S-transferase-2 is stably associated with them (Clayton et al., 1998). Then an alternative explanation is that these isoforms also exist in *Ctenacroscelis*, and they are alternately arranged and only one of them binds GST-2. The scattering object remains to be identified, but the alternating arrangement of tropomyosin isoforms is the most conceivable mechanism to provide the periodicity needed to create the peculiar meridional reflection.

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Effect Of Tropomyosin On The Binding Force Of Unphosphorylated Myosin To Actin

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Smooth muscle (SM) is unique in its ability to maintain force for long periods of time at low energy cost. This property is called the latch-state. One of the assumptions of the latch state model of Hai and Murphy is that myosin must first be phosphorylated in order to attach to the thin filament. However, we previously demonstrated that unphosphorylated (unPHOS) myosin can attach to unregulated actin filaments. The goal of this study was to measure the binding force of unPHOS SM myosin to tropomyosin-regulated actin filaments. A microsphere captured in a laser trap was attached to an actin filament decorated with SM tropomyosin-α and β. The filament was brought in contact with a pedestal coated with unPHOS pig antrum myosin. The pedestal was then moved away from the trap at constant velocity (0.5 $\mu m.s^{-1}$). Despite pulling the pedestal away, the microsphere did not move until the force exerted by the trap on the microsphere was sufficient to overcome the binding force of myosin on the actin/tropomyosin filament. At this point, the microsphere sprang back to its unloaded position. The force of unbinding was calculated as the product of the trap stiffness and the maximal displacement of the trapped microsphere, as assessed by displacement of its center of mass. The average force of unbinding per myosin molecule (F_{unb}) was obtained by dividing the measured force of unbinding by the number of myosin molecules estimated per actin filament length. We found that F_{unb} was greater in presence (0.222 pN $\pm$ 0.018; mean $\pm$ SE) than in absence (0.142 pN $\pm$ 0.019; $p<0.001$) of tropomyosin. These results demonstrate that tropomyosin strengthens the bond between unPHOS myosin and actin. Future studies will investigate the role of other regulatory proteins of the thin filament.

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Tropomyosin Flexibility Evaluated by Electron Microscopy Image Analysis

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Movement of tropomyosin (Tm) on thin filaments in response to Ca^{2+}-binding to troponin and myosin binding to actin is an inherent feature of muscle regulation. As part of this process, the cable-like mechanical properties of the Tm coiled-coil are thought to underlie cooperative on and off switching of contraction. In principle, movement of Tm over the flat surface of actin may not require significant molecular flexibility. However, local perturbations caused, for example, by myosin binding on actin may necessitate some plasticity of the Tm molecule. In contrast, any large-scale Tm flexibility might dampen its cooperative movement. In the current study, we directly assessed the flexibility of Tm by examining EM images of both rotary shadowed and negatively stained molecules. Single Tm molecules in both image sets showed no obvious signs of sharp bending or kinks, and displayed contours close to those predicted from a high-resolution 3D model of Tm (Lorenz *et al.*, 1995). Short multimeric strings of end-to-end bonded Tm were commonly observed in the rotary shadowed images. These showed no pronounced bending or joints at the intermolecular junctions. The persistence length of Tm was calculated to be over twice the length of the molecule based on these EM images. The data as a whole imply that Tm molecules exhibit an intrinsic stiffness sufficient to contribute to cooperativity on thin filaments and thus are consistent with models of muscle regulation (e.g. Lehrer & Geeves, 1998; Lehman *et al.*, 2000). A tropomyosin mutant in which the coiled-coil interface was destabilized by introduction of Ala clusters in the 2nd and 3rd periodic repeats (Y60A-L64A-L106A; Singh & Hitchcock-DeGregori, unpublished) was also examined by EM. No obvious extra bending was observed, although the calculated persistence length was significantly shortened, showing the sensitivity of the technique.

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The Fast Skeletal Troponin Activator, CK-1909178, Increases Skeletal Muscle Force *in-vitro* and *in-situ*

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Previously, we have discovered small molecules that increase cardiac contractility by directly activating the cardiac sarcomere; this mechanism is now being investigated as a therapy for treating systolic heart failure. Using this precedent, we have focused on the identification of compounds that directly increase skeletal muscle contractility for the potential therapy of diseases that result in muscle weakness and fatigue.

CK-1909178 is a member of a class of fast skeletal troponin activators that were identified by high throughput screening of skeletal sarcomere preparations. We sought to understand how this compound altered force development in isometric skinned and live muscle fibers. Treatment of skinned rabbit psoas fibers with 0.1 μM CK-1909178 caused a dose-dependent left-shift of the force-pCa relationship without altering the Hill slope or maximum force, consistent with a calcium sensitizing effect on force production. In living rat flexor digitorum brevis (FDB) preparations, CK-1909178 (10 μM) caused significant increases in subtetanic force (150% of control at 10 Hz) without altering maximum force. Similar experiments were then performed using a rat extensor digitorum longus (EDL) preparation *in-situ*, where nervous and vascular connections were left intact and the muscle was stimulated via the peroneal nerve. Infusions of CK-1909178 up to 10 mg/kg rapidly increased sub-tetanic isometric force (190% of control at 30 Hz). In summary, we have identified a skeletal troponin activator that sensitizes the sarcomere to calcium and results in increased submaximal muscle force development *in-vitro* and *in-situ.* We believe that this mechanism may translate to improved physical power and strength in diseases where muscle function is compromised due to injury, disease or age.

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The Small Molecule Skeletal Sarcomere Activator, CK-1909178, is a Calcium Sensitizer that Binds Selectively to the Fast Skeletal Troponin Complex

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Striated muscle contraction is tightly coupled to the release of Ca^{2+} from the sarcoplasmic reticulum by the sarcomeric calcium sensor, troponin. This complex of three proteins (troponins T, I, and C) undergoes calcium-sensitive conformational changes that regulate the accessibility of myosin binding sites along the actin filament. We used a high throughput screen to identify compounds that activate the ATPase activity of skinned fast skeletal myofibrils; optimization of the initial hit compounds has resulted in compounds with sub-micromolar affinity. A potent representative of this chemical series, CK-1909178, shifts the calcium sensitivity of detergent skinned skeletal myofibrils by >10-fold in a concentration dependent manner. This compound specifically activates fast skeletal myofibrils, with no effect on either slow skeletal or cardiac myofibrils. A reconstituted sarcomere assay using combinations of fast skeletal, slow skeletal, and cardiac components demonstrates that the activity of CK-1909178 requires the presence of fast skeletal troponin. Isothermal titration calorimetry indicates the compound binds directly to fast skeletal troponin with a sub-micromolar dissociation constant. Consistent with its calcium sensitization effects, CK-1909178 slows the dissociation of calcium from troponin as measured by Quin-2 fluorescence. Consistent with its mechanism of action, CK-1909178 sensitizes muscle *in vitro* and *in vivo*, suggesting this mechanism may increase power or strength in diseases where muscle function is compromised due to injury, disease or age.

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Modulation Of Human Cardiac Troponin C-troponin I Interaction By An Analogue Of Levosimendan, (2′,4′-difluoro(1,1′-biphenyl)-4-yl) Acetic Acid (dfbp)

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The binding of Ca2+ to cardiac troponin C (cTnC) triggers contraction in heart muscle. In diseased heart, the myocardium is often desensitized to Ca2+, leading to weak cardiac contractility. Compounds that can sensitize cardiac muscle to Ca2+ have therapeutic value in treating heart failure. Of the known drugs that are proposed to accomplish this, levosimendan is the best characterized and currently in clinical use. Levosimendan interacts with the regulatory domain of cTnC and promotes association of cTnC with troponin I (cTnI). Detailed understanding of the mechanism of levosimendan has been concealed by its unstable nature; however, the use of analogues that are more stable would