probe cantilever, while the other probe was attached to a rapid displacement generator; the Ca2+ pulse was applied by rapid translation of a double-barreled perfusion pipette (de Tombe, AJP, 2007). Activations were performed at long (SL=2.06 ± 0.03 μm) and short (SL=1.85 ± 0.01; n=5) length. As expected, SL modulated maximum Ca2+ saturated force ~20%. Both the rate of force redevelopment following a rapid release-restretch maneuver (kTr; ~26%) and Ca2+ activated force development (kCa;~14%) were faster at the long SL. In contrast, SL did not modulate any parameters of force relaxation following rapid removal of activator Ca2+. Our data suggest that length dependent myofilament activation in the heart may be the result of differential modulation of activation dynamics in response to changes in sarcomere length.

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Tropomyosin: Long Range Perturbations In The Hydrophobic Interface

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Skeletal tropomyosin, (Tm), is an α-helical coiled-coil which binds to actin, and with troponin, regulates muscle contraction. We previously demonstrated that a conserved Asp 137 in the hydrophobic interface produces a dynamic region in the middle of Tm, and that this region is involved in the myosin dependent activation of the thin filament at high Ca^{2+}, (Sumida et. al. JBC 283 2008). The current work characterizes a long-range interaction between positions 137 and 190. The thermodynamic properties of wild type (WT) Tm and two single mutants, C190A and D137L, are compared with those of the double mutant, D137L/C190A, using differential scanning calorimetry, (DSC), and circular dichroism, (CD). CD measurements show that Ala 190 increases the fraction of helix unfolding in the 40°C pre-transition, before the main transition. DSC measurements support this finding, indicating a large enthalpic pre-transition, (ΔH=150kcal/mol), for the C190A mutant relative to D137L, D137L/C190A, or WT, (average ΔH=20kcal/mol). Additionally, Ala 190 increases the ΔCp, (heat capacity), of Tm ~5 fold, reflecting an increase in solvent exposure of hydrophobic residues in the pre-transition during unfolding.

Since the D137L/C190A and D137L mutants do not exhibit the large enthalpic pre-transition observed for C190A, the Leu mutation at 137 must stabilize the alanine effect observed for C190A mutation 77Å away. This demonstrates how a locally dynamic region near 137 is able to produce global effects along the thin filament, and in this manner provide the proper regulation of the myosin dependent activation of the thin filament. This observation may also contribute to our understanding about the manner in which single point mutations significantly affect function in cardio-myopathies such as FHC and DCM.

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Localization of The Tropomyosin-Binding Sites in Troponin T And Functional Suppression of An Error Splicing of Its C-Terminal Variable Region

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The interaction between troponin T (TnT) and tropomyosin (Tm) is pivotal in the Ca2+-regulation of muscle contraction. It has been known for three decades that TnT has two binding sites for Tm. The conserved middle and the C-terminal regions of TnT each contain a Tm-binding site and both sites are critical for the function of muscle thin filament. However, the precise locations of the Tm-binding sites have not been identified. By mAb competition assays, we located the middle region Tm-binding site of TnT in the beginning of the conserved sequence. Previous data showed that deletion of the C-terminal 14 amino acids in TnT did not reduce Tm-binding. Cardiac TnT with a longer deletion of the C-terminal 28 amino acids also retained the C-terminal Tm-binding site as shown by its dominant cardiomyopathy phenotype that indicates effective myofilament incorporation. In contrast, a truncation of slow TnT deleting the C-terminal 83 amino acids due to a nonsense mutation significantly lowered Tm-binding affinity and causes a recessive form of nemaline myopathy. Considering the known crystal structure of partial troponin, we further tested additional deletions to locate the C-terminal region Tm-binding site of TnT in the beginning of the T2 segment. Different from the dominant C-terminal truncation mutation of cardiac TnT, an error-splicing of the mutually exclusive exon 16 and exon 17 in fast skeletal muscle TnT significantly lowered Tm-binding affinity by deleting the C-terminal 28 amino acids and replacing it with a long non-sense peptide. The suppression of potentially harmful effects of the splicing error provides a mechanism to protect muscle function.

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Identification And Characterization Of Cardiac Troponin I From The Trout Heart

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Trout cardiac myofibrils are ~10-fold more sensitive to Ca^{+2} than those from mammalian hearts when measured at the same temperature. It has been demonstrated that trout cardiac troponin C (ScTnC) has 2.3 fold the Ca^{2+} affinity of human cTnC and is responsible for a 2-fold increase in cardiac myofibril Ca^{+2} sensitivity. The contributions of trout cardiac troponin I (ScTnI) to the Ca^{+2} sensitivity of the trout heart is currently unknown. The cDNA for ScTnI has been cloned using RACE-PCR. Sequencing results indicate that ScTnI is 59% and 56% identical to human cTnI and human skeletal troponin I, respectively, at the amino acid level. Interestingly, ScTnI lacks the ~30-residue N-terminal sequence present in mammalian cTnI that contains two protein kinase A (PKA) target residues at positions 23 and 24. ScTnI has been expressed and the influence of it on the Ca^{2+} activation of human cardiac troponin is currently being characterized using steady state Ca^{2+} binding assays and stopped flow kinetic analysis. This work is supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canadian Foundation for Innovation (CFI) to TEG.

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PI3-Kinase Controls Smooth Muscle Contraction Via Regulation Of MLCP Activity

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We demonstrated for the first time that PI3-kinase plays a role in the regulation of smooth muscle contraction by controlling MLCP. The inhibition of PI3-kinase markedly inhibited Ca2+-induced contraction and GTPγS induced Ca2+ sensitization of α-toxin permeabilized vascular smooth muscle as well as K+-induced contraction of intact vascular smooth muscle. The contractile inhibition was accompanied by the decrease in MLC phosphorylation and MBS phosphorylation at Thr696 and Thr853, which are responsible for the inhibition of MLCP activity. On the other hand, the inhibition of PI3-kinase had no effect on MLCK activity. These results suggest that PI3-kinase is involved in the regulation of MLCP, thus regulating MLC phosphorylation. An Akt specific inhibitor, SH-6, had no effect on the contraction, suggesting that Akt, one of the major down-stream effecter of the PI3-kinase pathway is not involved in this mechanism. MBS phosphorylation at Thr853, a Rho kinase specific site, was decreased by the inhibition of PI3-kinase even at rest, when Rho kinase is not activated. These results suggest that PI3-kinase does not influence the MBS kinases, such as Rho kinase. In fact, we found that the PI3-kinase inhibition activated MBS phosphatase activity. Furthermore, we found that PI3-kinase inhibition increased MBS phsophorylation at the PKG site, suggesting the activation of PKG pathway. Since the activation of the cGMP/PKG pathway decreases MLC phosphorylation by activating MBS phosphatase (Nakamura et al., 2007), our results suggest that PI3-kinase regulates smooth muscle contraction by modulating the PKG pathway.

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Crossbridge-mediated Activation of Rabbit Skeletal Muscle Myofibrillar ATPase: a Role for the Calcium Binding Domains of Troponin C

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Activation of the thin filament in striated muscle is a cooperative process requiring both the binding of Ca^{2+} to troponin C (TnC) and the binding of myosin crossbridges to actin. The aim of this study was to assess the role of TnC domains in the crossbridge-mediated activation of rabbit skeletal muscle myofibrils in the absence of Ca^{2+}. Activation of myofibrillar ATPase was produced by addition of varying concentrations of myosin S1 modified by N-ethylmaleimide (NEM-S1), which facilitates crossbridge cycling by forcing tropomyosin into the open position on the filament. Comparisons were made of native myofibrils, myofibrils from which TnC was extracted, and myofibrils reconstituted with either a TnC mutant (TnC_{48-82}) in which Ca^{2+} activation was blocked by a disulfide bond in the N-terminal domain (Grabarek, et al, Nature, 345:132,1990) or a proteolytic fragment of TnC (TR2C) lacking the N-terminal Ca^{2+}- binding domain (Grabarek, et al, J. Biol. Chem. 265:13121, 1981). The ATPase activity of native myofibrils was increased ~170% by the addition of NEM-S1 (2-4μM). Following extraction of TnC the addition of the same concentrations of NEM-S1 produced ~60% activation. In both cases higher concentrations of NEM-S1 produced no further increase in activation. With the addition of either TnC_{48-82} or TR2C the degree of activation was higher (70-100%), but required higher NEM-S1 concentrations (4-8μM). These results suggest that both domains of TnC play a role in facilitating optimal crossbridge-mediated activation of the thin filament, presumably by providing alternative binding sites for troponin I.

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Structurally Unstable Regions in the Tropomyosin-Troponin Complex from Bovine Heart Muscle

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Regulation of contraction in striated muscles requires a semi-independent movement of various domains of the tropomyosin-troponin (TmTn) complex