The Regulatory Domain of the Myosin Head Behaves as a Rigid Lever†

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ABSTRACT: The regulatory domain of the myosin head is believed to serve as a lever arm that amplifies force generated in the catalytic domain and transmits this strain to the thick filament. The lever arm itself either can be passive or may have a more active role storing some of the energy created by hydrolysis of ATP. A structural correlate which might distinguish between these two possibilities (a passive or an active role) is the stiffness of the domain in question. To this effect we have examined the motion of the proximal (ELC) and distal (RLC) subdomains of the regulatory domain in reconstituted myosin filaments. Each subdomain was labeled with a spin label at a unique cysteine residue, Cys-136 of ELC or Cys-154 of mutant RLC, and its mobility was determined using saturation transfer electron paramagnetic resonance spectroscopy. The mobility of the two domains was similar; the effective correlation time (τ_{eff}) for ELC was 17 μs and that for RLC was 22 μs. Additionally, following a 2-fold change of the global dynamics of the myosin head, effected by decreasing the interactions with the filament surface (or the other myosin head), the coupling of the intradomain dynamics remained unchanged. These data suggest that the regulatory domain of the myosin head acts as a single mechanically rigid body, consistent with the regulatory domain serving as a passive lever.

According to the current working model for the mechanism of muscle contraction the torque generated in the catalytic domain is transmitted to the regulatory domain, which works like a lever arm swinging toward the Z-line (*1*−*6*). The atomic structure of the myosin head clearly identifies the involved domains, the globular catalytic domain that binds both actin (*7*, *8*) and ATP[1] and an elongated regulatory domain consisting of the calmodulin-like essential light chain (ELC) and regulatory light chain (RLC) bound to a long heavy chain helix originating in the catalytic domain. The above model implies two structural aspects which need experimental verification: that a hinge or region of high flexibility exists between the catalytic and regulatory domain, to allow the rotation of the regulatory domain, and that the regulatory domain is relatively stiff to allow transmission of the strain to the thick filament.

The existence of a flexible region between the catalytic and regulatory domains has been confirmed using a number of techniques: electron microscopy (*9*), X-ray crystallography of trapped intermediates (*2*), fluorescence spectroscopy (*10*), electric birefringence (*11*), and saturation transfer electron paramagnetic resonance (ST-EPR) (*12*). The second aspect, stiffness of the lever arm is more controversial. The linear relationship between the sliding velocities in the in vitro motility assay and the length of the regulatory domain supports the stiff, passive lever arm (*13*−*15*). This relationship was not always observed (*16*−*18*), suggesting a more active role for the regulatory arm (e.g., to store elastic energy) (*19*).

In this study we examine the relative mobility of the proximal (ELC containing) and distal (RLC containing) subdomains of the putative lever arm. One can reasonably expect that if the lever is flexible, the motions of the two subdomains occur on different time scales, but if the lever is rigid, then the motions will be similar. To accomplish this comparison a unique cysteine residue of ELC and a unique cysteine of a mutant RLC were labeled with a paramagnetic probe, indandione spin label (InVSL), and their motilities in synthetic myosin filaments were compared using ST-EPR. The ELC is located near the center of the myosin head, while the RLC forms the part of the head most distal from the actin−myosin interface. The mobilities of the labels on these

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[1] Abbreviations: ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; DMF, *N*,*N*′-dimethylformamide; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid aminoethyl ether; ELC, essential light chain; HMM, heavy meromyosin; LC1, skeletal light chain 1; RLC-C125R, recombinant light chain 2 with a single cysteine at position 154; LC3, skeletal light chain 3; MOPS, 3-(*N*-morpholino)propanesulfonic acid; RLC, regulatory light chain; S1, myosin subfragment 1; TRIS, tris(hydroxymethyl)aminomethane; InVSL, 2-(oxy-2,2,5,5-tetramethyl-3-pyrrolin-3-methynyl)indan-1,3-dione.

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sites were found to be very similar. Furthermore, the coupling of mobilities persisted under conditions known to modulate the global mobility of the head. These results strongly support the notion of the regulatory domain of the myosin head forming a stiff lever arm as required for the "swinging lever arm" model.

MATERIALS AND METHODS

Myosin was prepared using the method of Tonomura et al. (*20*) with modifications as described in ref *21* and stored at −20 °C in 50% v/v glycerol, 0.5 M KCl, 0.5 mM EDTA, 0.5 mM DTT, and 20 mM MOPS, pH 7.0, at a concentration of 12 mg/mL. The LC3 isoform of ELC was isolated from purified myosin using the guanidine hydrochloride method (*22*). The LC3 isoform was used to avoid possible complications that may be caused by the 41-residue extension of the LC1 isoform. For clarity we will refer to ELC, although strictly speaking we have used only LC3. The light chains were separated on a Cibacron Blue 3GA column (Sigma, St. Louis, MO) following the method of Toste and Cooke (*23*). The ELC was stored at −70 °C in 0.2 M KCl, 1 mM DTT, and 20 mM TRIS, pH 7.5, at 5 mg/mL. A mutant of the skeletal RLC, in which the cysteine 125 residue was replaced with an arginine residue (RLC-C125R), was expressed in *Escherichia coli* and purified by ion-exchange on Q-Sepharose (*24*). The RLC-C125R was stored at −70 °C at a concentration less than 1 mg/mL in 2 M urea, 0.5 mM $MgCl_2$, 100 mM KCl, and 25 mM TRIS, pH 7.5.

Labeling. Prior to labeling the proteins were incubated for 2 h at 22 °C in 40 mM KCl, 2 mM EDTA, 5 mM potassium phosphate, and 5 mM DTT, pH 7.0 (for ELC), or in 300 mM KCl, 5 M urea, 40 mM TRIS, and 5 mM DTT, pH 7 (for RLC). After extensive dialysis against their respective buffers minus the DTT the concentration of the light chain was adjusted to 3−6 mg/mL (ELC) or 1 mg/mL (RLC). The light chains were labeled using a 5-fold excess of InVSL, which was added from a stock solution (0.1 M in DMF), and allowed to react for 2 h at 22 °C. Free spin label was removed by exhaustive dialysis against labeling buffer. ELC was dialyzed into 300 mM KCl, 2 mM EDTA, and 100 mM MOPS, pH 7.0, and RLC was dialyzed into 500 mM KCl, 2 mM EDTA, and 40 mM TRIS, pH 8.0. A label/protein ratio of 0.9−1.0 was determined by spin quantitation using EPR and protein concentration determination by BCA assay (Pierce, Rockford, IL).

Exchange of Light Chains. ELC was exchanged onto myosin by incubating a 20-fold molar excess of labeled or unlabeled ELC over myosin in 4.7 M NH_4Cl, 5 mM ATP, 300 mM KCl, 2 mM EDTA, and 100 mM MOPS, pH 7.0, for 30 min at 0 °C. The exchanged myosin was precipitated by dialysis into 35 mM KCl and 10 mM MOPS, pH 7.0, and centrifuged for 20 min at 10 000*g*. To remove residual ELC, the myosin pellet was redissolved in high ionic strength buffer (500 mM KCl, 0.1 mM EDTA, and 10 mM MOPS, pH 7.0), then precipitated for 20 min by a 25-fold dilution in 35 mM KCl and 10 mM MOPS, pH 7.0, and centrifuged for 20 min at 10 000*g*. The myosin pellet was dissolved in 500 mM KCl, 0.1 mM EDTA, and 10 mM MOPS, pH 7.0. The efficiency of exchange was 50−70%.

RLC was exchanged onto myosin by incubating a 20-fold molar excess of RLC over myosin in 500 mM KCl, 5 mM ATP, 2 mM EDTA, and 40 mM TRIS, pH 8.0, for 10 min at 37 °C. The exchange was stopped by addition 10 mM $MgCl_2$ and cooling to 0 °C. Myosin was precipitated by dialysis against 1 mM $MgCl_2$ and 10 mM MOPS, pH 7.0, and centrifuged for 20 min at 10 000*g*. The excess of light chains was removed by two cycles of dissolving myosin in high ionic strength buffer and subsequent precipitation as described above. The efficiency of exchange was >80% as judged from the spin label/protein ratio. Notably, since the RLC used was an expressed recombinant protein, it was unphosphorylated, implying that essentially all of the exchanged myosin was not phosphorylated. Myosin with both the ELC and RLC exchanged was prepared by first exchanging the ELC followed by RLC exchange.

Synthetic Myosin Filaments. Synthetic myosin filaments were formed by dialysis against a buffer of 40 mM KCl and 10 mM MOPS, pH 7.0, for 10 h. After dialysis, the filaments were gently pelleted (5000*g* for 10 min) in a tabletop centrifuge at 4 °C and loaded into 50 μL fused silica capillary tubes (Wilmad Glass Co., Buena, NJ). Where necessary, preformed synthetic filaments were dialyzed against buffers with the desired pH and divalent ion concentrations prior to pelleting. The *high pH* buffer used was 40 mM KCl and 40 mM TRIS, pH 8.2, and the *high $MgCl_2$* buffer was 40 mM KCl, 10 mM $MgCl_2$, and 40 mM MOPS, pH 7.0.

ATPases. ATPase activity was assayed at 25 °C in 30 mM KCl, 3 mM $CaCl_2$, 1 mM $MgCl_2$, and 20 mM MOPS, pH 7.0, as described by Wikman-Coffelt et al. (*25*). The reaction was initiated by adding 1 mM MgATP. Aliquots were taken at 2, 4, 6, and 8 min, and the release of inorganic phosphate was determined by the method of Lanzetta et al. (*26*).

Myosin Phosphorylation. Myosin was phosphorylated in filamentous form. Myosin light chain kinase (MLCK, 1 nM) (gift of Dr. J. Stull), 100 nM calmodulin (CaM), 5 mM MgATP, and 1 mM $CaCl_2$ were added to approximately 4 mg/mL myosin in 40 mM KCl and 10 mM MOPS, pH 7.0, and incubated for 20 min at 25 °C. The phosphorylated myosin filaments were depolymerized to myosin monomers by the addition of 3 M KCl to a final concentration of 0.5 M KCl and extensively dialyzed in 0.5 M KCl, 0.5 mM EDTA, 0.5 mM DTT, and 20 mM MOPS, pH 7.0, to remove ATP. The extent of phosphorylation was determined by urea gels using the method of Szent-Gyorgyi and Niebieski (*27*). To quantify the extent of phosphorylation, gels were scanned on a page scanner (Microtek III) at 600 dpi resolution using Adobe Photoshop software. The images were stored as TIF files and were analyzed using SCION software from the National Institutes of Health.

EPR Spectroscopy. EPR and ST-EPR experiments were carried out on a Bruker ECS-106 X-band spectrometer as described in ref *28*, using a TE_{102} cavity with a microwave field strength (H_1) of 0.03−0.08 G and microwave modulation amplitude (H_m) of 2 G for EPR and $H_1 = 0.25$ G and $H_m = 5$ G for ST-EPR. The modulation frequency was 50 kHz. All experiments were carried out at 4 °C. Effective correlation times (τ_{eff}) were obtained from the ST-EPR spectra by comparison with spectra obtained from InVSL-labeled hemoglobin tumbling in media of known viscosity (87.55 wt %/wt, glycerol/water mixture and 37 wt %/wt, glycerol/water mixture).

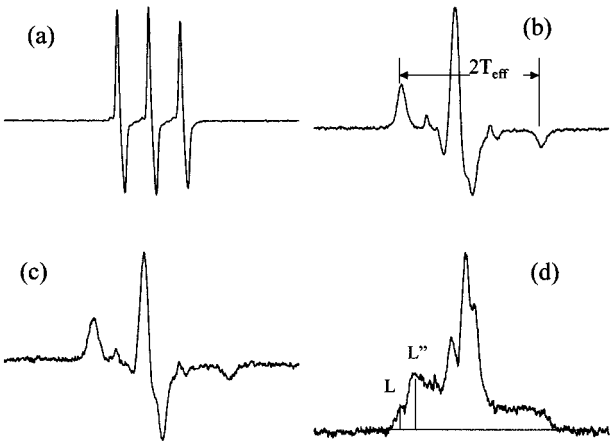

FIGURE 1: Spectra of InVSL-labeled RLC: (a) RLC in solution; (b) RLC exchanged into myosin filaments; (c) RLC immobilized; (d) ST-EPR spectrum of RLC immobilized on DITC beads.

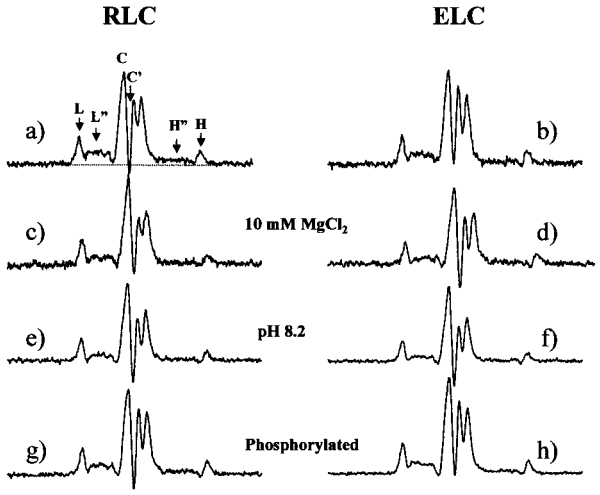

FIGURE 2: ST-EPR spectra of InVSL-labeled RLC and ELC myosin filaments: RLC (a) and ELC (b) at pH 7.0, 1 mM $MgCl_2$, and no phosphorylation; in 10 mM $MgCl_2$ (c) and (d) RLC and ELC, respectively; pH 8.2 (e and f); with RLC phosphorylated (g and h). A decrease in intensity of L″ (normalized to the intensity at L) indicates a lower τ_{eff} and a higher mobility ($1/\tau_{eff}$).

RESULTS

Spin Labeling of the ELC(LC3) and RLC. The single reactive cysteine residues Cys-136 of ELC(LC3) and Cys-154 of the RLC-C125R mutant were labeled with an indandione EPR spin label. It has been previously demonstrated that the spin label InVSL bound to ELC reports on the microsecond mobility of the ELC with no indication of faster nanosecond motions of the label with respect to the protein (*12*). To ascertain similar behavior for RLC, the protein was immobilized on DITC-coated glass beads, a widely used model system for determination of the rigid limit of protein mobility, and the EPR spectra were compared with those of labeled RLC exchanged into artificial myosin filaments.

The conventional EPR spectrum of artificial myosin filaments with InVSL-labeled RLC exchanged into it had a hyperfine splitting ($2T_{eff}$) of 70.8 ± 0.1 G, which compares well with the rigid limit spectrum of DITC, $2T_{eff}$ = 70.9 ± 0.2 G (see Figure 1). The high value of the ST-EPR diagnostic line-height ratio L″/L = 2.24 ± 0.07, obtained when bound to DITC beads, implies that the label does not execute motion on the sub-millisecond time scale of the ST-EPR technique. Taken together, the InVSL label attached to RLC-C125R does not execute motion with respect to the protein surface with correlation times shorter than ∼500 μs. Thus, the InVSL label is well suited to study the dynamics of the RLC subdomain.

Mobility of the ELC and RLC in Artificial Myosin Filaments. The dynamics of the regulatory domain was observed at the ELC and RLC vantage points using ST-EPR. The intensity of the ST-EPR spectra is a function of the spin label motion. The diagnostic regions L″, C′, and H″ (see Figure 2 a) are areas of high spectral diffusion and are most sensitive to rotational motion (*29*). The intensity of the spectra at the diagnostic regions is normalized to the intensity of the turning points L, C, and H for which spectral diffusion is minimal. This study used the values L″/L from the low-field region because of the high signal-to-noise ratio (see the low intensity in the H″ region of the spectrum) and the absence of spectral overlap as for the C′/C ratio.

Representative ST-EPR spectra of ELC and RLC are shown in Figure 2, RLC on the left column and ELC in the right column. Visual inspection indicates a considerable similarity of the line shapes irrespective of the vantage point. At low ionic strength, the L″/L ratios were 0.41 ± 0.02 for ELC and 0.46 ± 0.02 for RLC. The effective correlation times (τ_{eff}) corresponding to these values, obtained from comparison to hemoglobin, were 17.0 ± 0.2 μs for ELC and 22.5 ± 0.2 μs for RLC. This small, 25% difference in mobility ($1/\tau_{eff}$) implies either or both a limited degree of flexibility within the lever arm or a different orientation of labels with respect to the diffusional axis. The second possibility is not very likely due to large amplitudes of motion as observed by time-resolved phosphorescence of dyes attached to the same sites (*60*). The effects of restricted motion in ST-EPR approach that of isotropic motion at approximately 60° full amplitude which is the case here (*30*; Hustedt and Beth, personal communication).

Modulation of Global Mobility. Both high pH and the presence of divalent cations affect the interaction of the myosin head with the myosin filament (*31*−*33*) and are known to affect the mobility of the catalytic domain of the myosin head (*34*) and the proximal subdomain of the regulatory domain (*28*). These solution conditions have been shown previously to result in at least a 2-fold increase in mobility of the catalytic and regulatory domains. To investigate whether the intradomain flexibility can be unlocked by decreased interaction with the filament surface, ST-EPR measurements were performed on the ELC and RLC at $[Mg^{2+}]$ = 10 mM (Figure 2c,d) and at pH = 8.2 (Figure 2e,f). The 15-fold decrease of $[H^+]$ resulted in the τ_{eff} of RLC decreasing from 22.5 ± 0.2 to 11.1 ± 0.6 μs (L″/L = 0.34 ± 0.003) while the τ_{eff} for ELC decreased from 17.0 ± 0.2 to 7.1 ± 0.5 μs (L″/L = 0.29 ± 0.003) i.e., a 2-fold increase in the mobility for both the RLC and ELC subdomains of the regulatory domain. A 10-fold increase of $[Mg^{2+}]$ has a similar effect. The correlation time of RLC decreases from 22.5 ± 0.2 to 11.7 ± 0.8 μs (L″/L = 0.34 ± 0.004) accompanied by a similar decrease in ELC mobility from 17.0 ± 0.2 to 7.7 ± 0.4 μs (L″/L = 0.29 ± 0.002). Thus the mobility of the proximal and distal subdomains of

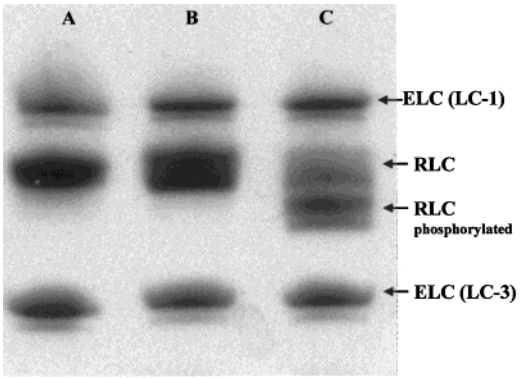

FIGURE 3: Urea gel of phosphorylated myosin filaments: lane A is native myosin, lane B is myosin exchanged with InVSL-RLC-C125R, and lane C is exchanged myosin phosphorylated with MLCK.

Table 1: Effective τ's of ELC and RLC in Myosin Filaments

	RLC		ELC	
sample conditions	τ_{eff} (SEM) (μs)	n^a	τ_{eff} (SEM) (μs)	n
low ionic	22.5 ± 0.2	23	17.0 ± 0.2	15
10 mM $MgCl_2$	11.7 ± 0.8	4	7.7 ± 0.4	4
pH 8.2	11.1 ± 0.6	4	7.1 ± 0.5	4
phosphorylated	15.2 ± 1.1	4	12.4 ± 0.4	4

[a] These were independent experiments using different protein preparations.

the regulatory domain remains tightly coupled, even when the global mobility of the head is increased.

Regulatory domain mobility was further modulated by the phosphorylation of Ser-16 of the RLC (*12*, *35*) by MLCK. The level of phosphorylation was evaluated using urea gels (Figure 3), and it varied from 69% to 90% for MLCK-treated samples and was less than 10% for native myosin.

Phosphorylation of myosin filaments resulted in a 1.5-fold increase of RLC mobility, $\tau_{eff} = 15.2 \pm 1.1$ μs ($L''/L = 0.40 \pm 0.004$), and a 1.4-fold increase for ELC, $\tau_{eff} = 12.4 \pm 0.4$ μs ($L''/L = 0.36 \pm 0.003$).

Table 1 summarizes the dynamics of ELC and RLC in all studied conditions. Despite 1.5−2-fold variation in the mobility of the regulatory domain as a whole the coupling between the two subdomains remains unchanged. The ratio of the ELC/RLC correlation time varied between 0.65 and 0.8 under all conditions (Figure 4).

DISCUSSION

The ELC subdomain of the regulatory domain of the myosin head was found to have a mobility similar to that of the RLC containing subdomain. This tight coupling persisted under conditions which increase the overall mobility of the myosin head: upon RLC phosphorylation, an increase of pH or the presence of divalent cations. Together, these results suggest that the ELC and RLC subdomains forming the regulatory domain act as a single, mechanically rigid body.

These basic results can be contrasted with the difference in flexibility between the catalytic and regulatory domains (*12*). For a motor which generates strain in one domain and transmits it to the other, it makes intuitive sense that there should be regions of flexibility between the domains while the domain transmitting the force, the lever arm, should be fairly rigid. These results are based on the observation of

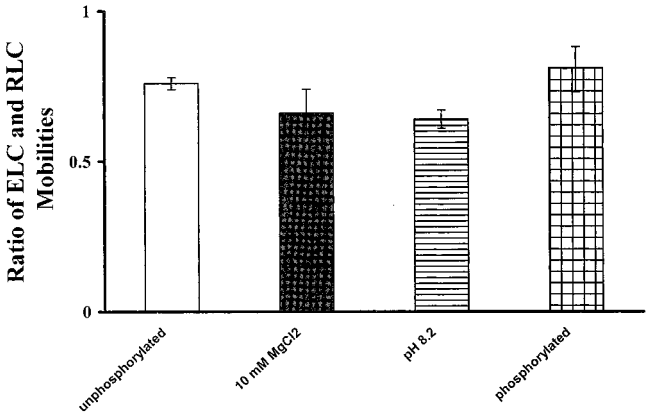

FIGURE 4: Subdomain mobility within the regulatory domain of myosin filaments. The ratio of the ELC and RLC correlation times unphosphorylated at pH 7.0 and 1 mM $MgCl_2$, 10 mM $MgCl_2$, pH 8.2, and for phosphorylated myosin.

mobility on two loci along the regulatory domain. Naturally, a larger number of vantage points would provide more detail and observe longer parts of the lever arm, but the basic result is unlikely to change. Modifying the existing cysteines with the spin label is not likely to change the structure of the regulatory domain. Functional assays involving force development and actomyosin ATPase seem to support this assertion (*36*−*39*). The recent determination of the crystal structure of a spin-labeled protein (*40*) with labels placed on the surface of the protein indicate no perturbation of protein structure.

The small difference in mobility within the lever arm can be explained in terms of the persistence length of a semiflexible cylinder, i.e., the length over which there is correlation in the direction of the cylinder axis. The 25% difference in correlation time values equates to the 15% ratio of the average angular amplitude as given by the definition of the rotational diffusion coefficient $\tau_{eff}^{ELC}/\tau_{eff}^{RLC} = \langle\theta^{RLC}\rangle^2/\langle\theta^{ELC}\rangle^2$. Assuming an amplitude of 25° for the half-cone angle for the regulatory domain in myosin filaments (obtained from phosphorescence probes at the same sites; L. Brown, personal communication), the average angular difference is 4°. This angular difference, $\Delta\theta$, detected over a distance (L) of 40 Å (ELC Cys-136 to RLC Cys-154) (*37*) translates into the persistence length value (p) according to $\langle\cos\Delta\theta\rangle = \exp(-L/p)$ (*41*). Hence, the persistence length is estimated to be about 1.5 μm, which is substantially longer than the regulatory domain (approximately 10 nm). This can be compared to the persistence length of 0.05 μm for DNA (*42*) and for collagen (*43*), and 6 μm for actin (*44*), as compiled in ref *45*. Furthermore, this persistence length translates into a stiffness of 30 pN/nm (*19*), which is considerably higher than that required to transmit the force of ~5 pN generated by the myosin head (*46*). This implies that the regulatory domain can be considered to be rigid under the forces that are generated by the myosin head. Whatever the small differences in the stiffness of the two subdomains might be, they are unlikely to play a role in transmission of force generated in the catalytic domain.

The function of the regulatory domain is somewhat controversial although there is an increasing body of evidence that it functions as a lever arm amplifying force generated in the catalytic domain. This role of the regulatory domain

was first proposed by Highsmith and Cooke (*47*) and hinted at by the presence of the changes in the hydrodynamic radius of the myosin head as evidenced by electric birefringence (*11*) and small-angle X-ray scattering (*48*) data. This idea gained momentum when the atomic structure of the head was solved by Rayment and co-workers (*8*) showing a clear delineation between the globular catalytic and an elongated regulatory domain. The relative orientation of the two domains was found to change in the putative pre- and postpower stroke states trapped in crystals of scallop and smooth muscle myosin heads (*1*, *2*). Significantly, such a reorientation was also observed in solution by luminescence and fluorescence energy transfer (*37*, *49*, *50*) and in muscle fibers by fluorescence anisotropy (*51*), EPR (*38*), EM (*52*), and X-ray diffraction (*53*).

Although the structural changes in the myosin head are well documented, the functional significance of the regulatory domain remains more controversial. The unitary force, the step size, and the rate of ATP hydrolysis have been measured for genetically engineered myosin motors with regulatory domains of varying length, for heads without any light chains, and for heads with one, two, and three light chains (*13*, *15*, *19*, *46*, *54*). The results of these experiments are not consistent. The interpretation of the linear relationship between the velocity of actin translocation and the length of the regulatory domain in in vitro motility assays (*55*) was complicated by the altered kinetics of head detachment.

Recent experiments by Yanagida and co-workers seem to indicate that the unitary step size is independent of the regulatory domain size. The authors concluded that the neck region mostly regulates the kinetics of the cross-bridge cycle and is not a lever arm amplifying force (*18*). One scenario that could account for the absence of the step size dependence would be the presence of residual flexibility in the regulatory domain, which would effectively decouple the catalytic domain from the S2 region. Force would then be generated in such a loosely coupled model by a biased Brownian ratchet mechanism (*18*, *56*−*58*). However, intradomain flexibility is not supported by the findings of this paper. The stiffness we calculate for the regulatory domain is substantially larger than the force generated by a myosin head. Moreover, Yanagida's findings are at odds with similar experiments in smooth muscle HMM reported by Warshaw, Trybus, and collaborators (*54*). The unitary step size and the unitary force measured with a laser optical trap showed a linear increase with the length of the regulatory domain (the latter suggesting that the regulatory domain is not a simple lever or cantilever arm for which a reciprocal relationship is expected). Warshaw and co-workers concluded that most likely the regulatory domain serves as a rigid lever arm and that much of the myosin elasticity is external to the neck.

Our finding that the two subdomains of the regulatory domain act essentially as a rigid body supports the lever arm hypothesis to the extent that the regulatory domain possesses the necessary mechanical characteristics of a lever arm.

Our observation that phosphorylation of RLC results in a uniform increase in the mobility of all parts of the myosin head is consistent with previous observations concerning RLC phosphorylation. Phosphorylation of myosin thick filaments results in a loss of the near-helical, periodic arrangement of myosin heads seen by EM in the relaxed state (*35*), suggesting greater head mobility, as we observed here. In turn, this increased mobility is believed to result in easier access to the thin filament, explaining the increased Ca^{2+} sensitivity of tension development that results from phosphorylation (*59*). Thus, our data support the hypothesis that phosphorylation alters either the interaction of the myosin head/S2 region with the thick filament and/or the flexibility of one or both hinges at either end of the S2 region.

In summary, a comparison of the mobility of proximal (ELC) and distal (RLC) subdomains of the regulatory domain indicates that it is essentially a rigid body with a persistence length equal to its physical dimensions. The regulatory domain remains rigid under conditions known to affect the global motion of the myosin head and behaves as a single body. This is consistent with the hypothesis that the regulatory domain serves as a lever to amplify the movement of the catalytic domain during the power stroke of striated muscle.

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