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The Specific Cleavage of Titin Springs to Quantify the Contribution of Titin to Myocardial Passive Stiffness

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The giant sarcomere protein titin bears passive load in cardiomyocytes (CMs) and altered titin stiffness occurs in heart failure. Contributions to CMs stiffness may also come from the actin and microtubular networks. Our aim was to quantify the contribution of titin to CMs viscoelasticity through specific cleavage of the titin springs in situ. We have developed a knock-in (KI) mouse model carrying a tobacco etch virus (TEV) protease-recognition site and a HaloTag in titin's elastic region. This HaloTag-TEV cassette allows for specific titin cleavage during mechanical measurements of CMs and visualization of successful cleavage. TEV-protease induced a complete, rapid, and specific cleavage of cardiac titin in skinned homozygous KI CMs, but not in wildtype. Confocal and atomic force microscopy were combined to quantify the transversal stiffness of CMs by nanoindentation. The Young's modulus of homozygous KI cells at a pre-set force of 3 nN was significantly reduced with titin-cleavage, by $26 \pm 4\%$ (n= 26 cells), indicating cell softening. The amplitude of this decrease was similar to that found in nanoindentation measurements on human CMs upon microtubule depolymerisation (Chen et al, Nat Med 2018;24:1225-1233). Furthermore, skinned KI CMs were stretched within physiological sarcomere lengths and the passive force recorded before and after TEV-treatment. TEV-protease reduced the steady-state force by $53 \pm 8\%$ and the viscous force by $56 \pm 7\%$ (n=9 cells). The skinned KI CMs were also incubated first with an actin-severing, Ca^{2+}-independent gelsolin fragment and then with TEV-protease, which reduced the passive force to ~82% and ~27% of the value before treatment, respectively (n=6 cells). Thus, titin is the main contributor to tensile force but the actin cytoskeleton contributes as well. High mechanical connectivity between the cytoskeletal elements is likely to occur in CMs, implicating a tensegrity structure.

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BAG3 Localizes to the Mature Sarcomere and Maintains Myofilament Function

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Bcl-2-associated athanogene 3 (BAG3) is a co-chaperone canonically involved in protein quality control (PQC). Decreased levels and mutations to BAG3 are linked with heart failure, though the mechanisms remain unclear. Neonatal myocyte studies showed BAG3 localizes to the Z-disk, however, the functional role of BAG3 at the sarcomere is unknown. First, we confirmed BAG3 co-localization with Z-disk α-actinin in the mature myofilament using immunofluorescence in human left ventricular (LV) cardiomyocytes. Furthermore, by western blot this myofilament-fraction of BAG3 decreased by ~20% in human heart failure. To test the hypothesis that decreased myofilament-bound BAG3 is detrimental to function, we used a mouse model of myocardial infarction (MI)-induced heart failure. Eight weeks post-MI, BAG3 was overexpressed via associated adenovirus-9 for three weeks. Myofilament function was assessed in LV cardiomyocytes. Compared with sham, MI decreased maximal calcium-induced force (F_{max}). However, BAG3 overexpression restored F_{max} to sham levels. To explore the mechanism responsible, we identified BAG3's myofilament interactome using immunoprecipitation and mass spectrometry. Interestingly, Hsp70 and HspB8, which together with BAG3 promote PQC in other cell types, were among the top hits. Association of this complex with the myofilament was confirmed by co-immunoprecipitation and immunofluorescence showing colocalization of HspB8 and Hsp70 at the Z-disk, suggesting a role for the complex in myofilament PQC. To support the functional significance of this complex, myofilament function was assessed in mice expressing the BAG3 P209L mutant, previously shown to impair client processing by the BAG3-Hsp complex. P209L mice displayed age-dependent depression of myofilament function. This work is the first to show BAG3 localizes to the mature sarcomere and modulates myofilament function in cardiomyocytes, which is disrupted by MI and mutation. Additionally, our data suggests that the maintenance of function following cardiac stress may be due to myofilament PQC.

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Impact of Mavacamten on Force Generation in Single Myofibrils from Rabbit Psoas and Human Cardiac Muscle

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Mavacamten (MYK-461, Axon Medchem BV) is an allosteric inhibitor of sarcomeric myosins presently used in preclinical/clinical trials for HCM treatment (Anderson et al., 2018)). Here, single or thin bundles of myofibrils from rabbit fast skeletal muscle (psoas) and human donor ventricle (frozen samples) have been used to study the effects of μmolar Mavacamten on maximal isometric force. Both myofibril types were mounted in relaxing solution (pCa 9; [Pi] ~ 200 μM , 15 °C,) and then fully activated (pCa 4.5) with and without selected concentrations of Mavacamten or subjected to rapid Mavacamten jumps in activating solution. Dose-response curves confirmed a higher sensitivity of maximal isometric force developed by cardiac muscle (IC_{50} ~0.5 μM) compared to fast skeletal (IC_{50} ~10 μM) as previously reported for pCa_{50}- activated ATPase of the same myofibrillar systems (Kawas et al., 2017). In fast skeletal myofibrils, the kinetics of force development were also strongly depressed by Mavacamten with a trend to a higher sensitivity compared to the effect on tension. This effect was undetectable in human ventricle. When submitted to Mavacamten jumps, both myofibril types responded with a rapid relaxation-like force drop (fully reversible) whose sensitivity to Mavacamten concentration was the same as that of the kinetics of force relaxation measured in the same conditions. The effect was unaffected by ADP (10 mM) and Pi (0-1 mM range). The results obtained are in agreement with the reported effect of Mavacamten on multiple stages of cross-bridge chemo-mechanical cycle (i.e. inhibition of Pi release rate and decrease of actin binding in M.ADP states) as well as on regulation state mediated by the availability of strongly actin binding heads. SilicoFCM EU H2020 grant agreement n. 777204.

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FRET Measurements of the Power Stroke in Human Cardiac Myosin

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We used a FRET strategy to investigate structural changes in the lever arm domain of human beta cardiac myosin subfragment 1 (M2B-S1). We exchanged Alexa488 labeled human ventricular regulatory light chain (V105C) onto M2B-S1 (M2B-S1 A.RLC) which served as a donor for Cy3ATP bound to the active site. The functional properties (ATPase and in vitro motility) of M2B-S1 A.RLC were similar to WT. The FRET efficiency was 2-fold higher in the presence of Cy3ATP compared to Cy3ADP. We monitored the FRET signal during the actin-activated product release steps using transient kinetic measurements. At lower actin concentrations the main component of the FRET transients was dependent on actin concentration and similar to the ATPase cycle time. At higher actin concentrations we observed a fast phase (8-10 s^{-1} at 30 μM actin) and a slower phase (2-3 s^{-1} at 30 μM actin) that was similar to the steady-state ATPase rate. Measurements as a function of temperature revealed that the slower phase was similar to the steady-state ATPase rate while the fast phase was 2-3 fold faster. We propose that the fast phase measured with our FRET probes represents the structural change associated with movement of the lever arm during the power stroke. We also performed measurements in the presence of the heart failure drug Omecamtiv Mercarbil which is proposed to inhibit the power stroke. We observed a lag in the fluorescence transients followed by a fluorescence change that was similar to the overall ATPase rate, suggesting little movement of the lever arm during actin-activated phosphate release. Our FRET approach can be utilized to examine the impact of disease mutations on the conformation of the lever arm in human cardiac myosin which will be crucial for establishing structure-based mechanisms of contractile dysfunction.

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Structure of the Actin-Tropomyosin-TNT Complex

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Thin filament-linked proteins actin, tropomyosin, and troponin are the major regulators of cardiac and skeletal muscle that activate contraction through calcium-mediated conformational changes. Single point mutations within the