of sarcomeres along the myocyte caused marked underestimation of sarcomere lengthening speed due to superpositioning of different timings for lengthening between sequentially connected sarcomeres. Then, we found that following the treatment with ionomycin, neonatal myocytes exhibited spontaneous sarcomeric oscillations (Cell-SPOC) at partial activation with blockage of sarcoplasmic reticulum functions, and the waveform properties were indistinguishable from those obtained in electric field stimulation. The myosin activator, omecamtiv mecarbil, markedly enhanced Z-disc displacement during Cell-SPOC. Finally, we interpreted the present experimental findings in the framework of our mathematical model of spontaneous sarcomeric oscillations (Sato, K. et al., Prog Biophys Mol Biol. 2011; Sato, K. et al., Phys. Rev. Lett. 2013). The present experimental system has a broad range of application possibilities for unveiling single sarcomere dynamics during excitation-contraction coupling in cardiomyocytes under various settings.

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Determining the Molecular Mechanisms that Link a Titin Mutation to Cardiomyopathy

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A mutation in the giant elastic protein titin has recently been linked to arrhythmogenic cardiomyopathy (AC), a disease primarily characterized by fibrofatty infiltration of the myocardium. Titin is the first sarcomeric protein linked to AC. In addition to largely unstructured segments, the extensible I-band region of titin contains ~40 immunoglobulin(Ig)-like domains that are natively folded as highly stable beta barrels. One of these domains, Ig10, contains a single Thr→Ile mutation that leads to AC. The first step in determining how this mutation leads to severe cardiac disease was to study the mutation at the single molecule level using atomic force microscopy (AFM) and in vitro degradation assays. With AFM individual proteins are mechanically stretched, and it was found that the mutation significantly reduces the force needed to unfold Ig10. Although no difference in refolding rate was found, by comparing the experimental AFM unfolding forces with computer simulated Ig unfolding, it was found that the mutation increases Ig10's unfolding rate fourfold. This effect predicts increased Ig10 unfolding (i.e. transition into a non-native state) under physiological conditions. It was also found that mutant Ig10 is more prone to protease degradation due to compromised local structure at the point mutation. This suggests that accelerated titin turnover in cardiomyocytes instigates the cardiac remodeling process in patients with titin-linked AC. This is the first time an Ig domain in titin's elastic I-band region has been linked to cardiac disease, and this study proposes a novel disease mechanism contingent on Ig domain unfolding.

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Cardiac Myofilament Sarcomere Shortening is Faster and Exhibits Greater Shortening-Induced Cooperative Deactivation after PKA Treatment

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Increased myocardial contractility by beta-adrenergic stimulation requires precise coordination between cardiac myocyte membrane potential, calcium handling, and myofilament mechanics. From a myofilament standpoint, more stoke volume at a given end-diastolic volume ideally would involve both faster loaded shortening for more ejection coupled with greater shortening-induced cooperative deactivation to assist in relaxation, which would help assure adequate filling at higher heart rates. We tested if myofilaments elicit these characteristics by direct measurement of myocardial sarcomere shortening during load clamps before and after PKA treatment, a downstream beta-adrenergic signaling molecule. Rat skinned ventricular cardiac myocyte preparations (n = 6) were attached between a force transducer and motor, calcium activated to elicit ~30% maximal force, and then light-to-moderate load clamps were induced by a servo-controlled feedback system. Initial sarcomere length was set at ~2.25 μm and sarcomere shortening was monitored during the load clamps using an IonOptix SarcLen system at 240 Hz. Interestingly, PKA seemed to result in the aforementioned ideal changes in sarcomere mechanics. First, loaded sarcomere shortening velocities were faster following PKA treatment (control = *0.838 ± 0.198 μm/sec/sarcomere* (n= 20) at loads of 0.17 ± 0.06 isometric force (P_o); PKA = *1.215 ± 0.071 μm*/sec/sarcomere (n = 31) at loads of 0.15 ± 0.07 P_o ($p < 0.001$) (means ± SD)). In addition, PKA appeared to yield greater shortening-induced cooperative deactivation during load clamps by exhibiting greater curvature of sarcomere length traces, which was indexed by the rate constant of sarcomere shortening ($k_{shortening}$), (Control $k_{shortening}$ = 4.27 ± 1.47; PKA $k_{shortening}$ = 6.93 ± 2.48 ($p < 0.001$) (means ± SD)). These findings illustrate the fine tuning of the myofilament "clock" to help match the resetting of the membrane and calcium clocks after beta-adrenergic stimulation.

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The Effect of α-Actin Mutant E361G on Force Generation and Cross-Bridge Kinetics in Thin-Filament Reconstituted Bovine Cardiac Muscle Fibers

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Recombinant human cardiac actin mutant E361G, known to cause FDC in humans, and its WT counterpart were expressed in baculovirus/insect cells. These actins were used to reconstitute the thin filament of bovine cardiac fibers, together with bovine cardiac tropomyosin (Tm) and troponin (Tn). Effects of [Ca^{2+}], [ATP], [phosphate], and [ADP] on tension and its transients were studied at 25°C. After actin filament reconstitution, tension of WT actin reconstituted fibers (T_a) reached 0.75 ± 0.06 T_0 (T_0=tension of native fibers), which was not different from rabbit skeletal actin reconstituted fibers (T_a=0.73 ± 0.07 T_0). E361G also generated similar tension after actin reconstitution (T_a=0.71 ± 0.12 T_0). After Tm and Tn reconstitution, tension at pCa 4.66 (referred to T_{HC}) for E361G reached 0.84 ± 0.09 T_0, which was similar with that of WT (0.85 ± 0.06 T_0). In the pCa-tension study, E361G increased the cooperativity (n_H=3.9 ± 0.7) without altering Ca^{2+} sensitivity (pCa_{50}=5.76 ± 0.03) compared with those of WT (n_H=2.6 ± 0.3, pCa_{50}=5.70 ± 0.03). E361G caused no relaxation dysfunction in the reconstituted fibers as shown by the unchanged T_{LC} (tension at pCa 8.0: 0.08 ± 0.02 T_0) compared to WT (0.08 ± 0.01 T_0). Tm's allosteric effect on the actin-myosin interaction is assessed from the ratio T_{HC}/T_a, which remained similar between E361G (1.28 ± 0.10) and WT (1.17 ± 0.08). Five equilibrium constants of the cross-bridge cycle were deduced using sinusoidal analysis. E361G caused a significant decrease in K_2 (cross-bridge detachment: 0.27 ± 0.05) compared to that of WT (0.78 ± 0.13). Other equilibrium constants did not change significantly. As a result, E361G formed significantly more (~20%) force generating cross-bridges than that of WT, indicating that force/cross-bridge is decreased by ~20% in E361G. We conclude that increased n_H and/or decreased force/cross-bridge may trigger the signal cascade that eventually results in FDC phenotype.

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The ACTC A295S Mutation Increases Heart and Skeletal Muscle Contractile Activity in Drosophila Models of Hypertrophic Cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disease characterized by thickened heart walls, cardiac arrhythmias, myocyte disarray and interstitial fibrosis. Over 10 mutations in alpha-cardiac actin (*ACTC*) are associated with HCM. The *ACTC* Ala295Ser substitution causes a highly penetrant disease with diverse phenotypes. To investigate the *in vivo* consequences of the ACTC mutation we generated *Drosophila* lines with muscle-specific expression of A295S actin. Our unique models limit genetic diversity and pathological complexity to help resolve molecular mechanisms of diseases. For expression in the fly heart we used *UAS-Act57B^{WT}* and *UAS-Act57B^{A295S}* actin transgenes in conjunction with the HandGal4 cardiac-specific driver. Confocal microscopy confirmed heart-restricted expression of UAS-Act57B^{WT}GFP and UAS-Act57B^{A295S}GFP and co-polymerization of transgenic and endogenous actin. High-speed video microscopy and motion analysis revealed A295S actin expression significantly reduced diastolic volumes and increased systolic intervals consistent with elevated contractile properties at rest and during contraction. For indirect flight muscle (IFM)-specific actin expression, we used the IFM targeted *Act88F^{WT}*and *Act88F^{A295S}* transgenes, in an IFM actin null background. Muscle mechanics on heterozygous IFM fibers revealed that the A295S actin mutation increased power, the frequency of maximum power generation (f_{max}), and stiffness relative to control fibers. The higher *fmax* and stiffness are likely causes of an observed increase in wing beat frequency (WBF) of *Act88F^{A295S}*heterozygous flies. The WBF increase had a negative impact on flight ability which decreased further as mutant: wildtype actin ratios were increased. Overall, enhanced contractile activity of actin HCM cardiac myocytes and IFM fibers is consistent with the hypercontractile properties frequently associated with HCM mutations.

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Dcm-Causing Mutation E361G in Actin Uncouples Myofibril Ca^{2+} Sensitivity from Protein Phosphorylation

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Experiments using the in vitro motility assay have shown that the myofilament Ca^{2+}-sensitivity is modulated by phosphorylation of troponin I and that the