MYOSIN IS AN ENZYME that catalyses the hydrolysis of adenosine triphosphate (ATP) and, in the presence of actin, has the ability to convert the energy of hydrolysis into directed movement. During muscle contraction, this enzymatic process causes an array of thick filaments, composed mostly of myosin, to actively slide past an array of thin filaments, containing primarily actin\(^1\). This 'sliding filament model' for muscle contraction has been known for many years but has proved difficult to understand on a molecular level, in part because the tertiary structure of myosin was unknown. The stumbling block in determining the three-dimensional structure has been the difficulty in obtaining X-ray quality crystals of the protein. This has been all the more frustrating because myosin is an exceedingly abundant molecule: for example, in skeletal muscle it comprises nearly 50% of the total cellular protein.

One reason for the difficulty in growing crystals of myosin can be ascribed to the highly asymmetrical shape of the molecule, which consists of two globular heads attached to a long tail (Fig. 1). It has a molecular weight of approximately 500 kDa and consists of six polypeptide chains: two heavy chains (total molecular mass = 400 kDa) and two sets of light chains, with each light chain species having a molecular mass of approximately 20 kDa. Each globular head is composed of approximately 850 amino acid residues contributed by one of the respective heavy chains and two of the light chains. The remaining portions of the two heavy chains assemble to form a coiled coil that is nearly 1500 Å in length. The globular heads contain the nucleotide-binding sites and the actin-binding regions, whereas the long rod-like portion of myosin forms the backbone of the thick filament. The myosin heads can be readily cleaved from the rest of the molecule by mild proteolysis to yield the so-called 'subfragments-1', which are very soluble. These subfragments, first identified in 1962 (Ref. 3), contain all the necessary elements to generate movement of actin during ATP hydrolysis\(^4\). Since the myosin heads can be prepared in very large quantities\(^5\), they seemed ideal candidates for pursuing an X-ray crystallographic analysis.

Unfortunately, they proved exceedingly difficult to crystallize by conventional crystallization techniques.

After many years of unsuccessful crystallization in various laboratories, this problem was overcome by the unusual approach of mild reductive methylation of lysine residues\(^6\). By this method, two methyl groups were successively attached to nearly all of the lysine residues contained in the myosin head through the steps shown in Box 1.

Given this unique strategy for crystallizing the myosin heads, it was necessary to demonstrate that chemical modification introduced no major structural or enzymatic changes in the molecule. Consequently, an X-ray study of chemically modified hen-egg-white lysozyme was undertaken and demonstrated that, although the crystallization conditions were radically different, there were very few changes in the three-dimensional structure of the protein\(^7\) (Fig. 2). Furthermore, previous biochemical studies had already demonstrated that hen-egg-white lysozyme retained most of its enzymatic activity following reductive methylation\(^8\). The kinetic properties of methylated myosin subfragment-1 were also investigated\(^9\) and the results indicated that, although the kinetic parameters for the hydrolysis of ATP had changed, the protein was still enzymatically active. Taken together, these investigations demonstrated that major structural changes in the myosin head were not introduced by reductive alkylation.

The three-dimensional structure

The myosin head is highly asymmetrical (Fig. 3). It has a length of over 165 Å and is approximately 65 Å wide and 40 Å deep at its thickest end. It is

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**Figure 1**

A schematic drawing of the myosin molecule (not to scale) showing the organization of the six polypeptide chains that form this macromolecular assembly. Each myosin head is composed of 840 amino acids of the heavy chain and a regulatory (RLC) and essential (ELC) class of light chain.

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Box 1. Reductive methylation of myosin to produce a crystallizable protein

The reductive methylation involves the initial formation of an adduct or Schiff’s base between an amino group of the lysine sidechain and formaldehyde, which is then reduced with sodium borohydride or a borane complex to form a secondary or tertiary amine.

\[
\text{R-NH}_2 + \text{CH}_2\text{O} \rightarrow \text{R-N=CH}_2 + \text{H}_2\text{O} \rightarrow \text{R-NH-CH}_3
\]

(1)

\[
\text{R-NH-CH}_3 + \text{CH}_2\text{O} \rightarrow \text{R-N=CH}_2\text{OH} \rightarrow \text{RN-(CH)}_3
\]

(2)

This reaction proceeds rapidly to form the dimethyllysine product since the pK_a of the monomethyl-lysine residue is lower than that of lysine itself.

clear that the secondary structure is dominated by many long α-helices. The longest of these extends for 85 Å from the thick end of the molecule towards the carboxyl terminus of the heavy chain fragment. This helix is enveloped by both the essential and the regulatory light chains, indicating that a major role of the light chains is the stabilization of this α-helix. This supports earlier observations by electron microscopy, which showed that the molecule collapses when the regulatory light chain is removed. In addition, these light chains might serve to amplify the effects of conformational changes associated with the binding and release of nucleotide at the thick portion of the head. The light chains have considerable sequence and structural similarity to both calmodulin and troponin. Since the structures of these light chains are observed in their natural setting, i.e. associated with the myosin heavy chain, they serve to illustrate two additional modes of interaction of this family of proteins with their target peptides.

The heavy chain forms the thick portion of the head. In Fig. 3, the heavy chain is displayed in green, red and blue to delineate the amino-terminal, central and carboxy-terminal fragments that extend over residues Asp4-Glu204, Gly216-Tyr626 and Gln647-Lys843, respectively. These segments are separated by disordered loops in the X-ray structure and were previously identified by mild tryptic cleavage of the myosin head as the 25 kDa, 50 kDa and 20 kDa fragments, respectively. While it is convenient to discuss the arrangement of the tertiary structural elements in the myosin head with respect to these three fragments, it is important to keep in mind that they are not structural domains as originally believed.

The thick portion of the myosin head contains both the nucleotide- and actin-binding sites and thus has all of the components necessary to generate movement. The fact that movement is possible with just the head region has been recently established by expression of a truncated myosin in Dictyostelium discoideum lacking the light-chain-binding region, which can move actin in an in vitro motility assay. The motor segment of the myosin head exhibits a complex arrangement of secondary structural elements centered around a large, seven-stranded, mostly parallel β-sheet. This motif is formed by components from all three trypptic fragments. The nucleotide-binding pocket is located at the end of the central β-strand of this sheet and is abutted on one side by the 25 kDa segment and on the other by the 50 kDa segment of the heavy chain. The location of this nucleotide-binding pocket was determined by the positions of amino acid residues originally identified from affinity photolabeling studies to be active-site residues, and by the position of the consensus sequence for phosphate-binding loops previously observed in adenylate kinase and Ras. As shown in Fig. 4, the pocket is approximately 13 Å deep and 13 Å wide and is empty, except for a sulfate ion which results because the crystals were obtained from ammonium sulfate. Since the crystals were grown in the absence of nucleotide, the subfragment-1 structure described here most probably represents the open conformation for the active site.

The myosin head is also characterized by several other prominent clefts and grooves that define structural domains in the subfragment. The most obvious cleft splits the 50 kDa region into an upper and a lower domain. This narrow cleft runs directly under the nucleotide-binding pocket towards the end of the molecule. These two parts of the molecule in the X-ray structure are not in Van der Waals contact with one another. It has been suggested that this cleft plays a central role in the functioning of myosin as a molecular motor, as discussed below. Before any model for muscle contraction can be proposed, however, the kinetic behavior of the protein and the structure of the actomyosin complex must be considered.

**Kinetic behavior of myosin**

The temporal relationship between ATP hydrolysis and the generation of movement has been established through an extensive series of kinetic studies.
The two most significant features of this process are: (1) that the hydrolysis of ATP occurs when myosin is either dissociated or only weakly bound to actin, and (2) that the power stroke occurs during product release. The contractile cycle can be summarized in the following manner. Starting in the absence of ATP, myosin is tightly bound to actin. Upon the addition of nucleotide, myosin is rapidly released from actin and, thereafter, ATP is hydrolysed to form a metastable myosin–products complex. Rebinding of myosin to actin catalyses the release of the γ-phosphate and ADP. Studies conducted on muscle fibers have shown that force is generated during the release of the hydrolysis products.

**Structure of the actomyosin complex**

The actomyosin complex is a very large macromolecular assembly that has been studied extensively by electron microscopy, with the result that the tightly bound form of the complex has been well characterized. Additionally, the three-dimensional X-ray structure of monomeric actin has been determined, and a model for the actin filament has been proposed. A preliminary atomic model for the actomyosin complex has been achieved by combining these two sources of information with the structure of myosin subfragment-1. Even though the resolution of the image reconstruction was approximately 30 Å, it was possible to assemble the model with an accuracy of 5–8 Å because only a few positional parameters had to be determined. As a consequence, the actomyosin model provides a reasonable estimate of the relationship between myosin and actin that is presumed to represent the end of the contractile cycle. It demonstrates clearly that the actin-binding site on myosin includes components from both the upper and lower domains of the 50 kDa heavy-chain fragment. Furthermore, it suggests that myosin binds to actin in a highly stereospecific manner.

One of the key features of this model for the actomyosin complex is a close contact between actin and the lower domain of the 50 kDa region of the structure, indicating that a conformational change might be induced in myosin when it binds to actin. This conformational change might result in closure of the long narrow cleft that divides the thick portion of the myosin head (Fig. 4), thereby allowing for communication between the actin- and nucleotide-binding sites, which are separated by about 35 Å.

**Molecular model for contraction**

The basic requirements for a molecular model of the contractile cycle are that there should be a change in the affinity of myosin for actin when ATP binds in the active site; that hydrolysis should only occur when myosin is dissociated or weakly bound to actin; and that rebinding of myosin to actin should catalyse the release of products and cause the movement of actin relative to myosin. The model must also conform to fundamental chemical principles of protein structure and function. These requirements can be readily met if it is assumed that the actin- and nucleotide-binding properties of myosin are mediated through a series of conformational changes between structural domains. A summary of the structural hypothesis for the contractile cycle is shown in Fig. 5.

The starting point for the contractile cycle is taken as the point where...
myosin is tightly bound to actin in the absence of nucleotide (Fig. 5a). In this state, it is assumed that the narrow cleft splitting the upper and lower domains of the 50 kDa region is closed. The first step in the contractile cycle thus requires a reduction of the binding affinity of myosin for actin when ATP binds. The myosin-head structure suggests that this might be accomplished by the opening of the narrow cleft when the γ-phosphate of ATP binds, which would serve to disrupt the actin-binding surface on myosin (Fig. 5b). The potential binding site for the γ-phosphate is located close to the confluence of the upper and lower domains of the 50 kDa region (Fig. 4). This model also predicts that the weak binding interaction of myosin with actin, which was deduced from kinetic studies and occurs even when nucleotide is bound to myosin, can be accounted for by the retention of an interaction between the flexible loop at the junction of the 50 kDa and 20 kDa tryptic fragments of myosin with actin. This latter interaction would then be nonstereospecific in nature and is predicted to help keep myosin close to actin during the hydrolysis step.

Kinetic and spectroscopic measurements suggest that nucleotide binding is a multistep process. In the first stage, it is hypothesized that only the α-, β-, and γ-phosphates, and perhaps part of the ribose moiety of the nucleotide, bind to subfragment-1 in the ‘P-loop’ at the base of the active-site pocket. It is not envisaged that the initial stage of nucleotide binding induces any major change in the curvature or overall shape of the protein. The major shape change is proposed to occur during the second stage of ATP binding, when the active-site pocket closes around the base of the nucleotide to produce a conformationally bent myosin head (Fig. 5c). Thus, in the proposed model, the major conformational change in the head occurs when the head is dissociated from actin. This two-stage process is necessary to avoid the problem of the molecule reversing its power stroke while it is tightly bound to actin in a stereospecific manner, which would result in no net movement of myosin relative to actin. It is further proposed that hydrolysis of ATP only occurs when the active-site pocket is in the closed conformation and results in a metastable state. At this stage, the molecule is thought to be in a conformationally bent state that is primed and ready for release of inorganic phosphate and ADP.

Rebinding of myosin to actin is also believed to be a multistep process. The initial model-building studies indicate that actin and myosin interact with each other in a stereospecific manner. This suggests that reformation of the tight complex between actin and myosin, after hydrolysis but before release of products, will only occur when the myosin head adopts a stereospecific orientation relative to actin. It must be emphasized that myosin is attached to the thick filament via a fairly rigid pair of intertwined α-helices, such that its degrees of freedom relative to actin are limited. As can be seen schematically in Fig. 5d, the myosin head must rotate (by diffusion) into a new position one or more actin subunits along the thin filament before it can adopt the appropriate stereospecific orientation.

Once this rotation has occurred, rebinding results in release of the γ-phosphate by closure of the narrow cleft (Fig. 5e). In this model, the presence of the γ-phosphate in the active site of myosin and tight binding of myosin to actin are mutually exclusive. The loss of the γ-phosphate triggers the start of the power stroke and allows the myosin molecule to reverse the conformational changes induced by the binding of the adenine portion of the nucleotide (Fig. 5e). As a result, the active-site pocket reopens and the myosin head returns to its rigor state (Fig. 5f), while concurrently moving the carboxy-terminal segment of the myosin molecule relative to actin. After this process, ADP is released, whereupon ATP can rapidly rebind and restart the contractile cycle. This analysis assumes that the conformation of myosin observed in the rigor state represents the end of the force-producing contractile cycle. It should be noted that this mechanism requires turnover of one ATP molecule per power stroke and is totally inconsistent with recent suggestions that myosin is capable of executing many power strokes per hydrolysis event.

Evidence for the model

One of the more frustrating aspects of the study of myosin over the past forty years has been the difficulty in detecting any major conformational changes in the subfragment-1 head dur-
ing the contractile cycle. It is only recently that very careful, low-angle scattering measurements have provided evidence for a change in the shape of the molecule upon nucleotide binding. Early chemical crosslinking studies, however, clearly suggested that significant movement in the head must occur, since the distance between two reactive cysteine residues (Cys697 and Cys707) changed dramatically from 19 Å to 2 Å when nucleotide was bound in the active-site pocket. Furthermore, these studies demonstrated that it was possible to trap ADP in the active site by crosslinking Cys697 and Cys707 (Ref. 36). In the X-ray structure, determined in the absence of nucleotide, these reactive sulfhydryl groups are separated by an α-helix and point to opposite sides of the molecule (Fig. 6). Evidence for closure of the active-site pocket was further provided by photolabeling studies in which amino acid residues, now known to lie on opposite sides of the active-site pocket and separated by about 13 Å, were chemically labeled by nucleotide analogs.

It is thus instructive to examine the subfragment-1 model to determine if there is a pathway by which the active-site pocket can be closed through a series of inter-domain movements. It is difficult to justify rearrangement within a domain, since this would be energetically unfavorable. The active-site pocket is abutted on one side by the amino-terminal segment of the heavy chain (Fig. 3). This region provides three of the seven β-strands of the major tertiary structural motif within the catalytic part of the myosin head, and also forms a small domain that constitutes one of the walls of the pocket. It is unlikely that the β-sheet bends when the active-site pocket closes around the nucleotide. However, rotation of this small domain around the end of the sheet might be an energetically feasible movement. Additionally, this small domain is characterized by a substantial protein-protein interface with the essential light chain (Fig. 3), such that any movement of this domain would be transduced to the light-chain and hence to the rest of the light-chain-binding region. When myosin is oriented onto the actin filament, the active-site pocket faces away from the filament and opens towards the center of the sarcomere or thick filament. Closure of the active-site pocket would cause the carboxy-terminal end of the heavy chain to rotate upwards relative to the actin-binding site. This is exactly the direction of movement of the tail necessary for priming the molecule for the power stroke. These movements are indicated in Fig. 6a.

There is one inherent problem with the proposed domain movement and that is due to the thickness of the myosin molecule. The base of the active-site pocket is located approximately
half way through the thickness of the head. Thus, closure of the nucleotide pocket by a domain rotation must be associated with a rearrangement below the active site, since the protein cannot be compressed or stretched without a large energetic penalty. It is thus noteworthy that this region below the active site contains the reactive cysteine residues discussed earlier. These residues represent a dividing line between those domains identified to bind to actin and those that might be associated with closing the active-site pocket and hence moving the carboxyl terminus of the heavy chain. Figure 6b shows that there is a deep cleft associated with one of the cysteine residues, and this is at the start of the long α-helix that constitutes the backbone of the light-chain-binding region. At this point, the long α-helix has little contact with the rest of the heavy chain. If it is true that the conformational change associated with closure of the active-site pocket is transduced to the light chains via contact with the essential light chain, then closure of this pocket would allow the helix to rotate relative to the actin-binding site without introducing any distortions within the domains themselves. These types of movement are consistent with those observed in other enzymatic reactions such as the active-site closure in hexokinase.37

Magnitude of the power stroke

The crystal structure of the myosin head predicts a minimum step size or power stroke of 60 Å based on simple closure of the active-site pocket. However, the physical dimensions of the protein place an upper limit on the size of the step size of approximately 200 Å, assuming that the molecule operates through a 90° power stroke. These lengths are consistent with earlier estimates deduced from the tension measurements of muscle fibers after a rapid change in their length.38,39 Likewise, an analysis of the kinetic properties of rapidly contracting muscle fibers yields an estimate of about 100 Å for the power stroke.39 By contrast, much larger values have been suggested based on the careful study of rapid length changes in muscle fibers induced by the photolysis of caged substrates, and from in vitro motility studies.34,40 These and other studies have given rise to the hypothesis that myosin can undergo several or many power strokes per ATP hydrolysed.41 It is difficult to reconcile the multiple-powerstroke hypothesis with the structure of myosin. If it is true that the source of movement is a series of distinct conformational changes induced by the sequential binding of nucleotide to myosin followed by hydrolysis and rebinding to actin, and that each of these steps leads to a decrease in free energy, then it is thermodynamically impossible for a single hydrolysis reaction to give rise to multiple power-generating steps. Resolution of this conflict between physiological measurements and the three-dimensional structure of myosin should lead to a greater insight into the biological mechanism of muscle contraction.

Concluding remarks

There are many questions that still remain to be answered concerning the structural and enzymatic basis of myosin-based motility. The most obvious are ‘what types of molecular conformations are exhibited by the myosin head upon nucleotide and actin binding?’ and ‘how does the subfragment-1 function within the context of a muscle fiber?’ Additional questions arise from the manner in which the ATPase activity is regulated in myosin from different biological sources. Skeletal-muscle myosin is only one member of a vast group of motor molecules that utilize the myosin-heavy-chain motif.42 There is still much to be learned about the cellular function of these proteins. Fortunately, the structure of the myosin head now provides a framework upon which to dissect these problems through the combination of molecular biology, biophysics and biochemistry. Indeed, this is an especially exciting time for the study of directed movement which is, after all, one of the essential characteristics of living organisms.

Acknowledgements

The study of muscle contraction has generated a vast literature over the past forty years and this information provides the foundation for the hypotheses described in this review. Unfortunately, through the confines of this short article, it is impossible to give adequate and due credit to the many outstanding researchers in the muscle field. This work was supported in part by funds from the NIH and the American Heart Association.

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