example, in the majority of families with LEOPARD Syndrome-associated HCM, mutations occur in the protein tyrosine phosphatase SHP2. We therefore hypothesized that the sarcomeric mechanical properties are altered by SHP2 mutations. Transgenic mice were generated with cardiac myocyte-specific expression of a loss-of-function mutation of SHP2 (Q510E-SHP2). These mice developed an early-onset form of HCM with increased myocyte size, heart-to-body weight ratios, and interventricular septum thickness. Interestingly, we found that permeabilized cardiac myocyte preparations from 1-month old Q510E-SHP2 mice displayed greater maximal Ca^{2+}-activated tension (SHP2 = 84 ± 2 kN/m^2; Wt = 45 ± 14 kN/m^2) and power generating capacity (SHP2 = 10 ± 2 μW/mg; Wt = 5 ± 1 μW/mg) compared to myocytes from littermate wildtype (Wt) mice. We also found PKA-mediated phosphorylation of both myosin binding protein-C (MyBP-C) and cardiac troponin I (cTnI) was increased in cardiac myofibrils from 1 month old Q510E-SHP2 mice. Taken together, these results implicate a compensatory increase in the contractile state of cardiac myofibrils in response to loss of SHP2 function.

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DCM-Linked D230N Tropomyosin Mutation Results in Early Dilatation and Systolic Dysfunction in Mice
Lauren Tal, Jesus Jimenez, Jil C. Tardiff.
Albert Einstein College of Medicine, Bronx, NY, USA.
Recently, a study in two large multi-generational families described a familial dilated cardiomyopathy (DCM) caused by a single amino acid substitution Asp230Asn (D230N) in tropomyosin. These families demonstrated a unique bimodal disease distribution in which infants presented with a severe form of DCM, while adults presented with a mild to moderate clinical phenotype. To determine the biophysical consequences of this mutation on tropomyosin and its effects on regulatory function in the sarcomere, we employed circular dichroism and the regulated in vitro motility assay. We found that while this mutation does not affect overall thermal stability of tropomyosin, it has a profound effect on regulatory function. As previously shown in solution, the presence of the D230N mutation decreases the maximal velocity of filament sliding and calcium sensitivity of thin filament activation compared to wild type filaments. Additionally, the D230N mutation increases the cooperativity of myofilament activation. In order to further explore our biophysical observations and the physiologic effects of the D230N mutation, we created a transgenic murine model. In mice carrying the D230N tropomyosin mutation we found evidence of early dilatation and systolic dysfunction by echocardiogram in the absence of histological changes such as fibrosis or inflammatory cell invasion. Ultrastructural analysis of transgenic left ventricular tissue demonstrated z-disk alterations. Finally, preliminary studies on isolated myocytes from transgenic mice loaded with fura-2AM demonstrate no discernible differences in calcium transients compared to non-transgenic siblings suggesting that functional impairments are not due to calcium handling defects. Collectively, these studies suggest that the D230N mutation in tropomyosin is responsible for alterations in structure and function of the thin filament that result in a primary dilatation of the cardiac left ventricle. *This work is supported by funding from the Children's Cardiomyopathy Foundation.*

Muscle: Fiber & Molecular Mechanics & Structure II

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Geometric Changes of Transverse Tubules in Rabbit Cardiac Myocytes during Contraction
Thomas G. McNary[1], Kenneth W. Spitzer[1], John H.B. Bridge[1], Hilary Holloway[2], Peter Kohl[3], Frank B. Sachse[1].
[1]University of Utah, Salt Lake City, UT, USA, [2]University of Auckland, Auckland, New Zealand, [3]Imperial College London, Harefield, United Kingdom.
Invaginations of the sarcolemma, called 'transverse tubules' (t-tubules), allow for rapid communication of electrical activation deep into the interior of ventricular cardiomyocytes. Given the length and radius of t-tubules, the rate of diffusion alone appears to be insufficient for homeostasis of the t-tubular content, especially during exercise [1]. Previously, we reported in rabbit ventricular myocytes that positive longitudinal strain modulates geometrical features of t-tubules [2] and their mouth-region [3] in a way that could support convective re-distribution of t-tubular content. Here, we test the hypothesis that cell contraction also affects t-tubular volume.
Isolated ventricular cardiomyocytes from adult New Zealand white (NZW; n=23) rabbits were imaged using an inverted confocal microscope [2] either at slack length or during negative strain to ~85%, caused by exposure to superfusate-induced tonic contracture. Image stacks of cell segments were deconvolved and t-tubules segmented. In addition, ventricular tissue from NZW rabbit hearts, fixed either at zero intra-ventricular pressure (n=2) or during contracture (n=2), was studied using transmission electron microscopy (TEM; [3]). T-tubular length and volume were assessed in confocal images, while ellipticity and orientation were explored both in confocal and TEM data. Statistical significance was determined using a two-tailed t-test with $p<0.05$ considered significant.
T-tubular cross-section changed, reducing ellipticity in contractured myocytes (compared to control), while apparent length and total volume of t-tubules increased. This suggests that both passive distension and active contraction may give rise to a convective component of fluid transport, mixing, and exchange of t-tubular content.
[1] Shepard, N, HB McDonough. Am J Physiol Heart Circ Physiol. 1998/275:H852-H860
[2] McNary, TG, JH Bridge, FB Sachse. Biophys J. 2011/100:L53-L55.
[3] Kohl, P, PJ Cooper, H Holloway. Prog Biophys Mol Biol. 2003/82:221-227.

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Mechanoregulation of Delayed Stretch Activation
Srboljub M. Mijailovich[1], Boban Stojanovic[2], Richard J. Gilbert[1], Thomas Irving[3].
[1]Tufts University, Boston, MA, USA, [2]Faculty of Science, University of Kragujevac, Kragujevac, Serbia, [3]Illinois Institute of Technology, Chicago, IL, USA.
Delayed stretch activation (SA) is a prominent feature in the function of the cardiac myocyte and plays an important role in regulating cardiac output. A mechanistic understanding of SA is essential for the development of models that quantitatively and causally connect molecular defects to global cardiac function. We propose a novel mechanism that defines how mechanical forces imposed by stretch affect troponin-actin and myosin-actin bonds and thereby modify calcium-modulated thin filament regulation. Tropomyosin molecules are assumed to form two continuous flexible chains (CFC) along each actin filament; tropomyosin movements are restricted by bound troponins and myosin heads bound to actin. Crossbridges transmit sarcomere forces to the thin and the thick filaments. A stretch applied on a sarcomere extends the thin filaments and associated CFCs imposing additional strain (via the CFC) on the TnI-actin and myosin-actin bonds. The spatial positions of these bonds were calculated using the computational platform, MUSICO (**MU**scle **SI**mulation **CO**de) and, hence, the forces acting on TnI-actin and myosin-actin bonds before and after stretch at different Ca^{2+} concentrations. These forces were assessed from finite element analysis of CFCs weakly interacting with the actin surface and strongly interacting with actin via Tn attachments to actin. An imposed stretch leading to sarcomere forces of ~50% of the maximum isometric force increased the forces on the bonds by more than 10 pN, sufficient to strongly tilt the energy landscapes and accelerate the rate of detachment of Tn from actin, even without Ca^{2+} bound to TnC. The maximum effect of this behavior is observed in muscle fibers at submaximal activation (pCa ~ 6). This analysis suggests a mechanism for observed modulation of cardiac myocyte contractility by SA based on altered mechanochemistry of thin filaments regulation via CFC. Supported by NIH R01 AR048776 and R01 DC 011528.

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A Model for Ca(2+)-Dependent Cooperative Activation in the Cardiac Thin Filament that Allows for Crossbridge Cycle Feedback
Daniel C. Rieck, King-Lun Li, Zhiqun Zhou, Wen-Ji Dong.
Washington State University, Pullman, WA, USA.
The mechanism underlying the apparent cooperativity of the cardiac thin filament was investigated by using FRET to follow the N-domain opening of recombinant cardiac troponin C (N-cTnC opening) passively exchanged into rat myocardial fiber bundles and tested under a variety of experimental conditions. Calcium titrations conducted in the presence of crossbridge cycle modulators and tension recovery experiments revealed: 1) N-cTnC opening occurred just as ''cooperatively'' with or without crossbridge cycle activity; 2) the calcium sensitivity of N-cTnC opening is enhanced by crossbridge cycling; 3) the rigor state results in no cooperativity and 10% of the cTnC ensemble being apparently open under resting conditions; 4) the breaking of crossbridges preceding a tension recovery phase results in a slight relaxation of the FRET distance associated with N-cTnC opening, which recovers during tension recovery. In light of this and other evidence from the literature, a model of apparent cooperativity based on the three state model is proposed wherein the steep force-Ca^{2+} relationship ultimately arises from a tunable cTnC binding affinity for Ca^{2+}. Activation may be mathematically modeled by a sum of weighted Hill-equation fractions (i.e. $[L]^n / (K_d + [L]^n)$) with each fraction governed by a hill coefficient of n=1 and representing a particular cTnC conformation with a unique affinity for Ca^{2+}, or ''affinity-state''. The affinity-state of an individual cTnC is influenced by the activation state of its neighboring