, and cell division are examples of the ability of cells to transform chemical free energy into mechanical work. Therefore, understanding the molecular basis for this type of free energy transduction is crucial to understanding the mechanism and regulation of many cellular processes.

Much of the available information about the mechanism of motile processes stems from structural, physiological, and biochemical studies on the mechanism of muscle contraction. Muscle contraction occurs when two sets of interdigitating filaments, the thin actin filaments and the thick myosin filaments, slide past each other (*1*). A widely accepted theory to explain this sliding process is the cross-bridge theory of muscle contraction (*1, 2*). This theory suggests that the sliding process is driven by cross-bridges that extend from the myosin filament and cyclically interact with the actin filament as adenosine triphosphate (ATP) is hydrolyzed (Fig. 1a). Although structural studies support the general concept of the cross-bridge theory, the exact mechanism whereby the chemical free energy of ATP hydrolysis is converted into mechanical work remains elusive. In this article we briefly review the development of cross-bridge models of muscle contraction from biochemical studies on the actomyosin adenosine triphosphatase (ATPase) activity. We also show how a current view of cross-bridge action is similar to the mechanism of other ATPase systems, such as active transport, and therefore illustrates the basic properties of ATP-driven free energy transduction.

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Structure of Myosin and Actin

Both the actin and myosin filaments are polymeric structures (Fig. 1b) (*3*). Each globular actin monomer in the actin filament has a diameter of about 5.5 nm and is composed of a single polypeptide chain with a molecular weight of 43,000. Each myosin monomer in the myosin filament is composed of two heavy chains, each with a molecular weight of about 200,000, and four light chains, each with a molecular weight of about 20,000 (*3*). These polypeptide chains fold into three separate domains that have different functions (Fig. 1b). The subfragment-1 domain forms the two globular cross-bridges, which are about 15 nm long and 7 nm wide; the light meromyosin domain is involved in the aggregation of the myosin molecule into filaments, and the subfragment-2 domain provides a flexible connection between the myosin filament and the cross-bridges (*3*). As

Summary. Muscle contraction occurs when the actin and myosin filaments in muscle are driven past each other by a cyclic interaction of adenosine triphosphate (ATP) and actin with cross-bridges that extend from myosin. Current biochemical studies suggest that, during each adenosine triphosphatase cycle, the myosin cross-bridge alternates between two main conformations, which differ markedly in their strength of binding to actin and in their overall structure. Binding of ATP to the cross-bridge induces the weak-binding conformation, whereas inorganic phosphate release returns the cross-bridge to the strong-binding conformation. This cross-bridge cycle is similar to the kinetic cycle that drives active transport and illustrates the general principles of free energy transduction by adenosine triphosphatase systems.

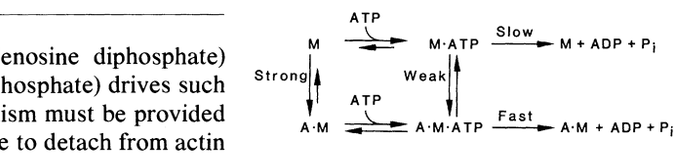
far as is known, the two cross-bridges that extend from each myosin molecule are functionally identical and act independently in their interaction with actin.

Structural studies (*4*) suggest that, in the absence of ATP, the myosin cross-bridges bind to actin at an angle of about 45° with a stoichiometry of one myosin cross-bridge per actin monomer (Fig. 1b). In contrast, in relaxed muscle the cross-bridges (apparently mostly detached from actin) appear to extend from the myosin filament at an angle of 90°. This has led to the concept that the cross-bridges go through repeated oarlike cycles as they push the actin filament past the myosin filament, first binding to the actin filament at a 90° angle, then rotating over toward a 45° angle as they push the actin filament past the myosin filament, and finally detaching and returning to a 90° angle before beginning a new cycle (*5*).

Although this structural model of cross-bridge action is speculative, it does seem likely that the myosin cross-bridge pushes, or pulls (*6*), the actin filament past the myosin filament by going through some type of oarlike cycle. Therefore the basic biochemical problem is to determine how the hydrolysis of ATP to ADP (adenosine diphosphate) and P_i (inorganic phosphate) drives such a cycle. A mechanism must be provided for the cross-bridge to detach from actin at the end of its work stroke, to reattach to actin with both a different conformation and a much higher free energy level at the beginning of its work stroke, and then to develop force and perform mechanical work during its work stroke.

Biochemical Studies

Most of the biochemical studies on the mechanism of the actomyosin ATPase cycle were performed with the isolated myosin subfragment-1 domain (myosin S1). Myosin S1 is prepared from the intact myosin molecule by proteolytic digestion; unlike myosin, myosin S1 is soluble at low ionic strength, which makes it useful for biochemical studies. Early studies (*7*) showed that actin has a profound effect on the myosin S1 ATPase activity, increasing it by a factor of more than 100 at high actin concentration (Fig. 2). They also demonstrated that the binding of ATP to the active site weakens the binding of myosin S1 to actin by several orders of magnitude. This is because in the presence of ATP, high concentrations of free actin are required to effect maximum activation of the myosin S1 ATPase, whereas in the absence of ATP myosin S1 binds essentially stoichiometrically to actin (*8*). These effects of ATP and actin were summarized in the kinetic model proposed by Eisenberg and Moos (*7*) as follows (M, myosin; A, one actin monomer in an actin filament).



The kinetic model of Eisenberg and Moos accounted for the basic steady-state properties of the actomyosin ATPase activity. However, it did not provide a mechanism to drive the myosin cross-bridge through an oarlike cycle. At high actin concentration the model predicted that the myosin cross-bridge might simply oscillate back and forth between states A·M and A·M·ATP without ever dissociating from actin. Even if the transition from A·M·ATP to A·M + ADP + P_i is associated with the work stroke of the cross-bridge, as was suggested at the time (*7*), a complete cross-bridge model has to provide a mechanism for cross-bridge detachment during each cycle of ATP hydrolysis.

Lymn-Taylor Model

A possible solution to the problem of cross-bridge detachment was first suggested by Lymn and Taylor (*9*). They observed that, in the absence of actin, ATP is hydrolyzed to ADP and P_i at the active site of myosin much more rapidly than the subsequent release of ADP and P_i into solution; that is, product release is rate-limiting (*10*). They also observed that not only does ATP weaken the binding of myosin S1 to actin but, in addition, ATP dissociates actomyosin into M·ATP and actin even more rapidly than the hydrolysis of ATP to ADP and P_i at the active site (*11*). On this basis, they suggested a kinetic model (Fig. 3a) in which ATP irreversibly dissociated A·M into M·ATP plus actin, and hydrolysis of ATP to ADP and P_i occurred only on the dissociated myosin; when myosin was bound to actin, hydrolysis of ATP did not occur (*9*).

This kinetic model led to a cross-bridge model (Fig. 3b) which was based on the assumption that each step in the oarlike action of the cross-bridge in vivo was associated with a separate biochemi-

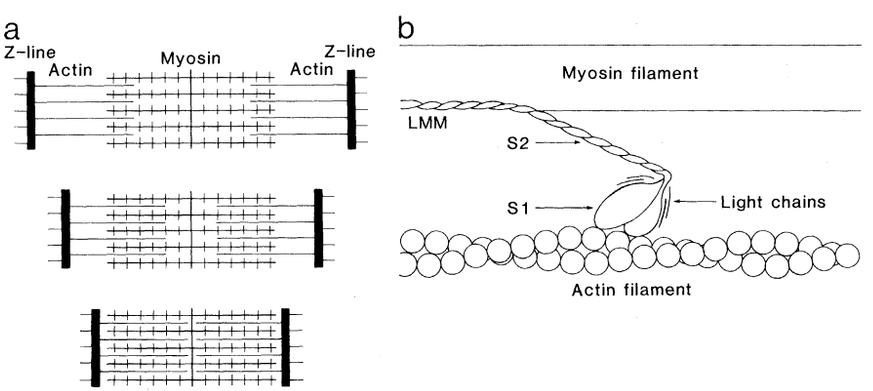


Fig. 1. (a) Diagram of a single segment (sarcomere) of a muscle fiber as it shortens. The shortening of thousands of segments in series along the muscle fiber causes the whole muscle to shorten. The short lines perpendicular to the myosin filament represent the myosin cross-bridges. (b) Diagram of a myosin molecule bound to an actin filament in the absence of ATP. Both the myosin and actin filaments are polymeric structures. Each circle in the actin filaments represents an actin monomer. Only one myosin molecule is shown in the picture; in vertebrate skeletal muscle there is one myosin cross-bridge present for every two actin monomers. During contraction, the actin filament moves to the left relative to the myosin filament.

cal step in vitro (*9*). Dissociation of the cross-bridge from actin was associated with the binding of ATP; the return stroke of the cross-bridge to a state binding weakly at a 90° angle was associated with the ATP hydrolysis step; and then the work stroke was associated with P_i release. In terms of energy changes in this model, the strong binding of ATP (*12*) weakened the binding of the cross-bridge to actin, very little free energy change occurred during the ATP hydrolysis step (*13*), and then a large drop in free energy was associated with the P_i release step as the cross-bridge rebound tightly to actin.

This cross-bridge model was important because it provided a plausible biochemical mechanism for driving an oar-like cycle of the cross-bridge. It was also of interest in view of the general role of ATP hydrolysis in free energy transduction because it linked ATP hydrolysis at the active site to a major structural change in the protein; that is, to the return stroke of the cross-bridge.

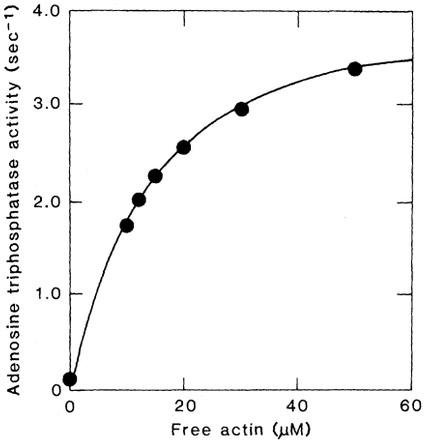


Fig. 2. ATPase activity of myosin S1 as a function of free actin concentration (given as the concentration of actin monomers present in the actin filaments). Conditions: 1.8 m*M* $MgCl_2$, 1 m*M* ATP, 2 m*M* imidazole, *p*H 7.0, 15°C. These data yield a linear double reciprocal plot with a V_{max} value of 4.7 sec^{-1} and a K_m of 17 μ*M*. The ATPase rate in the absence of actin is 0.04 sec^{-1}. Under the same conditions, the dissociation constant of myosin S1 from actin in the absence of ATP is several orders of magnitude smaller (*8*).

Test of the Lymn-Taylor Model

A key premise of the Lymn-Taylor model was that myosin had to dissociate from actin before ATP was hydrolyzed to ADP·P_i at the active site of the dissociated myosin. In 1979 Stein *et al.* tested this assumption by directly measuring the binding of M·ATP to actin at high actin concentration (*14*). Surprisingly, they obtained evidence that challenged two basic premises of the Lymn-Taylor model. First, Lymn and Taylor had suggested that ATP binding dissociated myosin from actin essentially irreversibly. However, Stein *et al.* showed that, at high actin concentration, not only does M·ATP bind to actin but it binds as well as M·ADP·P_i; M·ATP is in rapid equilibrium with A·M·ATP just as M·ADP·P_i is in rapid equilibrium with A·M·ADP·P_i. Second, Lymn and Taylor had suggested that the ATP hydrolysis step occurred only when myosin was dissociated from actin. However, Stein *et al.* found that the ATP hydrolysis step also occurs when M·ATP is bound to actin. This finding has since been confirmed by further studies of Stein *et al.* and also by studies on myosin subfragment-1 that is covalently cross-linked to actin (*15*).

On the basis of their data, Stein *et al.* proposed the kinetic model shown in Fig. 4 (*14*). The major difference between this new model and the Lymn-Taylor model is that, in the new model, there is no point during the in vitro ATPase cycle where myosin is required to dissociate from actin. Hence, the ATP hydrolysis step no longer occurs only when myosin is dissociated from actin as in the Lymn-Taylor model. Rather, in each overall ATP hydrolysis cycle, myosin alternates between two conformations, a weak-binding conformation and a strong-binding conformation. The binding of ATP transforms the myosin from the strong-binding conformation to the weak-binding conformation. It remains in the weak-binding conformation, with the free and actin-bound states in rapid equilibrium, while several kinetic steps occur [hydrolysis and a rate-limiting step (*16*)]. Then the release of P_i transforms it back into the strong-binding conformation (*17*).

There is general agreement on the major elements of this kinetic model. However, there is still a question as to whether there is a separate ATP hydrolysis step and rate-limiting step in the cycle. Eisenberg and his collaborators have supported this view (*18*), but Rosenfeld and Taylor (*19*) have suggested that the ATP hydrolysis step, itself, might be the rate-limiting step in the ATPase cycle. It has also been suggested that there are two A·M·ADP intermediates in the cycle rather than the single intermediate shown (*20*). Despite these questions, there is no disagreement on the basic facts that ATP hydrolysis occurs while myosin S1 is bound to actin, and that myosin S1 alternates between a weak-binding and strong-binding conformation during each cycle of ATP hydrolysis.

In developing a cross-bridge model from the kinetic model of Stein *et al.*, once again the problem arises of providing a mechanism for the cross-bridge to detach at the end of its work stroke and to rebind in a new configuration at the beginning of its work stroke. One possibility is to retain the basic premises of the Lymn-Taylor cross-bridge model, on the assumption that in vivo the function of ATP binding is to dissociate the cross-bridge from actin, and that the function of the ATP hydrolysis step is to transform the cross-bridge from the 45° conformation to the 90° conformation. However, this latter assumption is now completely arbitrary since the ATP hydrolysis step no longer has the special characteristic of occurring only when myosin is dissociated from actin, nor is there any evidence that M·ADP·P_i differs from M·ATP in its biochemical properties.

An alternative possibility more in line with the biochemical evidence is to assume that the states which are similar biochemically are also similar structurally, that is, to assume that all of the weak-binding states are in the 90° conformation while the strong-binding states are in the 45° conformation. This, of course, means that the binding of ATP would

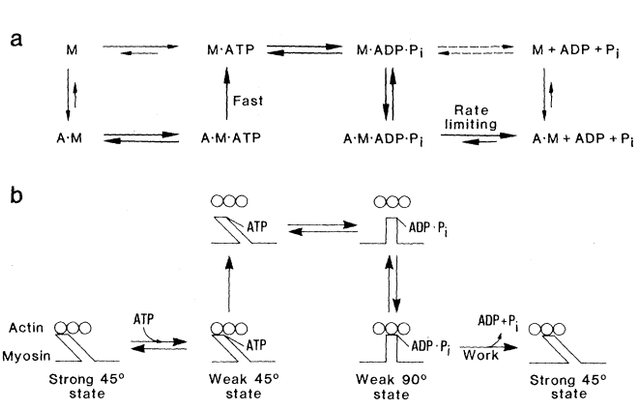


Fig. 3. (a) Kinetic model of Lymn and Taylor (*9*). Heavy solid arrows show the dominant pathway. The dashed arrows show the very slow rate-limiting step in the absence of actin. The relative lengths of the forward and reverse arrows indicate qualitatively the free energy change across a reaction. *M*, myosin; A, actin. (b) Cross-bridge model of Lymn and Taylor (*9*).

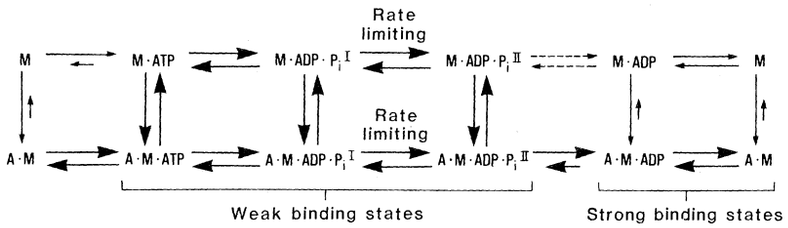


Fig. 4. Kinetic model of Stein *et al.* (*14*). The heavy solid arrows show the dominant pathway. The dashed arrows show the very slow rate-limiting step in the absence of actin. The relative lengths of the forward and reverse arrows indicate qualitatively the free energy change across a reaction. The rate-limiting steps $M{\cdot}ADP{\cdot}P_i^I \rightarrow M{\cdot}ADP{\cdot}P_i^{II}$ and $A{\cdot}M{\cdot}ADP{\cdot}P_i^I \rightarrow A{\cdot}M{\cdot}ADP{\cdot}P_i^{II}$ have about equal rates.

transform the cross-bridge from state A·M to state A·M·ATP just as in the earlier kinetic model of Eisenberg *et al.* Therefore the same question arises as in this earlier model; namely, if ATP binds to the actin-bound cross-bridge at the end of its work stroke and returns it to the 90° conformation before the cross-bridge detaches, why doesn't this reverse the work that is performed during the work stroke? In other words, what is the mechanism of cross-bridge detachment during each cycle of ATP hydrolysis?

Cross-Bridge Model of Eisenberg and Greene

In 1980, Eisenberg and Greene (*21*) suggested that the solution to this problem may lie in applying the physiological concepts of cross-bridge behavior developed by Huxley (*2*) and Huxley and Simmons (*22*) to the kinetic model of Stein *et al.* To do this, Eisenberg and Greene used the theoretical formalism of Hill, which provides the proper method to relate the biochemical and physiological properties of the cross-bridge (*23*). As in an earlier cross-bridge model developed by Eisenberg and Hill (*24*), the model of Eisenberg and Greene takes into account the basic fact, first noted by Huxley (*2*), that, in the muscle, there is a fixed lattice of actin and myosin filaments that cannot respond to the action of a single cross-bridge but only to the overall behavior of an ensemble of cross-bridges.

A complete cross-bridge cycle based on this model is shown in Fig. 5. The 90° and 45° conformations are just schematic representations of the real cross-bridge conformations that occur at the beginning and end of the work stroke; the detailed structure of these cross-bridge conformations is undoubtedly more complex (*25*). We begin the cycle in the upper left-hand corner, with the cross-bridge in the weak-binding 90° conformation. Just as in solution, the free and actin-bound cross-bridges are in rapid equilibrium; the exact value of the association constant in vivo is not known, but recent evidence suggests that it may be between one and ten (*26*). Two kinetic steps (hydrolysis and a rate-limiting step) occur while the cross-bridge is in the weak-binding 90° conformation. Because there is no evidence that these steps have a large effect on the properties of myosin S1 in vitro, Eisenberg and Greene assumed that neither of these steps has a large effect on the structure or binding affinity of the cross-bridge in vivo.

The next step in this cross-bridge cycle is the transition from the 90° to the 45° conformation (*27*). Unlike the previous steps in the cycle, this step must be different in muscle and in solution. In solution there is no restraint on the myosin S1 changing angle. But in the muscle, when the cross-bridge undergoes the transition from the 90° to the 45° conformation, the actin filament cannot respond by moving a short distance; the actin filament can respond only to the average behavior of all of the cross-bridges that are attached. This means that, if the cross-bridge undergoes the transition from the 90° to the 45° conformation, the result cannot be a stable 45° cross-bridge, as in solution, but rather is a strained 45° cross-bridge that exerts positive force as is symbolized by its curved shape (Fig. 5). This strain is gradually relieved only as the filaments slide past each other. Hence, in this model, the term "45° conformation" no longer refers to a single conformation of the cross-bridge, but rather to a continuous series of conformations with the cross-bridge always attached to actin at a 45° angle but with a variable amount of strain in the cross-bridge structure. The same is true for the 90° conformation.

The key postulate here, as in our earlier cross-bridge model (*24*), is that, in the muscle, the cross-bridge undergoes the transition from the 90° conformation to the 45° conformation in two stages. In the first stage, the cross-bridge passes over an activation-energy barrier in an all-or-none process that has a specific rate constant. However, once the cross-bridge passes through this first stage, it is unable to complete the change in state, that is, drop down to its new lower free energy level, until the actin and myosin filaments slide past each other. This second stage, then, is not an all-or-none process, but occurs gradually; it is in this second stage that work is done.

It is not known how much change occurs in the overall structure of the cross-bridge and in the structure of the active site during the first stage of the transition when the cross-bridge becomes strained. In Fig. 4, we have shown a fairly large change in overall structure occurring during this first stage, accompanied by release of P_i from the active site. However, it is possible that a much smaller overall change occurs in the first stage (*24, 28*), and P_i release may be slow until the work stroke is completed. Eisenberg and Greene did assume that ADP release from the cross-bridge is slow until the work stroke is completed. This is because, as was first described by Huxley (*2*), for this model (or any other) cross-bridge model to work, the cross-bridge must detach from actin slowly until after motion occurs and the strain in the cross-bridge is utilized to do work. Therefore, in the model in Fig. 5, ADP release becomes as rapid as it is in solution only after the filaments move and the cross-bridge reaches its stable 45° conformation.

After ADP is released, the next step is for ATP to rebind to the cross-bridge, just as in solution. In solution this would cause myosin S1 to return to the weak-binding 90° conformation. However, in the muscle, just as with the transition from the 90° to the 45° conformation, the cross-bridge is constrained by the filament lattice and, therefore, is not able to return to the stable 90° conformation. Instead, the cross-bridge enters a strained 90° conformation. Only here, instead of the cross-bridge exerting positive force as it did in the strained 45° conformation, it exerts negative force. We have now arrived at the question of why this negatively strained cross-bridge does not reverse the work already done in the cross-bridge cycle.

If, in fact, this negatively strained cross-bridge remained attached to actin long enough for significant motion of the filaments to occur, it would indeed reverse the positive work already done in the cross-bridge cycle. However, at this point Eisenberg and Greene made the

crucial assumption that, when the cross-bridge is in the negatively strained 90° conformation, it rapidly detaches from actin before it can do significant negative work. This assumption was based on the biochemical data showing that M·ATP attaches to, and detaches from, actin very rapidly (*9, 14*). The point is that the cross-bridge in the negatively strained 90° conformation detaches from actin much faster than the cross-bridge in the positively strained 45° conformation. Therefore, the transition from the 45° to the 90° conformation does not reverse the work that is performed during the work stroke even though this transition occurs before detachment of the cross-bridge from actin.

Finally, in the cross-bridge cycle in Fig. 5, once the cross-bridge detaches from actin it can immediately reattach to a new actin site and begin a new cycle. Since the binding of ATP has already returned it to the 90° conformation, the cross-bridge does not have to wait for the ATP hydrolysis step to take place before it reattaches to actin, as was required in the Lymn-Taylor model.

In the cross-bridge model of Eisenberg and Greene, in contrast to the Lymn-Taylor model, each step of an oarlike cross-bridge cycle is not linked to a separate biochemical step. Rather, the myosin cross-bridge alternates between a weak-binding conformation and a strong-binding conformation, each differing in the structural way they interact with actin. This simple alternating cycle performs mechanical work because the rate constants in the cycle are assumed to be sensitive to the mechanical strain in the cross-bridge.

Evidence for the Cross-Bridge Model

The cross-bridge model of Eisenberg and Greene is based on the assumption that the weak-binding and strong-binding states in the kinetic model of Stein *et al.* differ not only in their affinity for actin but also in the large-scale structural way they interact with actin. One line of evidence suggesting that this assumption is valid comes from studies with the ATP analog, AMP-PNP (adenyl-5′-yl imidodiphosphate). It has been shown that AMP-PNP can actually cause a muscle fiber to lengthen slightly, an effect that would occur if AMP-PNP were causing the cross-bridges to rotate from a 45° angle toward a 90° angle (*29*).

Other evidence supporting this assumption comes from studies on the mechanism of muscle relaxation. Skeletal muscle relaxation is caused by troponin-tropomyosin, a complex of proteins that lies along the actin filament (*30*). Structural studies suggest that, in the absence of Ca^{2+}, tropomyosin occupies a position on the actin filament where it sterically interferes with the binding of myosin to actin (*31*), and biochemical studies confirm that, in the absence of ATP, tropomyosin can greatly weaken the binding of the strong-binding states of myosin S1 to actin (*32*). Surprisingly, however, troponin-tropomyosin has almost no effect on the binding of the weak-binding states of myosin S1 to actin (*33*). This is not simply because they bind weakly. At high ionic strength M·ADP binds almost as weakly to actin as does M·ATP at low ionic strength, yet troponin-tropomyosin blocks the binding of M·ADP but not M·ATP (*34*). Thus, these studies suggest that there is a real structural difference in the way the strong-binding and weak-binding states interact with actin. Recently weakly bound cross-bridges have been detected in vivo (*35*) and their structure does indeed appear to be different from the strongly bound cross-bridges that occur in the absence of ATP (*36*). In addition, recent electron microscopy studies of myosin S1 chemically cross-linked to actin suggest that the cross-linked myosin S1 has a very different structure in the presence of ATP than in the absence of ATP (*37*).

Comparison of the Cross-Bridge Cycle with an Active Transport Cycle

The cross-bridge model of Eisenberg and Greene, regardless of whether or not it is valid in its entirety, does illustrate the general principles that are thought to underlie free-energy transduction in ATPase systems (*38, 39*). Before considering these general principles it is useful to compare the cross-bridge cycle of Eisenberg and Greene with the cycle proposed for the active transport of Ca^{2+}. There has been considerable speculation that active transport and muscle contraction have certain features in common (*38–40*), and the cross-bridge model of Eisenberg and Greene supports this view. As with myosin, the active transport enzyme also occurs in a conformation that binds ligand weakly and a conformation that binds ligand strongly (*41*). Only here, the ligand bound is Ca^{2+} rather than actin; and instead of the 90° and 45° conformations, the two conformations of the enzyme are open to the inside of the cell ("inside" conformation); and open to the outside of the cell ("outside" conformation).

The active transport cycle (*41*) (Fig. 6) begins with the enzyme in the strong-binding inside conformation. Ca^{2+} from the inside of the cell binds tightly to the enzyme and then an acyl group at the active site is phosphorylated by ATP.

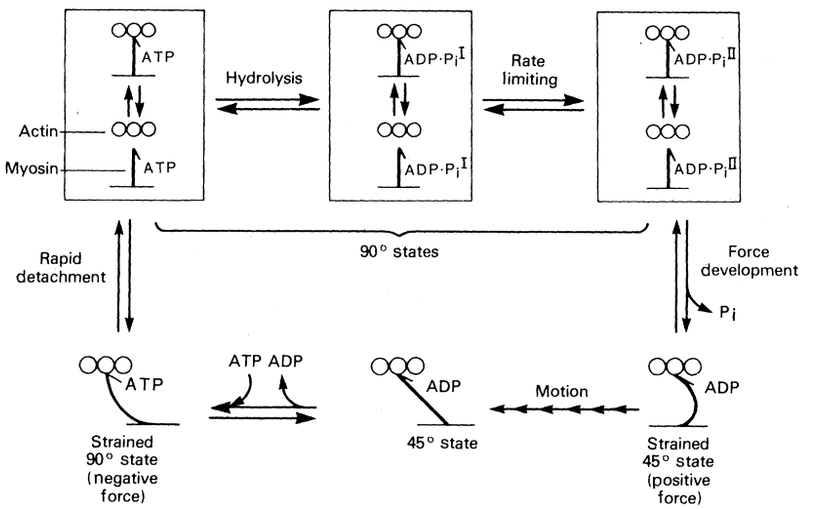


Fig. 5. Cross-bridge model of Eisenberg and Greene (*21*). The 90° states M·ATP, M·ADP·P_i^I, and M·ADP·P_i^{II} are in rapid equilibrium with A·M·ATP, A·M·ADP·P_i^I, and A·M·ADP·P_i^{II}, respectively. The symbol ⟵⟵ represents a transition that does not have an activation energy or a rate constant. Rather it is a continuous conformational change which occurs as the filaments slide past each other. This is stage 2 of the transition from the unstrained 90° conformation to the unstrained 45° conformation. Stage 1, which is represented by the P_i release step in this model, does have both an activation energy and a rate constant. The isometric pathway for ADP release and ATP rebinding is not shown but would occur as follows: in the isometric state there is no motion of the filaments and therefore the strain in the 45° cross-bridge is not relieved. The model assumes that ADP release from the strained 45° conformation can occur but is quite slow. After ADP release occurs in the isometric state, ATP binds directly to the strained 45° conformation and returns it to state A·M·ATP (see text). During this isometric cross-bridge cycle, the cross-bridge does not have to detach from actin.

This induces the enzyme to undergo the transition from the strong-binding inside conformation to the weak-binding outside conformation. The weakly bound Ca^{2+} is then released to the outside of the cell. After the Ca^{2+} is released, the acyl phosphate is hydrolyzed and P_i is released from the enzyme. This induces the enzyme to return to the strong-binding inside conformation ready to begin a new cycle. Thus, during its ATPase cycle, like myosin, the active transport enzyme also alternates between two conformations that differ in their structure and in their affinity for ligand (*42*).

Principles of Free Energy Transduction

In considering how ATP hydrolysis drives the active transport and cross-bridge cycles, there has been a tendency to think of the high free energy of ATP hydrolysis as actually being localized in the ATP molecule. Attempts are then made to determine how this free energy is transferred from ATP to the enzyme or the ligand bound to the enzyme. In effect, these ATPase systems are treated as if they were analagous to bacteriorhodopsin. Here light energy directly interacts with the protein, "energizing" it. The energized protein then undergoes a series of conformational changes that cause a proton to be transported across the membrane (*43*).

The problem with treating ATPase systems as if they were energized by ATP is that the free energy of ATP hydrolysis is not localized in the ATP molecule. Nor does it arise simply from a change in molecular structure when ATP is hydrolyzed to ADP and P_i; there is almost no change in free energy when ATP is hydrolyzed to ADP and P_i at the active site of myosin (*13*). Rather the free energy of ATP hydrolysis arises from a difference in the free energy of ATP and ADP + P_i in solution. This difference, in turn arises from a combination of three factors: a difference in the molecular structure of ATP and ADP + P_i, a difference in their interaction with water, and a difference in their concentration under physiological conditions. Since ATP and ADP + P_i are equally involved in these differences, free energy transduction requires a complete cycle that begins with ATP in solution and ends with ADP + P_i in solution (*44*).

The cross-bridge and active transport cycles we have described suggest that, during such a complete cycle, three essential events occur. First, at one point in the cycle, a ligand, such as Ca^{2+} or actin, binds to the enzyme with a very strong binding energy. In the cross-bridge cycle this strong binding allows the cross-bridge to exert significant force without detaching from actin, while in the transport cycle, it allows Ca^{2+} to bind to the enzyme despite its low concentration in the cell. Second, at another point in the cycle, the enzyme undergoes a major structural change that, either directly or indirectly, has the effect of changing the physical position of the ligand. Finally, at some point in the cycle the binding constant of the ligand markedly decreases, which allows the ligand to detach from the enzyme.

ATP is not involved in the first of these essential events because actin and Ca^{2+} bind very strongly to myosin and to the Ca^{2+} transport enzyme, respectively, in the absence of ATP. However, ATP is involved in both the detachment of bound ligand and in the structural change. A large input of free energy is required to detach a strongly bound ligand from the enzyme. This free energy does not come from ATP energizing the enzyme or the ligand bound to the enzyme in a manner analogous to the energization of bacteriorhodopsin by light. Rather, in the case of myosin, this free energy is supplied by the formation of strong bonds between ATP and myosin (*12*). This not only induces a structural change in the myosin but, in addition, the strong bonds between ATP and myosin replace the strong bonds between actin and myosin. This replacement causes almost no change in the total free energy of the system, in contrast to energization of bacteriorhodopsin by light, which causes a marked increase in the free energy of the protein.

At this point in the cycle, ATP has acted like a typical bound ligand inducing a structural change in a protein and weakening the affinity of a second bound ligand. However, replacing one tightly bound ligand with another tightly bound ligand is not free energy transduction; if it were, free energy transduction could be driven by an inert ligand like AMP-PNP. To complete the free energy transduction cycle, the strong bonds between ATP and myosin must be broken. Furthermore, this must be done at no cost in free energy so that all of the free energy available from the strong rebinding of the cross-bridge to actin can be used to do mechanical work rather than to reverse the tight binding of ATP.

In the first step in this process, ATP is hydrolyzed to ADP and P_i at the active site. As we pointed out, very little change in free energy is associated with this hydrolysis step; the M·ADP·P_i complex is just as stable as the M·ATP complex (*13*). However, in contrast to the release of ATP from myosin, which can only occur at a very high cost in free energy, the release of ADP and P_i from myosin is actually energetically favor-

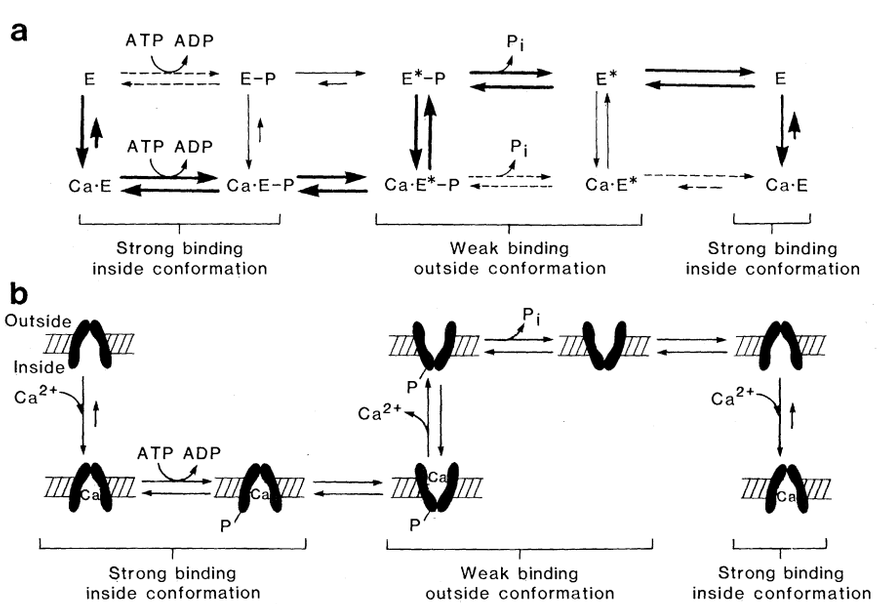


Fig. 6. Kinetic model for the active transport of Ca^{2+}. (a) Kinetic cycle. The heavy solid arrows show the dominant pathway. Dashed arrows show steps that are very slow. The relative lengths of the forward and reverse arrows indicate qualitatively the free energy change across a reaction. E represents the strong-binding inside conformation and E* represents the weak-binding outside conformation. E-P represents the covalent acyl-phosphate intermediate. For simplicity, the model shows one Ca^{2+} transported per ATP hydrolyzed; in reality two Ca^{2+} are transported per ATP hydrolyzed. (b) Diagrammatic representation of the Ca^{2+} transport cycle based on the kinetic cycle shown in (a).

able because the free energy of ADP + P_i in solution is so much lower than the free energy of ATP in solution. Therefore, ATP is released from myosin by changing it to a chemical form that is much more stable than ATP in solution; this allows all of the free energy drop associated with the rebinding of the cross-bridge to actin, as well as with P_i release itself, to be used to do mechanical work.

ATP hydrolysis drives active transport in a similar manner (*41*) (Fig. 6). Here, rather than ATP binding strongly to the enzyme, it first phosphorylates the enzyme, and then the covalently bound acyl phosphate group forms additional strong noncovalent bonds with the enzyme, changing the structure of the enzyme and greatly weakening its affinity for Ca^{2+}. Then, just as ATP is hydrolyzed and released from myosin at no cost in free energy, this strongly bound acyl phosphate group is hydrolyzed, and P_i released from the active transport enzyme at no cost in free energy, because free P_i is so stable in solution. Hence by binding tightly to an enzyme, a ligand like an acyl phosphate group or ATP can cause both a structural change and the detachment of another tightly bound ligand at one point in a free energy transduction cycle, and then itself can be easily released from the enzyme at another point in the cycle after it is chemically changed to a form that is more stable in solution. This simple phenomenon is at the heart of all free energy transduction processes driven by ATP hydrolysis.

The generality of this phenomenon is illustrated when we consider a system in which the driving force is provided by an ion gradient across a membrane, rather than by ATP hydrolysis. A prominent example is ATP synthesis by the F1 enzyme of mitochondria, which is driven by a proton gradient across the mitochrondrial membrane (*45*). In the first step in ATP synthesis, ADP and P_i bind to the enzyme and, with almost no change in free energy, combine to form a strongly bound ATP at the active site (*46*). Next, a proton acting as a typical ligand, that is, like ATP in the cross-bridge cycle, binds weakly to the enzyme from the side of the membrane where it is at high concentration. The enzyme then undergoes a conformational change which simultaneously makes the proton accessible to the other side of the membrane and greatly increases its strength of binding. This latter effect weakens the binding of the newly synthesized ATP and allows it to be released into solution (*45*). After this release, the strongly bound proton is, in turn, released from the enzyme, not by changing it to a different form, as occurs when ATP is hydrolyzed to ADP·P_i in the cross-bridge cycle, but simply by releasing it to the side of the membrane where it occurs at low concentration. Thus a proton, acting as a typical ligand, first induces the release of ATP by binding tightly to the enzyme and then, at no cost in free energy, is itself released, just as ADP and P_i are released from actomyosin at no cost in free energy.

Rate Constants in Free Energy Transduction

Although the alternate attachment and detachment of a ligand like Ca^{2+} or actin is a necessary part of the free energy transduction process, it is also necessary that this attachment and detachment occur at specific points in the cycle in relation to the structural change in the enzyme (*47*). We have emphasized earlier the importance of assuming that the release of ADP from the positively strained cross-bridge is slow so that actin remains attached to the cross-bridge until the work stroke is completed. Very slow rate constants in the Ca^{2+} transport cycle (dashed lines in Fig. 6a) have a similar function; they ensure that Ca^{2+} binds to the enzyme before the transition from the inside state to the outside state and is released from the enzyme before the transition from the outside state back to the inside state. Hence, in both the cross-bridge cycle and the active transport cycle, the timing of ligand attachment and detachment is controlled by the slow rate constants in the cycle.

This timing effect of rate constants works in tandem with the ATP-induced affinity changes to produce a free-energy transduction cycle that is both efficient and fast. The slow rate constants channel the enzyme along a pathway that provides tight coupling between ATP hydrolysis and transport or mechanical work. Then, as the enzyme moves along this pathway, the ATP-induced affinity changes ensure that neither attachment nor detachment of ligand are highly unfavorable energetically. This is important because, even though the overall cycles are energetically favorable, the occurrence of such unfavorable steps would make the cycles inoperably slow (*44*). Hence, the affinity changes optimize the rate of a cycle while the slow rate constants provide efficient coupling between ATPase activity and useful work.

Conclusion

We have described a simple model of muscle contraction in which the myosin cross-bridge alternates between two conformations that differ in their structure and in their affinity for actin. ATP binding induces the weak-binding conformation whereas release of P_i returns the cross-bridge to the strong-binding conformation. The only role of the ATP hydrolysis step in this cycle is to change the chemical form of ATP so it can be released from the enzyme at no cost in free energy. The sensitivity of specific rate constants in the cycle to mechanical strain ensures that actin attachment and detachment occur at the proper points in the cycle. A similar ATPase cycle appears to drive the active transport of Ca^{2+}.

Interestingly, there are also numerous regulatory cycles where nucleotide hydrolysis is linked to a steady-state cycle between two major protein conformations. In systems as diverse as the phosphorylation and dephosphorylation of regulatory enzymes, the polymerization and depolymerization of actin (*48*) and the guanosine triphosphatase cycles involved in regulating adenylate cyclase action (*49*), a nucleotide hydrolysis cycle causes a protein to alternate between two major conformations. There seems to be little fundamental difference between these regulatory cycles and the energy transduction cycles described in this article. Apparently, the ability of nucleotide hydrolysis to cause a steady-state cycle between two protein conformations can be used to facilitate the subtle regulation of cellular processes (*50*) as well as to drive free energy transduction. Further work on systems as diverse as ciliary motion and ATP synthesis is needed to determine whether the same general pattern occurs in all free energy transduction systems that are driven by nucleotide hydrolysis or ion gradients.

Even if we learn that the pattern is a general one, it will only be the first step in understanding the detailed mechanisms of free energy transduction systems. Comparison of the Ca^{2+} transport and cross-bridge cycles illustrates that, although they follow the same basic pattern, they have very different chemical intermediates. In the Ca^{2+} transport cycle, the covalently bound acyl phosphate group seems to play the same role that bound ATP plays in the cross-bridge cycle. This may be fortuitous but, more likely, it reflects differences that are necessary for the smooth operation and reg-

ulation of these systems. It remains to be determined how the chemical differences among various free energy transduction cycles, in concert with the numerous regulatory steps that occur in these cycles, are related to their specific functions in the cells.

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