The converter domain modulates kinetic properties of *Drosophila* myosin

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Littlefield, Kimberly Palmiter, Douglas M. Swank, Becky M. Sanchez, Aileen F. Knowles, David M. Warshaw, and Sanford I. Bernstein. The converter domain modulates kinetic properties of *Drosophila* myosin. Am J Physiol Cell Physiol 284: C1031–C1038, 2003. First published December 11, 2002; 10.1152/ajpcell.00474.2002.—Recentely the converter domain, an integral part of the “mechanical element” common to all molecular motors, was proposed to modulate the kinetic properties of *Drosophila* chimeric myosin isoforms. Here we investigated the molecular basis of actin filament velocity (V_{actin}) changes previously observed with the chimeric EMB-IC and IFI-EC myosin proteins [the embryonic body wall muscle (EMB) and indirect flight muscle isoforms (IFI) with genetic substitution of the IFI and EMB converter domains, respectively]. In the laser trap assay the IFI and IFI-EC myosins generate the same unitary step displacement (IFI = 7.3 ± 1.0 nm, IFI-EC = 5.8 ± 0.9 nm; means ± SE). Thus converter-mediated differences in the kinetics of strong actin-myosin binding, rather than the mechanical capabilities of the protein, must account for the observed V_{actin} values. Basal and actin-activated ATPase assays and skinned fiber mechanical experiments definitively support a role for the converter domain in modulating the kinetic properties of the myosin protein. We propose that the converter domain kinetically couples the P_i and ADP release steps that occur during the cross-bridge cycle. This specialized group of muscle fibers oscillates at the resonant frequency of the flight system, generating wing beat frequencies on the order of 220 Hz and enabling the insect to fly (for review, see Refs. 13, 19). Embryonic body wall muscle (containing the EMB isoform), on the other hand, is a slow muscle used for larval locomotion and is histolyzed in the later stages of morphogenesis. Muscle fibers isolated from transgenic flies expressing the IFI and EMB myosin isoforms in the IFM and subjected to sinusoidal length oscillation experiments show profound mechanical and kinetic differences that are also apparent in isolated myosin preparations (28, 29). These results suggest that IFI and EMB myosin isoforms confer very different biophysical and biochemical properties on the contractile characteristics of the IFM. Furthermore, because these isoforms differ only in the alternatively encoded regions, this suggests that the functional properties of the myosin are defined by at least one of these regions.

The converter domain, a specialized region within the enzymatic globular head of the MHC (see Fig. 1, A and B), is thought to be involved in coupling the energy of ATP hydrolysis to the mechanical events of the power stroke. In *Drosophila*, the converter domain is encoded by exon 11 (8). Exon 11 is one of the six alternatively spliced exon sets that code for variable regions in *Drosophila* myosin isoforms. Four of the variable regions are in the enzymatic S1 head (for review, see Ref. 3). The converter regions in the IFI and EMB myosin isoforms are encoded by the exon 11e and 11c isovariants, respectively, and are markedly different; with 23 of 39 nonconserved amino acid substitutions (Fig. 1C). Transgenic *Drosophila* were created by genetically swapping single alternative exons, including exon 11, between the IFI and EMB isoform backbones (29). The naturally occurring IFI and EMB myosin isoforms and the chimeric IFI-EC (IFI isoform backbone with the EMB converter domain) and EMB-IC (EMB isoform backbone with IFI converter domain) myosins provide us with a practical, integra-
A direct way to elucidate answers to complex structure-function questions and a powerful approach to ascribe a specific role to the converter domain in defining myosin function.

Alternative expression of IFI and EMB isoform-specific converter domains was recently shown to play a role in defining the functional properties of myosin. In vitro motility studies showed a dramatic influence on actin sliding velocity after genetic substitution of this region in the IFI-EC and EMB-IC chimeras (29). Similarly, mechanical studies on the IFM fibers expressing the chimeric proteins showed a dramatic influence on power-generating ability. The results of these studies led to the speculation that the converter domain modulates the kinetic properties of Drosophila myosin.

In the present study, we assessed the molecular basis for the apparent changes associated with converter domain substitution in the IFI-EC and EMB-IC chimeras. We determined the mechanical and enzymatic properties of the naturally occurring IFI and EMB isoforms and the chimeric IFI-EC and EMB-IC myosins with single-molecule, solution ATPase, and fiber mechanical assays. Using the single-molecule laser trap assay, we found that the previously observed changes in actin filament velocity ($V_{\text{actin}}$) (29), determined with the different myosin isoforms and chimeras, are not due to changes in the inherent mechanical capacity of the myosin molecule. This is definitive evidence that a kinetic mechanism must account for the converter domain-mediated changes in $V_{\text{actin}}$. In support of this, solution biochemical actin-activated ATPase assays (the first of their kind performed with native Drosophila myosin isoforms and chimeras) and skinned fiber data suggest that the converter domain modulates actomyosin kinetics, influencing the duration of several states in the cross-bridge cycle. We propose that interactions between the motor core (actin binding and ATP hydrolysis center) and the converter domain kinetically couple biochemical state transitions over the duration of the ATPase cycle.

**EXPERIMENTAL PROCEDURES**

*Fig. 1. Myosin converter domain and Drosophila myosin isoform amino acid sequences. A: chicken skeletal S1 fragment (25) with the converter domain (residues 712–779; Ref. 7) highlighted in red and green. Residues 724–764 (highlighted in green) of the chicken skeletal myosin correspond to those encoded by exon 11 in the Drosophila myosin isoforms. Residues 731–738 are not resolved in this structure. B: enlarged view of the converter domain. C: amino acid sequence of the indirect flight muscle (IFI) and embryonic body wall (EMB) isoforms encoded by exons 11e and 11c, respectively. * Nonconserved amino acid residues between the IFI and EMB converters.*
detail elsewhere (1, 9, 21). Briefly, MV analysis involves passing a time window over the displacement data point by point and calculating a position mean and variance for all points in that window. The mean and variance data are then compiled as a three-dimensional histogram: mean (x-axis), variance (y-axis), and counts (z-axis). For clarity, MV histograms are presented in two dimensions [i.e., mean (nm) and variance (nm²)] with the total counts at a given mean and variance color-mapped on the z-axis. Typically, MV histograms have two apparent regions of high density (populations) that can be attributed to baseline and unitary events (see Fig. 2B). The event population is statistically fit with a Gaussian distribution in the x-direction (mean) and a χ² distribution in the y-direction (variance). Resolving power is obtained through MV analysis, as event populations are offset from the baseline population by the reduction in variance that occurs during a unitary event. This decrease in variance occurs as myosin attaches to actin, resulting in a reduction of the inherent noise attributable to Brownian motion.

**Ca**²⁺-ATPase assays. High-salt Ca**²⁺**-ATPase assays were performed as previously described (28).

**Actin-activated ATPase assays.** Time course assays at a saturating actin concentration (5 µM) were performed before the actin-activated ATPase assays to determine the linear temporal range of phosphate (Pi) generation. These experiments (data not shown) were performed to ensure that the actin-activated ATPase assays were not substrate limited. Steady-state actin-activated ATPase assays were performed as previously described (15, 30) with the following modifications: *Drosophila* myosin (100 nM) was preincubated for 10 min with chicken F-actin (0.75 µM) in (mM) 20 KCl, 20 imidazole, 0.1 CaCl₂, 5 MgCl₂, pH 6.0, and 10 DTT at 25°C. The ATPase reaction was started with the addition of NaATP to a final concentration of 2 mM and allowed to proceed for 5 (IFI and IFI-EC) or 10 (EMB and EMB-IC) min. Blank reaction tubes containing only actin (0–7.5 µM) were assayed simultaneously. Pi generation was determined by extracting 50 µl of the ATPase reaction volume (150 µl total) into 500 µl of a 0.039% malachite green-1.1% ammonium molybdate-1 N HCl (Sigma, St. Louis, MO)-0.02% Sterox (Bacharach, Pittsburg, PA) solution. After 1 min the color development reaction was quenched by the addition of 50 µl of 34% sodium citrate. Optical densities (OD) were read at 650 nm with a Beckman Coulter DU640B spectrophotometer (Fullerton, CA). Total Pi generated was calculated by subtracting the appropriate actin blank OD from the actin-myosin reaction OD and using the parameters generated in the linear regression analysis of a Pi standard curve.

**Muscle isolation and skinned fiber preparation.** A bundle of IFMs was removed from a *Drosophila* half-thorax as described previously (28). To avoid the negative influence of a deteriorating ultrastructure in the EMB and EMB-IC lines, we used fibers from flies younger than the age at which myofibril deterioration starts (<2 h old) (29). Using fibers from young flies did not affect kinetics, as shown by the identical shapes of the viscous modulus from 2-day-old and 2-h-old IFI control lines (see Fig. 5). Thus we can compare kinetics across all four fiber types. However, the amplitude of the viscous modulus is reduced in young flies because of the smaller myofibrillar cross sectional area per muscle cross-section (26).

The fibers were separated, and a single fiber was split lengthwise to improve diffusion of ATP into the fiber during mechanical experiments. The preparations were ~100 µm in diameter and ~0.6 mm in length. Fibers were chemically demembranated (skinned) in a relaxing solution containing 5 mM MgATP, 15 mM creatine phosphate, 240 U/ml creatine phosphokinase, 1 mM free Mg²⁺, 5 mM EGTA, 20 mM N,N-bis(z-hydroethyl)-zaminoethane sulfonic acid (BES) (pH 7.0), 1 mM DTT, and a protease inhibitor cocktail (Roche Biochemicals) containing 0.5% Triton X-100 and 50% glycerol for 1 h at 4°C. The ionic strength was adjusted with sodium methane sulfonate to 200 mM. Aluminum T clips were used to mount the fibers on the mechanical rig.

**Determination of rate of tension redevelopment.** To determine the rate of tension redevelopment, fibers were subjected to a series of four identical 0.5% muscle-lengthening steps. The force response was averaged over the four steps. The resulting phase 3 of the force response, rₑ (30) [equivalent to rate of tension development after a quick stretch (KTR) (5)], was fit with an exponential rise to a maximum: y = yo + a (1 – e⁻ᵏᵇ), where b is rₑ.

**Determination of frequency of maximal work per cycle.** To determine the frequency of maximal work per cycle (Wmax), fibers were subjected to sinusoidal length oscillations. In experiments, the details of which were previously described (6). Briefly, the fiber was activated stepwise by progressively exchanging the initial relaxing solution with activating (pCa 4.0) solution, up to pCa 4.5. Sinusoidal length changes of 0.25% muscle length (full amplitude) were applied over 47
frequencies from 1 to 1,000 Hz. For each frequency, elastic and viscous moduli were calculated from the force response to sinusoidal length perturbations by computing the amplitude ratio and the phase difference for force and length and dividing the ratio by fiber cross-sectional area. Temperature was 15°C for all mechanics experiments.

RESULTS

Single-molecule mechanics show that unitary step displacement amplitudes of IFI and IFI-EC myosins are the same. We determined the amplitude of the unitary step displacement, \( d \), generated by both the IFI and IFI-EC myosins with the laser trap assay. Figure 2A shows 3 s of raw displacement data obtained with IFI-EC myosin in 3 \( \mu \)M ATP. Arrows denote single unitary events. The MV histogram (see EXPERIMENTAL PROCEDURES for detailed description) generated from the complete raw data set partially illustrated in Fig. 2A is shown in Fig. 2B. Baseline (the time during which myosin is detached from actin) and event (duration for which actin and myosin are strongly associated) populations are denoted by B and e, respectively. Baseline data have a higher position variance (owing to the effects of Brownian motion) and an average displacement of 0 nm. Event populations, on the other hand, are characterized by a lower variance due to stiffening of the system when myosin attaches to actin. Average \( d \) values (Fig. 2C) were 7.3 ± 1.0 (\( n = 9 \)) and 5.8 ± 0.9 (\( n = 11 \)) nm (means ± SE) for the IFI and IFI-EC isoforms, respectively, and are not significantly different by Student’s t-test.

ATPase activity increased in both chimeras. The enzymatic activity of the native myosin isoforms and chimeras was determined both in the presence (actin-activated ATPase) (Fig. 3 and Table 1) and absence (basal \( \text{Ca}^{2+} \)- and \( \text{Mg}^{2+} \)-activated ATPase; Table 1) of actin. The actin-activated ATPase activity of the IFI myosin isoform was significantly greater than the EMB isoform. This difference in actin-activated ATPase activity is inherent to the IFI and EMB myosins as reflected in both their basal \( \text{Ca}^{2+} \)- and \( \text{Mg}^{2+} \)-activated ATPase activities (Table 1). Interestingly, however, the \( V_{\text{max}} \) of EMB myosin was potentiated 10-fold by actin, whereas IFI activity was potentiated only ~4-fold. There was no difference in the \( K_m \) between these myosins.

In contrast, the chimeric myosins had actin-activated ATPase activities that were, in both cases, greater than their respective control myosins (Fig. 3, A and B). We observed similar increases in both the basal \( \text{Ca}^{2+} \)- and \( \text{Mg}^{2+} \)-activated ATPase activities for the IFI-EC relative to the IFI myosin. However, basal rates for the EMB-IC chimera, relative to the EMB isoform, did not increase. We observed approximately twofold decrease in the \( K_m \) for actin in the EMB-IC myosin as well as the IFI-EC chimeric myosin (see Table 1 for all data).

Skinned fiber data support a kinetic role for the converter domain. All fibers exhibited the classic delayed tension rise following a quick stretch that promotes work generation in IFM (Fig. 4). However, fibers expressing EMB, EMB-IC, and IFI-EC myosin vary dramatically in the rate of tension redevelopment after stretch (\( r_3 \)) (Table 2 and Ref. 29). The \( r_3 \) values are 11.8-fold lower in fibers containing EMB myosin compared with the IFI fibers. The \( r_3 \) values were lower for IFI-EC fibers relative to the IFI and higher in the EMB-IC fibers compared with EMB.

Fibers were also subjected to sinusoidal length oscillation experiments (Fig. 5). Because we are interested in the kinetics of active force generation by myosin cross bridges, we focused on the viscous modulus, which reveals the optimal frequency range for work generation by cross bridges. The four fiber types differ

<table>
<thead>
<tr>
<th>Myosin isoform</th>
<th>Basal ( \text{Ca}^{2+} ) ATPase rate ( \text{s}^{-1} \text{head}^{-1} )</th>
<th>Basal ( \text{Mg}^{2+} ) ATPase rate ( \text{s}^{-1} \text{head}^{-1} )</th>
<th>Maximal ATPase rate ( V_{\text{max}} ), ( \text{s}^{-1} \text{head}^{-1} )</th>
<th>( K_m ), ( \mu )M</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFI</td>
<td>7.6 ± 0.3 (6)</td>
<td>0.2 ± 0.04</td>
<td>0.9 ± 0.04 (5)</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>IFI-EC</td>
<td>11 ± 1.3 (6)</td>
<td>0.4 ± 0.04</td>
<td>1.5 ± 0.05 (5)</td>
<td>0.4 ± 0.04†</td>
</tr>
<tr>
<td>EMB</td>
<td>3 ± 0.2 (7)</td>
<td>0.07 ± 0.01</td>
<td>0.7 ± 0.05 (5)</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>EMB-IC</td>
<td>2 ± 0.1 (6)</td>
<td>0.06 ± 0.03</td>
<td>1.1 ± 0.02 (5)</td>
<td>0.4 ± 0.03†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Nos. in parenthesis indicate no. of individual experiments. \( V_{\text{max}} \) and \( K_m \) are calculated parameters generated when the ATPase data presented in Fig. 3, A–C, were fit to the Michaelis-Menten equation with SigmaPlot (SPSS, Chicago, IL). Statistical significance determined by Student’s t-test (\( P < 0.05 \)) unless otherwise noted: *Indirect flight muscle isoform (IFI) vs. embryonic body wall muscle (EMB); †IFI vs. IFI-EC (IFI isoform backbone with EMB converter domain) with substitution of EMB converter domain (IFI-EC). \( P < 0.05 \) by Z-test on the parameter fits of \( V_{\text{max}} \) and \( K_m \). ‡IFI vs. EMB; ††IFI vs. IFI-EC; ‡‡EMB vs. EMB-IC (EMB isoform backbone with IFI converter domain) with substitution of IFI converter domain (EMB-IC).

![Fig. 3. Steady-state rates of actin-activated myosin ATPase. A: IFI vs. IFI-EC. B: EMB vs. EMB-IC (EMB isoform backbone with IFI converter domain). \( P < 0.05 \) by Z-test on the parameter fits for IFI vs. IFI-EC (††) and EMB vs. EMB-IC (‡‡).](Image)
dramatically in the frequency range over which useful work is generated (by convention, where the viscous modulus is negative). For example, in EMB fibers the modulus is negative from 2 to 25 Hz, in EMB-IC fibers from 5 to 80 Hz, in IFI-EC fibers from 8 to 150 Hz, and in wild-type fibers from 15 to 230 Hz. The lowest point of the viscous modulus curve corresponds to the $W_{f_{\text{max}}}$.

The $W_{f_{\text{max}}}$ values agree in relative order and magnitude with the $r_3$ measurements.

**DISCUSSION**

Our purified chimeric myosin and fiber preparations had the converter region exchanged between two naturally occurring *Drosophila* myosin isoforms (IFI and EMB). The relay helix, converter domain, and light chain-binding domain (putative lever arm) make up the proposed “mechanical element” (32). A series of structural transitions within these specialized domains results in the generation of the power stroke. The ability to express chimeric myosin, where changes in primary sequence and (possibly) structure are limited to a defined region of the molecule, offers the opportunity to ascribe specific functional properties to a particular structural domain. In this study we provided direct evidence that the converter domain influences myosin’s kinetic properties by performing single-myosin molecule step displacement, enzymatic

![Graph](https://example.com/graph.png)

**Table 2. Skinned fiber kinetic data**

<table>
<thead>
<tr>
<th>Myosin Isoform</th>
<th>Rate of Tension Redevelopment ($r_3$), s$^{-1}$</th>
<th>Frequency of Maximum Work ($W_{f_{\text{max}}}$), Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFI</td>
<td>1059 ± 63(6)</td>
<td>109 ± 5</td>
</tr>
<tr>
<td>IFI-EC</td>
<td>729 ± 30(6)</td>
<td>47 ± 1</td>
</tr>
<tr>
<td>EMB</td>
<td>90 ± 5(7)</td>
<td>17 ± 1</td>
</tr>
<tr>
<td>EMB-IC</td>
<td>367 ± 26(6)</td>
<td>35 ± 1</td>
</tr>
</tbody>
</table>

The rate of tension redevelopment ($r_3$) was determined by curve fitting the rate of tension rise after the initial spike and decline following a quick stretch (see Fig. 4 and EXPERIMENTAL PROCEDURES). The frequency at which the viscous modulus was lowest ($W_{f_{\text{max}}}$) is the frequency at which maximum work is produced (see Fig. 5 for details).
ATPase, and skinned fiber length oscillation experiments.

Mechanical properties of IFI and IFI-EC myosin proteins. We have demonstrated that the 2.4-fold attenuation of $V_{\text{actin}}$ previously observed with the IFI-EC chimera (29) cannot be accounted for by a change in the inherent mechanical capacity of the myosin molecule. At the molecular level $V_{\text{actin}} = \frac{d}{t_{\text{on}}}$ (12) where $d$ is the unitary step displacement and $t_{\text{on}}$ is the duration of the strong actin-myosin interaction. Given that the amplitude of myosin’s unitary step is constant for three of the four isoforms characterized in this and a previous study (28), the observed differences in $V_{\text{actin}}$ must be related to differences in the time spent in the strongly bound state following the power stroke ($t_{\text{on}}$), as has been observed in other myosin isoforms (16, 21, 22, 30). Thus swapping converter domains does not perturb the mechanical capabilities of the Drosophila myosin but rather the kinetics of steps in the actomyosin ATPase cycle that contribute to the duration of $t_{\text{on}}$.

$t_{\text{on}}$ is determined by the rates of both ADP release ($k_{\text{-ADP}}$) from, and ATP binding ($k_{\text{+ATP}}$) to, the myosin active site (1, 15, 21, 31). For Drosophila myosin the ADP release rate is most likely faster than that of chicken skeletal muscle myosin (~500 s$^{-1}$) (18, 27). This would correspond to an average $t_{\text{on}}$ of <2 ms at saturating ATP concentrations in the laser trap assay. Given that this duration is less than the temporal resolution of the assay, it would be impossible to determine whether the $t_{\text{on}}$ values for the IFI and IFI-EC myosins differ at saturating ATP, thus preventing us from relating any potential differences in $t_{\text{on}}$ to changes in the ADP release rate.

Solution kinetic measurements. Characterizing the actin-activated ATPase activity of the IFI and EMB isoforms and the two chimeras provides additional, novel insight into the kinetic properties of the myosin isoforms and chimeras. The low rate of ATPase activity observed specifically with the IFI isoform is consistent with previous actin-activated ATPase rates (~2 s$^{-1}$) determined in Drosophila skinned fiber preparations (34). The expected differences in ATPase activity observed with the IFI and EMB myosins may represent functional tuning of the myosin specific for the muscle environment in which they are expressed. The enhanced actin-activated ATPase activities of both chimeric myosins relative to their controls may reflect the ability of actin to provide a greater level of activation relative to either the EMB or IFI myosin. Alternatively, it is likely that genetic swapping of the fast IFI and slow EMB converter domains perturbs normal structural interactions that constrain the rate of ATP hydrolysis and presumably $P_i$ release. Finally, the increase in ATPase activity of the IFI-EC chimera, above that of the fastest IFI isoform, was unexpected but other instances of myosin mutations causing an increase in ATPase have been reported (30, 35).

Duty ratio. A kinetic parameter frequently used to characterize myosin is its duty ratio ($f$), defined as the fraction of the total cycle time ($t_{\text{cycle}}$) that myosin remains strongly bound to actin after the power stroke, where

$$f = \frac{t_{\text{on}}}{t_{\text{cycle}}}$$

Although $t_{\text{on}}$ and $t_{\text{cycle}}$ vary across the vertebrate class II muscle myosin isoforms, the ratio of $t_{\text{on}}$ to $t_{\text{cycle}}$, $f$, is constant (31). Both $t_{\text{on}}$ and $t_{\text{cycle}}$ can be estimated from $V_{\text{actin}}$ and the ATPase rate, respectively, where

$$t_{\text{on}} = \frac{d}{N_{\text{actin}}}$$

$$t_{\text{cycle}} = \frac{1}{V_{\text{ATPase}}}$$

so that

$$f = \frac{d}{N_{\text{actin}}} \times \frac{1}{V_{\text{ATPase}}}$$

Because $d$ is constant across the various Drosophila myosin isoforms, then

$$f \approx \frac{V_{\text{ATPase}}}{N_{\text{actin}}}$$

Using this relationship, we estimated a relative duty ratio for the two isoforms and two chimeras (see Table 3). In contrast to the vertebrate muscle myosins (31), our estimates suggest that the duty ratio is not constant across Drosophila isoforms and chimeras. Given that these estimates are model dependent, it is still possible that differences between the IFI and EMB isoforms may have evolved so that actomyosin kinetics are adapted to match the functional demands on the myosin isoform. In both chimeras, the duty ratio estimates suggest that the kinetic parameters that define $f$ are uncoupled. Interestingly, exchanging converter domains resulted in both chimeras having a duty ratio closer in value to their respective donor isoform (Table 3).

Average force generation. Changes in $f$ may have important implications for average force generation ($F$) in skinned fiber preparations as

$$F = N \times F_{\text{uni}} \times f$$

where $N$ is the number of cross bridges and $F_{\text{uni}}$ is the cross-bridge unitary force (33). If we assume that $N$ and $F_{\text{uni}}$ are constant for the various fibers, then the average fiber force will be directly proportional to the myosin duty ratio. This prediction is surprisingly accurate with the rank order of duty ratios following that of the maximum isometric force measured in skinned fiber preparations, i.e., $F_{\text{EMB}} > F_{\text{IFI-EC}} > F_{\text{IFI}}$ (29).

Table 3. Relative duty cycle predictions

<table>
<thead>
<tr>
<th>Myosin Isoform</th>
<th>Relative Rate of ATP Hydrolysis</th>
<th>Relative Velocity ($V_{\text{actin}}$)</th>
<th>Relative Duty Ratio ($f$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFI</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>IFI-EC</td>
<td>1.6</td>
<td>0.4</td>
<td>4.0</td>
</tr>
<tr>
<td>EMB</td>
<td>0.7</td>
<td>0.1</td>
<td>7.0</td>
</tr>
<tr>
<td>EMB-IC</td>
<td>1.3</td>
<td>0.8</td>
<td>1.6</td>
</tr>
</tbody>
</table>

All relative values were normalized to the appropriate values obtained with the IFI isoform (for $V_{\text{actin}}$ values see Refs. 28 and 29). Because unitary step displacement ($d$) did not vary across the myosin isoforms, the relative $d$ is 1 for all myosins.
tions concerning isometric parameters based on measurements in the motility assay or in solution where the actomyosin interaction occurs under unloaded conditions.

**Kinetic correlations.** When particular experimental parameters measured for myosin and muscle fibers are coupled, or otherwise related (limited by the same biochemical rate constants), they should correlate. For example, Tyska and Warshaw (31) show a strong linear correlation between $V_{\text{actin}}$ and ATPase activity in the vertebrate class II muscle myosins as was originally described in whole muscle (2). Although the kinetic steps that govern $V_{\text{actin}}$ and ATPase activity are different (i.e., cross-bridge detachment rate for $V_{\text{actin}}$ vs. attachment rate for ATPase), a strong correlation between $V_{\text{actin}}$ and the ATPase rate for the various vertebrate myosins argues that changes in the kinetics of these steps are coupled as a result of evolutionary selection. As illustrated in Fig. 6A, this does not appear to be the case for the *Drosophila* myosin isoforms and chimeras. The lack of correlation between $V_{\text{actin}}$ and ATPase activity ($R^2 = 0.009$) suggests that the converter can modulate the kinetic properties of multiple rate-limiting steps in the actomyosin ATPase cycle to various extents. However, conclusions about coupling in the native isoforms are premature until additional native isoforms have been characterized.

To understand how muscle fiber kinetics are influenced by the kinetic changes seen at the molecular level, we correlated the rate of tension recovery ($r_3$) and the frequency for maximum work production ($W_{\text{max}}$) in skinned fibers to myosin ATPase activity and $V_{\text{actin}}$. A strong linear correlation ($R^2 = 0.87$) between $W_{\text{max}}$ and $r_3$ (Fig. 6B) is expected because these parameters are governed by similar cross-bridge kinetic rate constants [$r_3 \times 2\pi(W_{\text{max}})$] (14). Using the classic two-state model (11), $r_3$ has been proposed to be influenced by both cross-bridge attachment ($f$) and detachment ($g$) rates ($r_3 = f + g$; Ref. 4). When correlating fiber and molecular level parameters, we see no correlation ($R^2 = 0.26$) between $r_3$ and ATPase (Fig. 6C) activity, although a weak correlation ($R^2 = 0.47$) does exist between $r_3$ and $V_{\text{actin}}$ (Fig. 6D). This suggests that although both attachment and detachment kinetics determine $r_3$ (4), $r_3$ is more strongly influenced by the same kinetic step that governs $V_{\text{actin}}$, i.e., the rate of ADP release or cross-bridge detachment. Again, although dramatic changes in both molecular and fiber level properties can be achieved by exchanging the converter domain, correlations between isolated myosin and fiber kinetics should be made with caution given the influence of strain and a constraining lattice on fiber kinetics.

In conclusion, we have shown, by determination of the single-molecule mechanical properties and enzymatic ATPase activity of native and chimeric *Drosophila* myosin proteins, that the converter domain influences myosin’s kinetic properties rather than its mechanical capabilities. These kinetic changes translate to the fiber level, resulting in a significant alteration in the kinetic properties of skinned fiber preparations. However, the structural mechanism by which changes to the converter domain modulate myosin’s kinetic properties remains to be elucidated. It is apparent from both the isolated myosin and fiber studies that the converter domain is not the sole determinant of *Drosophila* myosin kinetics, because swapping converter domains does not fully switch the myosin kinetics of either chimera to that of the IFI or EMB isoform. Because there are three other variable domains located...
in the myosin S1 head that could influence myosin kinetics (for review see Ref. 3), additional chimeras with single or combinations of alternative exons will help identify which are required for interconversion between the IFI and EMB isoforms.

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REFERENCES