Molecular mechanism of the contractile cycle of muscle

Sunil Nath* and Divya Khurana

Department of Biochemical Engineering and Biotechnology,
Indian Institute of Technology, Hauz Khas, New Delhi 110 016, India

Motility is a characteristic feature of all living organisms. Myosin and actin together constitute the contractile machinery of the cell. Myosin is an asymmetric protein composed of two globular heads attached to a long tail and forms the thick filament, while actin is a double-helical protein making up the thin filament. Considerable effort has been spent over the past several decades to understand the physicochemical basis of energy conversion by the actomyosin system of muscle; yet the molecular mechanism of muscle contraction remains one of the outstanding enigmas in biology. Here we propose a novel, comprehensive molecular mechanism of the contractile cycle of muscle. Specifically, we address the mechanisms of force generation, energy transduction and storage, and subsequent utilization of the stored energy to perform useful work. Our original approach and insights elucidate the molecular mechanism of muscle contraction.

MORE than 130 years have elapsed since the actomyosin complex was isolated from muscle[1], yet the molecular origin of the force produced during muscle contraction is unknown and remains one of the most outstanding enigmas in biology[2–4]. Force generation in muscle involves interactions between actin, a helical protein, and myosin, a highly asymmetric protein molecule[5,6]. The myosin molecule consists of two globular heads attached to a long tail. Its molecular weight is approximately 500,000 Da and it is made up of six polypeptide chains: two heavy chains with a mass of 400,000 Da and two sets of light chains, each weighing about 20,000 Da. Each globular head, composed of approximately 850 amino acid residues, contains one heavy chain associated with two light chains. Each heavy chain forms the bulk of one head and intertwines with its neighbour to form the nearly 150 nm long rod[5–8]. Limited proteolytic digestion studies have shown that myosin head (subfragment 1 or S-1) contains an ATP, actin and two light chain binding sites, and the myosin rod consists of a coiled-coil of two α-helices. The myosin molecule constitutes the thick filament in a muscle sarcomere.

The structure of skeletal muscle myosin S-1 indicates that the molecule has several deep clefts and pockets (Figure 1)[5–8]. Another key structural feature of S-1 is an 8.5 nm long α-helix (called the regulatory domain or the lever arm) composed of more than 60 amino acid residues, which extends from the thick part of the head (the catalytic domain containing the nucleotide and actin binding sites) to the COOH terminus of the S-1 fragment. The head is composed of three major regions: a 25 kDa NH_2 terminal domain which contains the nucleotide binding site, a 20 kDa COOH terminal domain and a central 50 kDa segment that is split into an upper and a lower domain by a prominent cleft (50 K cleft) that extends from the base of the nucleotide binding pocket to the actin binding interface (Figure 1). This, along with the 20 kDa domain constitutes the actin binding site of the molecule, which lies on the opposite side of the nucleotide binding site[2,7]. The 50 kDa cleft serves as the communication between the actin binding interface and the nucleotide binding site by means of domain movements associated with it. These domain movements are such that binding of actin to myosin closes the 50 K cleft and opens the nucleotide binding site, while binding of nucleotide closes the nucleotide binding site and disrupts the actin binding interface by opening the 50 K cleft. Apart from the myosin head (consisting of the regulatory and catalytic domains), the molecule is made up of a fibrous rod-like tail region whose amino acid sequence is highly repetitive, showing cycles of a 28-residue repeat pattern composed of four heptapeptides characteristic of α-helical coiled coils[2–8].

Actin is the major constituent of thin filaments in the muscle sarcomere[5,6,9,10]. At physiological ionic strengths, actin exists as F-actin, a polymer of individual monomers of G-actin. The F-actin structure can be described as a right-handed double helix in which two threads of actin twist around each other[5]. The helix has a diameter of about 9 nm and a pitch of about 36 nm[9,10].

In this paper, we propose a novel, comprehensive molecular mechanism of the contractile cycle of muscle. In our mechanism, we address some of the most fundamental aspects of this problem: How is the force generated? How is the chemical energy of ATP hydrolysis transduced and stored? How is this stored energy utilized to cause movement?

We begin the exposition of our molecular mechanism from the point where the ATP is bound to the nucleotide binding site of the myosin molecule and the myosin molecule is detached from actin and is primed for hydrolysis. Insight into the molecular mechanism of ATP hydrolysis and the conformational changes accompanying it can be gained from the pioneering X-ray structural studies of the Rayment group[2,7,8,11–14]. They solved crystal structures of the truncated myosin head containing only the catalytic domain in the presence of various nucleotide analogues such as MgADP·BeF_x, MgADP·AlF_4, MgADP·VO_4, $MgPP_i$, MgAMPPNP and MgATPγS (refs 11–14). Their studies reveal that the beryllium fluoride complex mimics the pre-hydrolysis (ATP) state and adopts an overall protein conformation which is almost identical to that of chicken skeletal myosin S-1, while the aluminum fluoride and the vanadate complexes are realistic analogues of the transition state of ATP hydrolysis

*For correspondence. (e-mail: sunath@dbeb.iitd.ernet.in)

(ADP·P_i state)[2]. The BeF_3O moiety exhibits a tetrahedral coordination similar to that expected for ATP, while the metal atom in the AlF_4^-/VO_4^{3-} complex exhibits octahedral coordination. Overlap of the beryllium and vanadate complex structures shows that the ribose sugar, adenine base and α- and β-phosphate groups adopt almost identical locations in the nucleotide binding site. This implies that the major conformational change between the two states occurs in the γ-phosphate pocket and that the MgADP leaving group experiences a very similar environment in the two states. Rather, the conformational change in myosin is such that it orients a water molecule for nucleophilic attack on the γ-phosphate. Thus, ATP hydrolysis takes place by an associative mechanism in which the phosphorus to oxygen distance increases on transition from ATP to the ADP·P_i state[15]. The conformational change in the γ-phosphate pocket is seen as a movement of the lower 50 kDa domain relative to the upper 50 kDa domain, which results in partial closure of the 50 K cleft; this change is propagated to the COOH terminal region of myosin and results in a major rearrangement of the polypeptide chain beyond Lys-690. This suggests that the top of the regulatory domain of the myosin head rotates by ~ 20°. Moreover, the conformational changes associated with myosin–vanadate complex[2] as well as oxygen exchange studies[16–18] suggest that after hydrolysis, bound MgADP and P_i do not leave the site by themselves and require a conformational change which, in our view, is initiated by binding of actin to myosin.

We now address some of the original and key aspects of our molecular mechanism of muscle contraction and elucidate how the energy of ATP hydrolysis is converted to mechanical work. A central tenet of our molecular mechanism is storage of the energy of ATP hydrolysis as internal energy within the myosin molecule. The fact that ATP hydrolysis takes place in the state where myosin is detached from actin[19], but movement requires the attachment of myosin with actin filament necessitates, in our view, the need for storage of the energy of ATP hydrolysis in the myosin molecule, in the absence of which the energy would be dissipated, without being employed for the performance of useful work. The presence of a time lag between the hydrolysis event and motion is an implicit feature of all kinetic schemes to date[19–23], which offers further support to our energy storage feature. We make the novel proposal that the energy of ATP hydrolysis is stored as an increase in twist of the two α-helices forming the coiled-coil of the S-2 region of the myosin rod. Rotation of the top of the regulatory domain of the myosin head caused by ATP hydrolysis leads to this increase in twist in the myosin rod and to the rotation of the myosin head. The latter motion is a consequence of the fact that the myosin head is detached from actin and is free to rotate, i.e. the catalytic domain rotates along with the

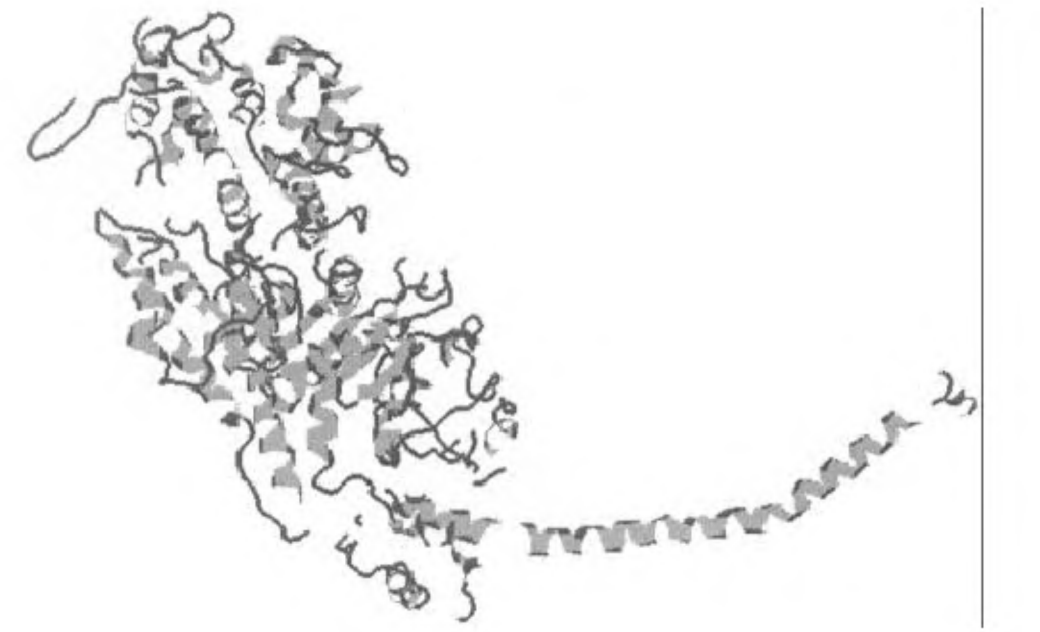

Figure 1. Ribbon representation of the X-ray structure of chicken skeletal muscle myosin subfragment S-1. The orientation is such as to face the actin binding interface looking into the 50 K cleft, while the nucleotide binding site lies on the opposite side. The molecule is divided into the catalytic domain (thick part) containing the actin and the nucleotide binding sites and a long helix (regulatory domain) associated with two light chains. Based on trypsin digestion studies, the myosin molecule can be divided into three regions: the 25 kDa NH_2 terminal domain, 50 kDa central domain and the 20 kDa COOH terminal domain. These domains are identified by the presence of disordered loops between them in the X-ray crystal structure. The 50 kDa central region serves as a communication between the actin binding interface and the nucleotide binding site formed by both the NH_2 terminal and 50 kDa central domains, while the COOH region serves as the regulatory domain.

regulatory domain as one unit. Increase in twist in the coiled-coil is accompanied by tilt of the myosin head. This tilt takes place about the region connecting the myosin head to the rod region (i.e. connecting S-1 to S-2). Thus, rotation of the top of the regulatory domain caused by ATP hydrolysis leads to three effects: (i) increase in twist of the α-helices forming the myosin rod; (ii) rotation of the myosin head; and (iii) tilt of the myosin head. These motions can be understood more clearly by reference to Figures 2 and 3. We define the twist angle ϕ, as the angle between the axis of the rod and the tangent to one of the α-helices constituting the coiled-coil. Increase in twist of the α-helices forming the coiled-coil leads to a decrease in ϕ as depicted in Figure 2, which is drawn for both pre-hydrolysis and post-hydrolysis states. The tilt angle, θ is defined as the angle formed by the axis of the actin filament with the lever arm (Figure 3). In our proposed molecular mechanism, ATP hydrolysis leads to a decrease in θ as represented in Figure 3. According to our molecular mechanism, decrease in ϕ stores the energy released due to the enthalpy change upon ATP hydrolysis, while decrease in θ and rotation of the myosin head are responsible for bringing the myosin head in proximity to the actin filament and facilitate the attachment and binding of myosin to actin. All three motions (tilt, twist and rotation) are essential and play critical roles in our molecular mechanism.

Thus, by the time the ATP hydrolysis event is complete, the energy of hydrolysis has been stored as an increase in twist and the myosin head is bound to the actin. Binding of myosin to actin leads to complete closure of the previously partially closed myosin 50 K cleft and thereby opens the nucleotide binding site and releases the bound P_i and ADP. In our molecular mechanism, release of bound nucleotides is not the driving force for movement and hence is not directly coupled to movement; therefore, the order of release of P_i and ADP is not of great consequence to our mechanism. However, kinetic studies indicate that P_i leaves the site before ADP[16,18,24].

The power stroke takes place when the S-2 region (coiled-coil) untwists, i.e. ϕ increases (Figure 2). This decrease in twist leads to release of stored energy which is utilized to move the actin filament. Concomitant with untwisting, the myosin head untilts about the contact between S-1 and S-2, i.e. the angle θ increases (Figure 3). This untilting motion generates the force that drags the actin filament along with the myosin head. The overall motion of the actomyosin complex is similar to the motion of a shovel shovelling its load (mud or coal), except that in the case of the actin–myosin system, the shovel (myosin) remains attached to the load (actin) throughout the power stroke. Figure 4 illustrates the state of the actomyosin complex before and after the power stroke. In our conception, the myosin head tilts and untilts as a single entity, i.e. there is no bending of one region of myosin head with respect to another. An implication of our molecular mechanism is that in the absence of a proper frame of reference (i.e. without S-2), it is impossible to detect the envisaged changes in tilt, twist or rotation. Thus, techniques such as attachment of spectroscopic probes to S-1, which fix the frame of reference to the S-1 (refs 25 and 26) itself, will not measure these changes.

We now predict the changes in the tilt angle θ required for the execution of the power stroke according to our proposed molecular mechanism. The length of the myosin head is approximately 20 nm as measured by electron microscopy and X-ray crystallography[27–29]. The myosin step size in the power stroke measures 5.3 nm as determined by single-molecule total internal reflection fluorescence microscopy[30]; this also coincides with the distance between two adjacent myosin binding sites on the actin filament. Representing the myosin head as a rigid entity tilting about a fulcrum lying at the base of the head, the change in the tilt angle $\Delta\theta$ can be obtained from the equation:

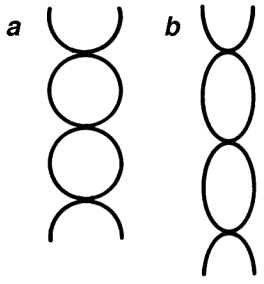

Figure 2. Pre-hydrolysis and post-hydrolysis states of the myosin rod formed by the coiled-coil of two α-helices, each coil belonging to a different myosin head. The coiled-coil has a 28-residue or a 4 heptapeptide repeat pattern characteristic of all coiled-coil structures. (*a*) In the pre-hydrolysis state, the coiled-coil structure is relaxed with a larger twist angle, ϕ; (*b*) In the post-hydrolysis state, an increase in the twist of the two α-helices forming the coiled-coil stores the energy of ATP hydrolysis. This increase in twist causes decrease in the twist angle ϕ of the coiled-coil, which arises from rotation of the top of the regulatory domain of myosin head, a conformational change caused by ATP hydrolysis.

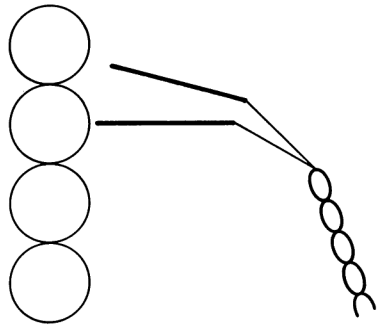

Figure 3. Tilt of the myosin head in the pre-hydrolysis and post-hydrolysis states. (Top) The pre-hydrolysis state. The lever arm makes an angle of ~ 110° (θ) with the axis of the actin filament. (Bottom) The post-hydrolysis state. Due to ATP hydrolysis the molecule tilts about the contact between S-1 and S-2 by an angle of approximately 15° (θ ~ 95°), sufficient to bring the myosin head close enough to bind to the myosin binding site of the next actin monomer.

$$20 \cdot \sin(\Delta\theta) = 5.3, \quad (1)$$

which yields a $\Delta\theta$ value of approximately 15°. From the crystal structure of S-1 (ref. 7), the post-power stroke value of θ is determined to be 111°. Thus, the pre-power stroke value of θ measures approximately 96°. These values of the tilt angles are in complete consonance with recent fluorescence polarization measurements on intact muscle fibers[31]. Note that depending on the experimental system, the pre-power and post-power stroke tilt angles may vary; however, the difference between them is expected to be of almost the same magnitude as predicted in this work.

At the end of the power stroke, the energy stored in the myosin has been converted to useful work, the twist in the myosin rod and the tilt in the myosin head have reached their pre-hydrolysis values and the 50 K cleft is closed. The myosin head had experienced a rotation after ATP hydrolysis prior to its binding with actin. Upon commencement of the power stroke, the head would try to follow a trajectory that is the exact reversal of its previous motion; however the binding of the myosin head to actin monomer places constraints on this from happening. As a result, the myosin head unrotates while still bound to actin. Therefore, the interactions between residues forming the actin binding interface of the myosin head and the myosin binding site of the actin monomer are strained. We name this state the rigor state. Although the actomyosin interactions are strained in rigor, they are not sufficient to detach myosin from actin. Thus, additional interactions are needed to break the actomyosin complex.

In the rigor state, no nucleotide is bound to the myosin nucleotide binding site. The next cycle can commence only after another molecule of ATP from the medium binds and supplies the additional (binding) energy required to break the actomyosin interactions, thereby detaching myosin from actin and priming it for hydrolysis. How exactly this takes place will now be explained in detail. First, Mg^{2+} interacts with its ligands at the top of the tunnel leading from the nucleotide binding site to the 50 K cleft. The Mg^{2+} is directly coordinated to the Oγ1 atom of Thr-186 (distance 2.1 Å) and the Oγ atom of Ser-237 (distance 2.4 Å) and to two water molecules (2.1 Å each). These water molecules in turn interact with the carboxy side chain of Asp-454 (distance 2.5 Å) and with the main chain carbonyl oxygen of Asn-235 (distance 2.8 Å). Thus, this interaction of Mg^{2+} with its ligands makes the sub-region acquire the correct conformation for binding. Now Mg^{2+} binds and the binding energy is utilized to modify the site to a conformation conducive to the binding of the adenine ring and the phosphate groups, a process in which each of them incrementally facilitate the other's binding to the nucleotide binding site. Since the myosin–actin interactions are already strained, the binding energy of MgATP is sufficient to break the actomyosin complex. Thus, nucleotide binding opens the 50 K cleft which in turn disrupts the actin binding site and reduces the affinity of myosin for actin. This leads to detachment of myosin from actin; concomitantly, the nucleotide binding site adopts a conformation that is closed around MgATP (i.e. the motion that opens the 50 K cleft also closes the site). Thus the nucleotide binding site in myosin is in a state that is conducive for hydrolysis. This brings us back to the state that we started with and completes the contractile cycle (Figure 4).

The fundamental questions in the field of motility (e.g. how is the hydrolysis of ATP coupled to motion?) have proved very difficult to answer, despite decades of intense research, and the molecular mechanism of muscle contraction has remained a mystery. The 1970s and 1980s saw the use of sophisticated biophysical techniques such as electron paramagnetic resonance, X-ray scattering, fluorescence spectroscopy, electric birefringence, etc. to detect the rocking of the myosin head about its contact point with actin, as predicted by the swinging crossbridge model[32,33] of the 1960s. The results were negative; however, since it was felt that the model was never very clear about how the crossbridge moved and no detailed molecular mechanism of the process was presented, it was refined to the swinging lever arm model[34–38] in the 1980s. This model has gained the maximum general support over the years. The model envisages the bulk of the cross-

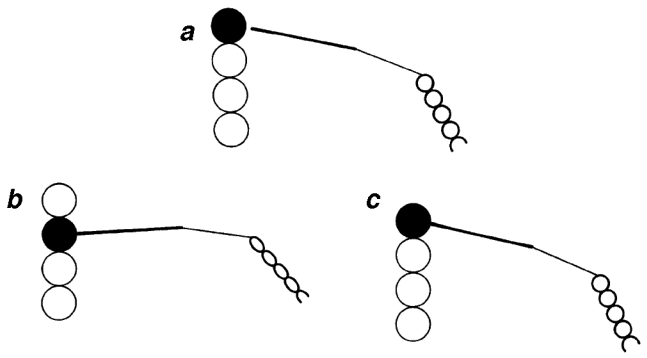

Figure 4. Schematic representation of the contractile cycle of muscle according to our molecular mechanism. (*a*) One contractile cycle has been completed and ATP has already bound to the nucleotide binding site in the myosin head and disassociated the myosin head from the actomyosin complex. The 50 K cleft is open and the nucleotide binding site is closed around ATP. The tilt angle θ is approximately 110° and the molecule is primed for hydrolysis; (*b*) ATP hydrolysis has taken place leading to rotation of the top of the regulatory domain, which causes tilt in the myosin head and energy storage as increase in twist in the myosin rod. The rotation and tilt of the head brings myosin sufficiently close to the myosin binding site on actin, so that an actomyosin complex can form. Binding of actin to myosin leads to release of the products P_i and ADP; (*c*) State of the actomyosin complex after the power stroke is complete. The power stroke is initiated by untwisting of the coiled-coil of S-2. Simultaneously, the myosin head untilts about the contact between S-1 and S-2, generating a force that drags the actin filament along with the myosin head, as explained in the text. During the power stroke, the unrotation of the myosin head generates strain in the actin–myosin interactions because the myosin head cannot rotate freely when it is bound to actin. The binding energy of MgATP is sufficient to break the strained actin–myosin interactions. Thus, dissociation of myosin from actin takes place, leading to the state of the system shown in (*a*), and completing the contractile cycle.

bridge to bind to actin with a fixed geometry (i.e. the orientation of the catalytic domain does not change) and only the carboxy-terminal part of myosin head (the regulatory domain) swings ~ 10 nm with respect to both the catalytic and rod domains[34–38]. However, there is no direct experimental evidence that such large conformational changes take place during force generation in the muscle. It is a prediction of our proposed molecular mechanism of the contractile cycle of muscle that the large amplitude motions of the regulatory domain relative to both the catalytic and rod domains of myosin have not been detected experimentally because they do not exist, and our molecular mechanism clearly shows that there is, in fact, no need for such motions.

Recently, in a series of papers, we have proposed the torsional mechanism of energy transmission and ATP synthesis[39–41] – the first complete, unified mechanism of ATP synthesis by ATP synthase, a *nonequilibrium* molecular machine (the smallest known) and a remarkable mechano-(electro)chemical transducer. Our mechanism of ATP synthesis has been quantified[42–46], a novel kinetic model has been formulated and mathematically analysed[42,43,45,46] and a detailed analysis based on the concepts of irreversible thermodynamics[44] has been carried out. It is instructive to note that storage of energy within a single molecule (as internal energy) plays a central role in the torsional mechanism of ATP synthesis also. Energy storage within single molecules and its subsequent utilization via specific mechanisms of the type proposed and detailed in earlier papers[39–43,45,46] and in this work may prove to be one of the great unifying principles of biology – in oxidative phosphorylation, muscle contraction and other related energy transductions. These properties make the above topics very attractive for contemporary interdisciplinary research. Finally, in our view, the aspects dealt with in this work constitute the *key* elements whose lack of detailed consideration has held back the progress of research in the important field of motility.

We refer to our molecular mechanism as the Rotation–Twist–Tilt (RTT) energy storage mechanism of muscle contraction. As in lever arm models, velocity is proportional to length of the lever arm (l); however, a key prediction and distinguishing feature of the RTT energy storage mechanism lies in the fact that the force (f) that drags the actin filament is independent of l, or, at least, it has no *direct* relationship with l, unlike in the lever arm model[34–36,38] (where $f \propto l^{-1}$) or the modified lever arm model[37] (in which $f \propto l^{-2}$).

1. Kühne, W., Untersuchungen uber das Protoplasma und die Contractilität, Engelmann, Leipzig, 1864.
2. Rayment, I., *J. Biol. Chem.*, 1996, **271**, 15850–15853.
3. Block, S. M., *Cell*, 1996, **87**, 151–157.
4. Goldman, Y. E., *Cell*, 1998, **93**, 1–4.
5. Vander, A. J., Sherman, J. H. and Luciano, D. S., *Human Physiology*, McGraw-Hill, New York, 1994, 6th edition.
6. Stryer, L., *Biochemistry*, W. H. Freeman, New York, 1988, 3rd edition, chapter 36.
7. Rayment, I. *et al.*, *Science*, 1993, **261**, 50–58.
8. Rayment, I., Holden, H. M., Whittaker, M., Yohn, C. B., Lorenz, M., Holmes K. C. and Milligan, R. A., *Science*, 1993, **261**, 58–65.
9. Mannherz, H. G., *J. Biol. Chem.*, 1992, **267**, 11661–11664.
10. Holmes, K. C., Popp, D., Gebhard, W. and Kabsch, W., *Nature*, 1990, **347**, 44–49.
11. Fisher, A. J., Smith, C. A., Thoden, J. B., Smith, R., Sutoh, K., Holden, H. M. and Rayment, I., *Biochemistry*, 1995, **34**, 8960–8972.
12. Smith, C. A. and Rayment, I., *Biochemistry*, 1995, **34**, 8973–8981.
13. Smith, C. A. and Rayment, I., *Biochemistry*, 1996, **35**, 5404–5417.
14. Gulick, A. M., Bauer, C. B., Thoden, J. B. and Rayment, I., *Biochemistry*, 1997, **36**, 11619–11628.
15. Senior, A. E., Nadanaciva, S. and Weber, J., *J. Exp. Biol.*, 2000, **203**, 35–40.
16. Trentham, D. R., Eccleston, J. F. and Bagshaw, C. R., *Q. Rev. Biophys.*, 1976, **9**, 217–281.
17. Webb, M. R., Hibberd, M. G., Goldman, Y. E. and Trentham, D. R., *J. Biol. Chem.*, 1986, **261**, 15557–15564.
18. Goldman, Y. E., *Annu. Rev. Physiol.*, 1987, **49**, 637–654.
19. Lymn, R. W. and Taylor, E. W., *Biochemistry*, 1971, **10**, 4617–4624.
20. Ishijima, A., Kojima, H., Funatsu, T., Tokunaga, M., Higuchi, H., Tanaka, H. and Yanagida, T., *Cell*, 1998, **92**, 161–171.
21. Geeves, M. A., *Biochem. J.*, 1991, **274**, 1–14.
22. Dantzig, J. A., Barsotti, R. J., Manz, S., Sweeney, H. L. and Goldman, Y. E., *Biophys. J.*, 1999, **77**, 386–397.
23. Spudich, J. A., *Nature*, 1994, **372**, 515–518.
24. Yount, R. G., Lawson, D. and Rayment, I., *Biophys. J.*, 1995, **68**, 44s–49s.
25. Thomas, D. D., *Annu. Rev. Physiol.*, 1987, **49**, 691–709.
26. Toyoshima, Y. Y., Kron, S. J., McNally, E. M., Niebling, K. R., Toyoshima, C. and Spudich, J. A., *Nature*, 1987, **328**, 536–539.
27. Elliott, A. and Offer, G., *J. Mol. Biol.*, 1978, **123**, 505–519.
28. Winkelmann, D. A., Mekeel, H. and Rayment, I., *J. Mol. Biol.*, 1985, **181**, 487–501.
29. Winkelmann, D. A., Baker, T. S. and Rayment, I., *J. Cell Biol.*, 1991, **114**, 701–713.
30. Kitamura, K., Tokunaga, M., Iwane, A. H. and Yanagida, T., *Nature*, 1999, **397**, 129–134.
31. Corrie, J. E. T. *et al.*, *Nature*, 1999, **400**, 425–430.
32. Huxley, H. E., *Science*, 1969, **164**, 1356–1366.
33. Huxley, A. F., *Prog. Biophys. Biophys. Chem.*, 1957, **7**, 255–318.
34. Cooke, R., *Curr. Biol.*, 1999, **9**, R773–R775.
35. Holmes, K. C., *Curr. Biol.*, 1997, **7**, R112–R118.
36. Vale, R. D. and Milligan, R. A., *Science*, 2000, **288**, 88–95.
37. Uyeda, T. Q. P., Abramson, P. D. and Spudich, J. A., *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 4459–4464.
38. Highsmith, S., *Biochemistry*, 1999, **38**, 9791–9797.
39. Rohatgi, H., Saha, A. and Nath, S., *Curr. Sci.*, 1998, **75**, 716–718.
40. Nath, S., Rohatgi, H. and Saha, A., *Curr. Sci.*, 1999, **77**, 167–169.
41. Nath, S., Rohatgi, H. and Saha, A., *Curr. Sci.*, 2000, **78**, 23–27.
42. Nath, S. and Jain, S., *Biochem. Biophys. Res. Commun.*, 2000, **272**, 629–633.
43. Jain, S. and Nath, S., *FEBS Lett.*, 2000, **476**, 113–117.
44. Nath, S., *Pure Appl. Chem.*, 1998, **70**, 639–644.
45. Nath, S., *Adv. Biochem. Eng. Biotechnol.*, 2001 (in press).
46. Jain, S. and Nath, S., *Thermochim. Acta*, 2001 (in press).

ACKNOWLEDGEMENTS. This research has been generously funded by a special five-year Swarnajayanti Research Fellowship (2001–2006) to S. N. by the Ministry of Science and Technology, New Delhi.

Received 17 October 2000; accepted 5 March 2001