Molecular engineering of myosin

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Protein engineering and design provide excellent tools to investigate the principles by which particular structural features relate to the mechanisms that underlie the biological function of a protein. In addition to studies aimed at dissecting the communication pathways within enzymes, recent advances in protein engineering approaches make it possible to generate enzymes with increased catalytic efficiency and specifically altered or newly introduced functions. Here, two approaches using state-of-the-art protein design and engineering are described in detail to demonstrate how key features of the myosin motor can be changed in a specific and predictable manner. First, it is shown how replacement of an actin-binding surface loop with synthetic sequences, whose flexibility and charge density is varied, can be employed to manipulate the actin affinity, the catalytic activity and the efficiency of coupling between actin- and nucleotide-binding sites of myosin motor constructs. Then the use of pre-existing molecular building blocks, which are derived from unrelated proteins, is described for manipulating the velocity and even the direction of movement of recombinant myosins.

Keywords: *Dictyostelium*; X-ray structure; protein design; actin; myosin

1. INTRODUCTION

The cyclical interaction between myosin and the actin filament drives muscle contraction and many other types of cellular movement. The conserved myosin motor domain is the active partner in this interaction, and undergoes a series of conformational changes, 'rowing' the actin filament past the myosin. It is this part of the myosin molecule that corresponds to the cross-bridges that decorate the myosin filaments in the 'sliding-filament' model (Huxley & Hanson 1954; Huxley & Niedergerke 1954). The cross-bridges were first visualized almost 50 years ago (Huxley, H. E. 1957) and a quantitative cross-bridge theory of muscle contraction with coupled ATP hydrolysis was proposed within the same year (Huxley, A. F. 1957). Further studies led to the 'swinging cross-bridge' model, visualizing the cross-bridges as active partners in the interaction with filamentous actin and producers of a 'power-stroke' (Reedy *et al.* 1965; Huxley 1969). Lymn & Taylor (1971) showed that myosin is a product-inhibited ATPase that is strongly stimulated by actin, and produced a first model of the cyclical interaction between myosin and actin filaments that associates structural with biochemical states. Spectroscopic and structural observation refined this 'swinging cross-bridge' hypothesis into a 'swinging lever arm' model, in which the bulk of the cross-bridge binds to actin with almost constant geometry, and movement is mostly produced by the distal part of the cross-bridge moving as a lever arm (see review by Holmes & Geeves 2000).

More recently, the availability of methods for the production of recombinant myosin motors (Manstein *et al.*

1989; Trybus 1994; Matsu-ura & Ikebe 1995), direct functional assays for motor activity (Sheetz & Spudich 1983; Yanagida *et al.* 1984; Kron & Spudich 1986; Finer *et al.* 1994; Funatsu *et al.* 1995; Molloy *et al.* 1995b; Ishijima *et al.* 1998) and crystal structures of the myosin head (see reviews by Geeves & Holmes 1999; Houdusse & Sweeney 2001) have greatly advanced our understanding of how particular structural features relate to the mechanism of chemo-mechanical coupling in the myosin motor domain. As biochemical and molecular genetic approaches have grown ever more powerful, new questions have become interesting and addressable. Here, we will focus on current models of the mechanism of energy transduction by myosin and how these models relate to state-of-the-art protein design and engineering approaches.

2. CURRENT MODEL OF THE MECHANISM OF ENERGY TRANSDUCTION

Crystallographic studies of myosin head fragments show that two conserved sequence motifs at the nucleotide-binding pocket, termed switch-1 and switch-2, which act as γ -phosphate sensors, play a central role in the mechanism of force and movement generation (figure 1a). Similar to the G-proteins, the transition of switch-1 and switch-2 between 'closed' and 'open' states is nucleotide dependent and associated with specific intramolecular movements (Vale 1996; Goody & Hofmann-Goody 2002). Based on structural models of myosin motor domains in different nucleotide states, it has been suggested that myosins amplify the 'open' to 'closed' transition of switch-2 to a swinging motion of the adjacent lever-arm from an initial 'up' to a 'down' position (Geeves & Holmes 1999). Movement of switch-1 is communicated by the three edge β -strands of the central β -sheet to the actin-binding residues of the upper and lower

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Figure 1. Structural features of the myosin motor domain. (a) Ribbon diagram of the nucleotide-free structure of the *Dictyostelium* myosin II motor domain (Reubold *et al.* 2003). The upper 50 K domain (red), the actin-binding domain (blue) and the N-terminal domain (green) contribute to the seven-stranded β -sheet. (b)(i) Nucleotide-dependent changes in the active site of myosin. Structural elements surrounding Mg-ADP-BeF₃ in a complex with both switch-1 and switch-2 in the CA conformation (Dominguez *et al.* 1998). The P-loop β -strand and loop (green) coordinate the Mg²⁺ ion through Thr186 (residue numbers are derived from the *Dictyostelium* myosin II sequence). Switch-I (red) interacts directly with the nucleotide γ -phosphate (BeF₃ in this structure) by interactions with Ser236 (not shown) and indirectly through Mg²⁺, which is coordinated by Ser237. Switch-II (cyan) interacts with the γ -phosphate directly by a hydrogen bond from Gly457 (blue sphere) and indirectly with the Mg²⁺ from Asp454 and a bridging water molecule (waters shown as cyan spheres). The salt bridge between Arg238 and Glu459 is formed and binding interactions are shown as dashed lines. (b)(ii) Structural elements surrounding the nucleotide-binding site in a nucleotide-free structure showing a conformation that is similar to that assumed for the SA state (Reubold *et al.* 2003). The P-loop is essentially unchanged, and switch-II has moved away by *ca.* 7 Å from its position in (a). The tip of switch-I has moved away from the P-loop by *ca.* 8 Å. Despite these local changes the salt bridge between Arg238 and Glu459 is also present in the nucleotide-free structure.

50 K domains (myosin subdomains), leading to changes of up to 20 Å in the distance between elements of the actin-binding region that are positioned in the upper and lower 50 K domains (Coureux *et al.* 2003; Reubold *et al.* 2003). Additionally, the conformational changes that are associated

with the transition of the switch motifs are tightly coordinated and integrated. There is growing evidence from electron microscopy and spectrophotometric studies of the actomyosin complex that the resulting opening and closing of the large cleft that separates the upper and lower 50 K domains is coupled to large changes in the affinity of myosin for actin filaments (Volkmann *et al.* 2000; Conibear *et al.* 2003; Holmes *et al.* 2003).

According to these results, the myosin motor domain appears to cycle between two key states, which we will refer to as the CA-state and the SA-state. In the CA-state, a hydrogen bond can be formed between G457 and the γ -phosphate, and the γ -phosphate is coordinated by the Mg²⁺ ion, the invariant P-loop lysine K185, and S181. The large cleft that separates the upper and lower 50 K domains is open or in the low-affinity conformation for F-actin, and the lever-arm in the 'up' or pre-powerstroke position. In the SA-state, corresponding to the rigor form, the large cleft is closed and in the high-affinity conformation for F-actin binding, and the lever-arm is in the 'down' or post-powerstroke position. Virtually all essential interactions and almost all coordination between the protein and the Mg²⁺ ion, direct or indirect, are lost.

Both the CA- and SA-states are stabilized by the formation of a salt bridge between R238 in switch-1 and E459 in switch-2 (figure 1b). Additionally, the salt bridge acts to drive the reaction forward by orienting the critical catalytic residues from switch I and switch II (Furch *et al.* 2000). This facilitates ATP hydrolysis and ATP rebinding, which limit the lifetimes of the CA- and SA-states, respectively. Additionally, there exist an as yet undefined number of states with intermediate actin-affinity and ADP-Pi, ADP or Pi at the active site. Structural models for several of these intermediate states show the salt bridge always broken, and indicate that similar to the paired salt-bridge residues, the unpaired residues play a critical role in coordinating ATP binding and facilitating product release (Fisher *et al.* 1995; Houdusse *et al.* 1999, 2000; Gulick *et al.* 2000).

3. ARTIFICIAL REPLACEMENTS FOR ACTIN-BINDING LOOPS

There exists good experimental evidence showing that several surface loops on the myosin motor domain play important roles in modulating the functional and kinetic characteristics that differentiate one myosin isoform from another (Goodson *et al.* 1999; Van Dijk *et al.* 1999). Thus differences in ADP affinity to acto-S1 and in *in vitro* motility can be attributed to differences in loop 1, a surface loop situated near the nucleotide-binding site (Kurzawa-Goertz *et al.* 1998; Sweeney *et al.* 1998; Murphy & Spudich 2000). That it is actually possible to increase the activity of a myosin motor through changes in a surface loop was first demonstrated in studies of chimeric myosins, where loop 2 was replaced (Uyeda *et al.* 1994). Loop 2 forms part of the actin-binding region. To understand how changes in the structure and charge of loop 2 affect the $K_{M(\text{actin})}$ and V_{max} , we analysed a series of mutant constructs in which the charge and length of loop 2 were systematically changed (Furch *et al.* 1998). This work reveals a clear correlation between the charge of the loop, the activation by actin of the mutant constructs' ATPase rates, and the strength of the interaction between mutant

Figure 2. ATPase activity of myosin II constructs with synthetic insert in the loop 2 region. Actin-activated ATPase activities of wild-type construct M765 (filled squares), M765(5/−1) (diamonds) with a loop 2 region that is five residues longer and lacks one positive charge, and M765(8/+4) (open squares) with eight extra residues and four additional positive charges measured at varying F-actin concentrations are shown.

motor domains and actin. The addition of 4 to 12 positive charges to loop 2 increased the affinity for actin up to 120-fold, $V_{\max}$ up to fivefold and the efficiency of coupling between the actin- and nucleotide-binding sites, as expressed by $V_{\max}/K_{M(\text{actin})}$, 30–70-fold (figure 2).

Differential scanning calorimetry confirmed that length and charge changes in loop 2 do not significantly affect nucleotide-induced structural changes in the myosin motor domain, but they affect structural changes that occur when the motor domain is strongly bound to actin and affect the coupling between the actin- and nucleotide-binding sites. In the presence of nucleotide, most mutants with synthetic inserts displayed transition temperatures similar to or higher than those of the wild-type construct (Levitsky *et al.* 1998; Ponomarev *et al.* 2000).

Parameters including length, conformational flexibility, charge and charge density can be easily adjusted for synthetic insert sequences and their use thus provides a straightforward means to fine-tune the catalytic activity of recombinant myosin motor constructs.

4. ARTIFICIAL LEVER ARMS

(a) *Extending the lever arm*

Myosins move unidirectionally along polymer tracks formed by filamentous actin, producing movement and force in an ATP-dependent fashion. They achieve this by amplifying small conformational changes in the motor domain by the use of the LCBD as conformational amplifier or lever arm (Rayment *et al.* 1993; Schröder *et al.* 1993; Holmes 1997). An important feature of the lever arm model is that it has the LCBD rigidly attached to the converter domain and not by a mobile joint. Converter and lever arm swing together in a rigid body motion with its axis of rotation close and almost parallel to the relay helix. The replacement of the LCBD with artificial lever arms is facilitated because of the limited number of contacts between the motor domain and the LCBD. Therefore, the main technical problems that need to be solved upon

Figure 3. Structure of a backward-moving myosin motor. (a) The four-helix bundle derived from hGBP is an ideal building block to rotate the direction of the myosin lever arm by 180°. (b) The artificial pointed (−)-end directed motor E698-Ω2R (Tsiavalieris *et al.* 2004) consists of a myosin I motor domain (gold), a four-helix bundle derived from hGBP (red), and an extended lever arm consisting of *Dictyostelium* α-actinin repeats 1 and 2 (green).

replacement of the LCBD are the creation of a stiff junction, tight control over the direction in which the lever projects away from the motor domain, and a means to produce constructs with lever arms of different but well-defined length.

Anson *et al.* (1996) pioneered the use of genetically engineered domains of similar rigidity and dimensions for replacement of the native LCBD. The resulting single-chain constructs have potential for use in nanotechnological applications. They are more efficiently produced, display unchanged kinetic properties compared with the parental myosin, and produce force and movement (Ruff *et al.* 2001). The crystal structure of the *Dictyostelium* myosin II motor domain fused to the spectrin-like repeats 1 and 2 of α -actinin indicates that the artificial lever emerges from the motor domain in the proper orientation with little angular deviation relative to the natural amplifier domain (Kliche *et al.* 2001). Recently, we have extended this approach to produce a variety of unconventional myosin motor domains with artificial lever arms consisting of one to six spectrin-like repeats (S. Fujita-Becker, G. Tsiavaliaris, U. Dürrwang and D. J. Manstein, unpublished results). So far, the fastest myosin construct generated by this method is a *Chara corallina* myosin XI motor domain fused to two α -actinin repeats, which moves actin filaments with a mean velocity of $16.2 \mu\text{m s}^{-1}$ (Ito *et al.* 2003). These results show that spectrin-like repeats do provide a convenient means to replace and extend the lever arm by 6 nm modules. When the fusion site was chosen one to four residues downstream of the conserved pentapeptide Glu-Glu-X-Arg-Glu motif at the boundary between motor domain and LCBD, the addition of spectrin-like repeats produced in every instance functional motors with the expected motile activity. We have focused entirely on the use of spectrin-like repeats, but there are certainly other proteins and protein domains that can be used as building blocks to extend the lever arm in a controlled fashion.

(b) *Molecular engineering of a backward-moving myosin motor*

A direct consequence of the lever arm theory is that the generation of a myosin motor with reversed direction of movement can be achieved simply by rotating the lever arm through 180° (Wells *et al.* 1999). If the lever arm points in the opposite direction, the same rotation of the converter region that produces (+)-end directed movement in the native myosin will now lead to the opposite translational movement of the tip of the lever arm and therefore (–)-end directed movement in the mutant constructs. The following points need to be considered in engineering a construct with a lever arm rotated by 180° . First, a suitable protein or protein domain needs to be identified whose insertion leads to a near 180° rotation of the lever arm. Second, steric clashes between the lever arm and the motor domain must be avoided. Finally, rigid junctions have to be created and the individual building blocks joined in the proper orientation. The successful generation of an artificial backward moving myosin was achieved using the following pre-existing molecular building blocks: a directional inverter formed by a four-helix bundle motif derived from hGBP, an artificial lever arm formed by *Dictyostelium* α -actinin repeats 1 and 2, and a forward-moving class I myosin

motor domain derived from *Dictyostelium* MyoE (Tsiavaliaris *et al.* 2004).

The first building block, the hGBP four-helix bundle (Prakash *et al.* 2000), is an ideal building block for achieving rotation of the lever arm. It is a highly rigid structure whose orientation, after fusion with the C-terminal helix emerging from the converter domain, can be accurately predicted. Additionally, the hGBP four-helix bundle can be attached to the converter domain in such a way that the plane in which the lever arm moves during the working-stroke is shifted away from the motor domain by *ca.* 3.5 nm (figure 3a). This helps to prevent steric clashes with the motor and makes it possible to rotate the lever arm the full 180° . Spectrin-like repeats, in particular α -actinin repeats, the second type of building block, have been used as artificial lever arms in a wide range of constructs, where they always performed reliably (Anson *et al.* 1996; Kurzawa *et al.* 1997; Ruff *et al.* 2001; Ito *et al.* 2003). The third building block, the 698 residue *Dictyostelium* MyoE motor domain (E698), resembles the motor domain of conventional myosins (Kollmar *et al.* 2002). Importantly, E698 lacks an N-terminal SH3-like domain, which helps to prevent steric clashes between the motor domain and the engineered lever arm. The structures of all three building blocks used for the generation of the backward-moving motor were known, which considerably facilitated designing a functional construct. Although several technical problems had to be overcome, this strategy led to the generation of a molecular motor that moves actin filaments backwards with normal speed (Tsiavaliaris *et al.* 2004).

The generation of backward- and forward-moving motors that display increased thermal stability, optimized kinetic properties and tight regulation by external signals will be important for integrating them in nanotechnological devices. Because of the high degree of structural conservation between motor domains from different myosin classes and because all building blocks can be connected by the direct fusion of α -helical regions, it appears as if most myosins can be used to produce backward-moving motors with minimal trial and error. However, electron microscopy studies with myosin I isozymes of *Acanthamoeba castellanii* suggest that exceptions may exist. In the case of *A. castellanii* myosin-IB, the lever arm appears to move in a plane that is almost perpendicular to the plane of the power-stroke of other myosins for which structural information has been obtained (Carragher *et al.* 1998).

5. CONCLUSION

Considerable progress has been made in applying molecular engineering approaches to the design and production of myosin motors with enhanced and specifically altered functions. Myosins motor domains are ideal model systems for developing and advancing protein engineering and design because they produce mechanical changes that can be directly followed by a large variety of techniques, in addition to spectroscopic signals and chemical changes during their catalytic cycle. This facilitates the testing and direct confirmation of the correct stereospecific attachment of engineered modules using *in vitro* motility assays and single molecule approaches (Kron & Spudich 1986; Finer *et al.* 1994; Molloy *et al.* 1995a; Ishijima *et al.* 1998).

The successful generation of a backward-moving myosin motor by the attachment of two unrelated building blocks to

a myosin I motor domain shows that directionality is primarily a function of the neck region. The motor core is preserved in all members of the myosin family, and direction of movement depends on how the same nucleotide- and actin-binding-dependent conformational changes are subsequently interpreted by attached amplifier domains.

So far, the functional characterization of engineered myosins has focused on detecting the motile activity of the constructs at low load. Future work will focus on the ability of the constructs to produce force against external loads, the incorporation of switches that allow tight control of motor activity, the attachment of domains that mediate cargo binding, and the addition of modules for the ordered assembly on specific substrates. Progress in these areas will advance protein-engineering approaches generally and eventually lead to the incorporation of custom-built molecular motors into nanotechnological devices.

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REFERENCES

- Anson, M., Geeves, M. A., Kurzawa, S. E. & Manstein, D. J. 1996 Myosin motors with artificial lever arms. *EMBO J.* **15**, 6069–6074.
- Carragher, B. O., Cheng, N., Wang, Z. Y., Korn, E. D., Reilein, A., Belnap, D. M., Hammer, J. A. & Steven, A. C. 1998 Structural invariance of constitutively active and inactive mutants of *Acanthamoeba* myosin IC bound to F-actin in the rigor and ADP-bound states. *Proc. Natl Acad. Sci. USA* **95**, 15 206–15 211.
- Conibear, P. B., Bagshaw, C. R., Fajer, P. G., Kovacs, M. & Malnasi-Csizmadia, A. 2003 Myosin cleft movement and its coupling to actomyosin dissociation. *Nat. Struct. Biol.* **10**, 831–835.
- Coureux, P. D., Wells, A. L., Menetrey, J., Yengo, C. M., Morris, C. A., Sweeney, H. L. & Houdusse, A. 2003 A structural state of the myosin V motor without bound nucleotide. *Nature* **425**, 419–423.
- Dominguez, R., Freyzon, Y., Trybus, K. M. & Cohen, C. 1998 Crystal structure of a vertebrate smooth muscle myosin motor domain and its complex with the essential light chain: visualization of the pre-power stroke state. *Cell* **94**, 559–571.
- Finer, J. T., Simmons, R. M. & Spudich, J. A. 1994 Single myosin molecule mechanics: piconewton forces and nanometre steps. *Nature* **368**, 113–119.
- Fisher, A. J., Smith, C. A., Thoden, J. B., Smith, R., Sutoh, K., Holden, H. M. & Rayment, I. 1995 X-ray structures of the myosin motor domain of *Dictyostelium discoideum* complexed with MgADP.BeFx and MgADP.AIF₄-. *Biochemistry* **34**, 8960–8972.
- Funatsu, T., Harada, Y., Tokunaga, M., Saito, K. & Yanagida, T. 1995 Imaging of single fluorescent molecules and individual ATP turnovers by single myosin molecules in aqueous solution. *Nature* **374**, 555–559.
- Furch, M., Geeves, M. A. & Manstein, D. J. 1998 Modulation of actin affinity and actomyosin adenosine triphosphatase by charge changes in the myosin motor domain. *Biochemistry* **37**, 6317–6326.
- Furch, M., Rimmel, B., Geeves, M. A. & Manstein, D. J. 2000 Stabilization of the actomyosin complex by negative charges on myosin. *Biochemistry* **39**, 11 602–11 608.
- Geeves, M. A. & Holmes, K. C. 1999 Structural mechanism of muscle contraction. *A. Rev. Biochem.* **68**, 687–728.
- Goodson, H. V., Warrick, H. M. & Spudich, J. A. 1999 Specialized conservation of surface loops of myosin: evidence that loops are involved in determining functional characteristics. *J. Mol. Biol.* **287**, 173–185.
- Goody, R. S. & Hofmann-Goody, W. 2002 Exchange factors, effectors, GAPs and motor proteins: common thermodynamic and kinetic principles for different functions. *Eur. Biophys. J.* **31**, 268–274.
- Gulick, A. M., Bauer, C. B., Thoden, J. B., Pate, E., Yount, R. G. & Rayment, I. 2000 X-ray structures of the *Dictyostelium discoideum* myosin motor domain with six non-nucleotide analogs. *J. Biol. Chem.* **275**, 398–408.
- Holmes, K. C. 1997 The swinging lever-arm hypothesis of muscle contraction. *Curr. Biol.* **7**, R112–R118.
- Holmes, K. C. & Geeves, M. A. 2000 The structural basis of muscle contraction. *Phil. Trans. R. Soc. B* **355**, 419–431. (doi: 10.1098/rstb.2000.0583)
- Holmes, K. C., Angert, I., Kull, F. J., Jahn, W. & Schröder, R. R. 2003 Electron cryo-microscopy shows how strong binding of myosin to actin releases nucleotide. *Nature* **425**, 423–427.
- Houdusse, A. & Sweeney, H. L. 2001 Myosin motors: missing structures and hidden springs. *Curr. Opin. Struct. Biol.* **11**, 182–194.
- Houdusse, A., Kalabokis, V. N., Himmel, D., Szent-Gyorgyi, A. G. & Cohen, C. 1999 Atomic structure of scallop myosin subfragment S1 complexed with MgADP: a novel conformation of the myosin head. *Cell* **97**, 459–470.
- Houdusse, A., Szent-Gyorgyi, A. G. & Cohen, C. 2000 Three conformational states of scallop myosin S1. *Proc. Natl Acad. Sci. USA* **97**, 11 238–11 243.
- Huxley, A. F. 1957 Muscle structure and theories of contraction. *Prog. Biophys. Biophys. Chem.* **7**, 255–318.
- Huxley, A. F. & Niedergerke, R. 1954 Structural changes in muscle during contraction; interference microscopy of living muscle fibres. *Nature* **173**, 971–973.
- Huxley, A. F. & Hanson, J. 1954 Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature* **173**, 973–976.
- Huxley, H. E. 1957 The double array of filaments in cross-striated muscle. *J. Biophys. Biochem. Cytol.* **3**, 631–648.
- Huxley, H. E. 1969 The mechanism of muscular contraction. *Science* **164**, 1356–1365.
- Huxley, H. & Hanson, J. 1954 Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature* **173**, 973–976.
- Ishijima, A., Kojima, H., Funatsu, T., Tokunaga, M., Higuchi, H., Tanaka, H. & Yanagida, T. 1998 Simultaneous observation of individual ATPase and mechanical events by a single myosin molecule during interaction with actin. *Cell* **92**, 161–171.
- Ito, K., Kashiwayama, T., Shimada, K., Yamaguchi, A., Awata, J., Hachikubo, Y., Manstein, D. J. & Yamamoto, K. 2003 Recombinant motor domain constructs of *Chara corallina* myosin display fast motility and high ATPase activity. *Biochem. Biophys. Res. Commun.* **312**, 958–964.
- Kliche, W., Fujita-Becker, S., Kollmar, M., Manstein, D. J. & Kull, F. J. 2001 Structure of a genetically engineered molecular motor. *EMBO J.* **20**, 40–46.
- Kollmar, M., Dürrwang, U., Kliche, W., Manstein, D. J. & Kull, F. J. 2002 Crystal structure of the motor domain of a class-I myosin. *EMBO J.* **21**, 2517–2525.
- Kron, S. J. & Spudich, J. A. 1986 Fluorescent actin filaments move on myosin fixed to a glass surface. *Proc. Natl Acad. Sci. USA* **83**, 6272–6276.

- Kurzawa, S. E., Manstein, D. J. & Geeves, M. A. 1997 *Dictyostelium discoideum* myosin II: characterization of functional myosin motor fragments. *Biochemistry* **36**, 317–323.
- Kurzawa-Goertz, S. E., Perreault-Micale, C. L., Trybus, K. M., Szent-Gyorgyi, A. G. & Geeves, M. A. 1998 Loop I can modulate ADP affinity, ATPase activity, and motility of different scallop myosins. Transient kinetic analysis of S1 isoforms. *Biochemistry* **37**, 7517–7525.
- Levitsky, D. I., Ponomarev, M. A., Geeves, M. A., Shnyrov, V. L. & Manstein, D. J. 1998 Differential scanning calorimetric study of the thermal unfolding of the motor domain fragments of *Dictyostelium discoideum* myosin II. *European J. Biochem.* **251**, 275–280.
- Lymn, R. W. & Taylor, E. W. 1971 Mechanism of adenosine triphosphate hydrolysis by actomyosin. *Biochemistry*, 4617–4624.
- Manstein, D. J., Ruppel, K. M. & Spudich, J. A. 1989 Expression and characterization of a functional myosin head fragment in *Dictyostelium discoideum*. *Science* **246**, 656–658.
- Matsu-ura, M. & Ikebe, M. 1995 Requirement of the two-headed structure for the phosphorylation dependent regulation of smooth muscle myosin. *FEBS Lett.* **363**, 246–250.
- Molloy, J. E., Burns, J. E., Kendrick-Jones, J., Tregear, R. T. & White, D. C. 1995a Movement and force produced by a single myosin head. *Nature* **378**, 209–212.
- Molloy, J. E., Sparrow, J. C., Tregear, R. T., Kendrick-Jones, J. & White, D. C. 1995b Single-molecule mechanics of heavy meromyosin and S1 interacting with rabbit or *Drosophila* actins using optical tweezers. *Biophys. J.* **68**(Suppl.), 298S–303S.
- Murphy, C. T. & Spudich, J. A. 2000 Variable surface loops and myosin activity: accessories to a motor. *J. Muscle Res. Cell Motil.* **21**, 139–151.
- Ponomarev, M. A., Furch, M., Levitsky, D. I. & Manstein, D. J. 2000 Charge changes in loop 2 affect the thermal unfolding of the myosin motor domain bound to F-actin. *Biochemistry* **39**, 4527–4532.
- Prakash, B., Renault, L., Praefcke, G. J., Herrmann, C. & Wittinghofer, A. 2000 Triphosphate structure of guanylate-binding protein 1 and implications for nucleotide binding and GTPase mechanism. *EMBO J.* **19**, 4555–4564.
- Rayment, I., Holden, H. M., Whittaker, M., Yohn, C. B., Lorenz, M., Holmes, K. C. & Milligan, R. A. 1993 Structure of the actin-myosin complex and its implications for muscle contraction. *Science* **261**, 58–65.
- Reedy, M. K., Holmes, K. C. & Tregear, R. T. 1965 Induced changes in orientation of the cross-bridges of glycerinated insect flight muscle. *Nature* **207**, 1276–1280.
- Reubold, T. F., Eschenburg, S., Becker, A., Kull, F. J. & Manstein, D. J. 2003 A structural model for actin-induced nucleotide release in myosin. *Nature Struct. Biol.* **10**, 826–830.
- Ruff, C., Furch, M., Brenner, B., Manstein, D. J. & Meyhofer, E. 2001 Single-molecule tracking of myosins with genetically engineered amplifier domains. *Nature Struct. Biol.* **8**, 226–229.
- Schröder, R. R., Manstein, D. J., Jahn, W., Holden, H., Rayment, I., Holmes, K. C. & Spudich, J. A. 1993 Three-dimensional atomic model of F-actin decorated with *Dictyostelium* myosin S1. *Nature* **364**, 171–174.
- Sheetz, M. P. & Spudich, J. A. 1983 Movement of myosin-coated structures on actin cables. *Cell Motil.* **3**, 485–489.
- Sweeney, H. L., Rosenfeld, S. S., Brown, F., Faust, L., Smith, J., Xing, J., Stein, L. A. & Sellers, J. R. 1998 Kinetic tuning of myosin via a flexible loop adjacent to the nucleotide binding pocket. *J. Biol. Chem.* **273**, 6262–6270.
- Trybus, K. M. 1994 Regulation of expressed truncated smooth muscle myosins. Role of the essential light chain and tail length. *J. Biol. Chem.* **269**, 20 819–20 822.
- Tsiavaliaris, G., Fujita-Becker, S. & Manstein, D. J. 2004 Molecular engineering of a backwards-moving myosin motor. *Nature* **427**, 558–561.
- Uyeda, T. Q., Ruppel, K. M. & Spudich, J. A. 1994 Enzymatic activities correlate with chimaeric substitutions at the actin-binding face of myosin. *Nature* **368**, 567–569.
- Vale, R. D. 1996 Switches, latches, and amplifiers: common themes of G proteins and molecular motors. *J. Cell Biol.* **135**, 291–302.
- Van Dijk, J., Furch, M., Lafont, C., Manstein, D. J. & Chaussepied, P. 1999 Functional characterization of the secondary actin binding site of myosin II. *Biochemistry* **38**, 15 078–15 085.
- Volkman, N., Hanein, D., Ouyang, G., Trybus, K. M., DeRosier, D. J. & Lowey, S. 2000 Evidence for cleft closure in actomyosin upon ADP release. *Nature Struct. Biol.* **7**, 1147–1155.
- Wells, A. L., Lin, A. W., Chen, L. Q., Safer, D., Cain, S. M., Hasson, T., Carragher, B. O., Milligan, R. A. & Sweeney, H. L. 1999 Myosin VI is an actin-based motor that moves backwards. *Nature* **401**, 505–508.
- Yanagida, T., Nakase, M., Nishiyama, K. & Oosawa, F. 1984 Direct observation of motion of single F-actin filaments in the presence of myosin. *Nature* **307**, 58–60.

GLOSSARY

- ADP: adenosine diphosphate
 ATP: adenosine triphosphate
 CA: catalytically active
 hGBP: human guanylate binding protein-1
 LCBD: light-chain binding domain
 MyoE: *Dictyostelium discoideum* class-I myosin
 Pi: phosphate
 SA: strongly attached