by switches of myosin isoforms from IIa to IIx and IIb, with no change in type I myosin, and to more low molecular weight fast troponin T splice forms. During reloading, soleus muscle mass, fiber size and contractile force gradually recovered and reached the control level by 15 days. The levels of type IIa, IIx and IIb myosins recovered at 15 days of reloading. However, fatigue tolerance and post fatigue force recovery showed a trend of worsening during this period with significantly inflammatory cell infiltration at 3 and 7 days, indicating reloading injuries. The reloading injury was accompanied by up-regulations of filamin-C and alpha-crystallin-B, which returned to control level after 30 days. During the course of slow recovery, we observed later increases of type I myosin expression and the number of type I fibers to levels significantly higher than that in normal adult mouse soleus muscle at 30 to 60 days of reloading, which may contribute to the recovery of fatigue tolerance. The data demonstrated the value of increased slow fiber contents as a secondary adaption to compensate for muscle reloading injury, serving as an indicator for monitoring muscle function during the chronic recovery from unloading and disuse conditions.

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Minimum and Maximum Limit to Number of Myosin II Motors Participating in an Ensemble Motility

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Myosin II is an ensemble motor that participates in muscle contraction, actin cortex remodeling and cytokinesis ring constriction. It has been an interesting puzzle for scientific community to understand the allosteric rules between motors that regulate/co-ordinate when they work in group. We have performed classical *In Vitro* motility assay at different heavy meromyosin (HMM) density and ATP concentrations. By changing immobilized HMM density and ATP concentration, we change the number of HMM molecules available for interaction/unit length of actin filament. Actin filaments of >10 micron length were added in flow cell and assay was initiated by addition of ATP. Actin filaments breaks down to smaller pieces within minutes after addition of ATP. Average length of sliding filaments correlates well with motors density and ATP concentrations in solution. At 633 head/μm^2 density and 2 mM ATP concentration the average filament length was 1747.6 ± 614 nm and further reduced to 1290.5 ± 385.5 nm at 0.1 mM ATP. At 4000 heads/μm^2 density and 2 mM ATP concentration the average filament length was 935.5 ± 286 nm and reduced to 599.5 ± 154.2 nm at 0.1 mM ATP. Any filament below this average length does not slide continuously, they detach from surface with time and any filament above this average length fragments to smaller pieces with time. Assuming Poisson distribution of immobilized heads, we calculated maximum number of molecules that can interact for a given actin length. Number of molecules required for continuous sliding is independent of motor density and depends only on ATP concentration. For 2 mM ATP concentration, 123.6 ± 28.9 heads and for 0.1 mM ATP concentration 62.3 ± 19.5 heads are required for continuous sliding of actin. All values are mean $\pm$ SD.

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Muscle Shortening Velocity is Modulated by Alternative Myosin Converters

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The myosin converter has been proposed to be critical for setting muscle shortening velocity by influencing the amplification of small conformational changes at the ATPase site into the larger lever arm swing. To test this hypothesis, we exploited the natural variation of the converter region found in *Drosophila* muscle types. In *Drosophila*, a single myosin heavy chain gene is alternatively spliced to generate myosin isoforms. The converter region is encoded by five alternative versions of exon 11 that are expressed in different muscle types. Through genetic modification we forced the expression of the alternative versions in the *Drosophila* jump muscle and found that two of the three versions tested to date, 11a (native to indirect flight muscles) and 11e (embryonic muscles), caused faster maximum shortening velocities of skinned jump muscle fibers relative to the native control 11c. Increases in velocity were primarily responsible for 70.9% and 79.0% higher power outputs for 11a and 11e fibers, respectively, because maximum force generation was not significantly different from control fibers. The fibers expressing 11a and 11e also exhibited a straighter force-velocity curve, as shown by increased Hill equation parameters *a* (95.4% and 84.5% higher, respectively) and *b* (72.6% and 65.5% higher, respectively) relative to control fibers, indicating that the converter modulates load dependent cross-bridge kinetics. The higher power and shortening velocity of 11a and 11e fibers enabled increased jumping ability, 76.0% and 48.8% farther, respectively. Converter homology models, using the new *Drosophila* myosin S1 crystal structure (pdb 4QBD), revealed minimal tertiary structure differences suggesting residue specific interactions with the relay, N-terminus, or essential light chain are critical. Overall, our data support the hypothesis that the converter helps set muscle shortening velocity under loaded and unloaded conditions and thus contributes to muscle fiber type diversity.

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Actomyosin Regulation by Conserved Sites of Tm5NM1 (Tpm3.1), a Nonmuscle Tropomyosin

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Actin filaments carry out diverse cellular functions including cell division, intracellular transport, and muscle contraction. Tropomyosin (Tm) is an α-helical coiled-coil protein that regulates actin in most eukaryotic muscle and nonmuscle cells. Nonmuscle isoforms of Tm, particularly Tm5NM1 (Tpm3.1) (short isoform, 247 a.a., TPM3 gene), play an important role in cellular functions such as cell migration, and cytokinesis as well as in the transformation and metastasis of cancer cells. In previous work, mutation of evolutionarily-conserved residues in striated muscle αTm (Tpm1.1) (long isoform, 284 a.a., TPM1 gene) revealed the regions important for actomyosin regulation. In the present study, we have mutated evolutionarily-conserved residues in Tpm3.1 to determine the molecular basis for isoform-specificity of actomyosin regulation by Tms. We mutated surface residues in nonmuscle Tpm3.1 in periods P1-P7 at positions homologous to the residues that are important for actomyosin regulation by striated muscle Tpm1.1 (Barua et al., 2012). In vitro motility assays were carried out to determine the effect of mutations on actin filament velocities. Actin-Tm velocities with skeletal myosin are inhibited by Tpm1.1 (~60%) but activated by Tpm3.1 (~60%) relative to actin alone. The Tpm3.1 mutants had little or no effect on velocity, except for the P3 and P6 mutants that showed a ~50-60% inhibition in filament velocity relative to WT Tpm3.1. In comparison, amongst the Tpm1.1 mutants, the P3 and P6 mutants also showed the largest inhibition (~70-80%) in filament velocity relative to WT Tpm1.1. These results indicate that the same regions of Tm (periods 3 and 6) are important for regulation of skeletal myosin by two different isoforms that have contrasting effects (inhibition by Tpm1.1 vs. activation by Tpm3.1) on filament velocity. Supported by NIH.

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The Binding of S1A1-ADP to Skeletal Thin Filaments is Insensitive to the Presence of Covalently Bound Phosphate in Tropomyosin

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Striated muscle tropomyosin is phosphorylated at a single site. Effects of this covalent modification include an increase in myosin activation by thin filaments (+Ca(II)) under steady state conditions (Vmax, 16 vs. 8 sec-1; ionic strength, ~ 30 mM; T, 25 oC). Thin filaments were assembled using skeletal muscle troponin and alpha-tropomyosin (either unphosphorylated or phosphorylated). Each type of thin filament was mixed with varying concentrations of myosin-S1A1 (4uM F-actin; buffer, 5.5 mM MgCl2, 50 mM KCl, 10mM imidazole, 3mM MgADP, 1mM dithiothreitol, pH 7 plus 0.5mM EGTA or Ca(II)) and incubated at 4 oC for 1hr with 20uM myokinase inhibitor. The mixtures were then centrifuged for 30min at 150,000 x g at 25 oC. The unbound S1 was determined using a NH4/EDTA ATPase versus [myosin-S1A1] standard curve. The zero time samples showed no colour development, consistent with the absence of ATP. At a given pCa the isotherms obtained with each type of reconstituted thin filament are virtually superimposable (in terms of steepness and mid-point). The apparent binding constants range from 0.1 to 0.4 uM. Added Ca(II) strengthens affinity and EGTA weakens it. Pre-steady state experiments using double-mixing fluorescent stopped-flow are in progress to investigate further the effect of this modification.

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The Functional Consequences of Hypertrophic Cardiomyopathy Troponin C Mutations in the Regulation of Slow Skeletal Muscle Contraction: The Protective Role of Slow Skeletal Troponin I

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Mutations in cardiac troponin C (cTnC) have been linked to hypertrophic cardiomyopathy (HCM) in humans. The cTnC subunit of the troponin complex is