Localized Nitric Oxide Signaling Mediates Cardiac Mechanismo-Chemo-transduction
Zhong Jian, Huilan Han, Tiejiao Zhang, Jose Puglisi, Leighton T. Izu, John Shaw, Yi-Je Chen, Rafael Shimkus, Nipavan Chiamvimonvat, Donald M. Bers, Kit S. Lam, Ye Chen-Izu.

Cardiomyocytes contract against a mechanical afterload during each heartbeat. We have leveraged Cell-in-Gel systems to impose 3D mechanical stresses on single cardiac myocytes during contraction. Using this new technique, we identified key molecules involved in transducing mechanical stress to alter Ca\(^{2+}\) dynamics. Increasing mechanical load causes enhanced contractility and elevated systolic Ca\(^{2+}\) transient that is mediated by nitric oxide synthase (NOS). Increased load also causes a marked increase of diastolic spontaneous Ca\(^{2+}\) sparks, and their suppression is only effected by inhibition of nNOS but not eNOS. The differential effects on Ca\(^{2+}\) sparks may stem from the two-fold closer physical proximity of nNOS vs. eNOS to ryanodine receptors. In addition to NOS, NOX2 and CaMKII are also involved in the mechano-chemotransduction pathways, which together fine-tune cardiac contraction under mechanical load.

Cardiomyopathy Ctn Mutation in Patient Derived Cardiomyocytes from Induced Pluripotent Stem Cells Affects Sarcomere Structure and Function
Kathleen M. Broughton, Veronica Sanchez-Freire, Joseph C. Wu, Beata M. Wolska, Ross J. Solaro, Brenda Russell

Cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CM) are useful to understand basic structural and functional characteristics of normal and diseased human heart cells. We investigated mechanical properties of hiPSC-CM derived from unaffected and affected members of a family harboring a dilated cardiomyopathy (DCM) mutation in cardiac troponin T (cTnT), the troponymosin binding unit of troponin. Patients with a cTnT point mutation (R173W) develop DCM, which commonly leads to diastolic and systolic dysfunction and progressive heart failure. To study the shortening and shortening velocity of normal and cTnT mutant hiPSC-CM cells, cells were plated on substrates with a relatively soft stiffness (160 kPa) molded from polydimethylsiloxane and measured using a line scan method. Data were collected using a Zeiss 710 confocal microscope. Visual observation of cells after 5 days maturity on the PDMS substrates indicate a smaller percent of mutant (8%) compared to normal (87%) hiPSC-CM were spontaneously beating. Immunohistochemistry showed that myofilibr structure was better developed in normal compared to cTnT mutation cells. Day 5 line scans revealed normal hiPSC-CM cells shorten more (0.47 \(\mu\)m) than mutant cTnT cells (0.32 \(\mu\)m); shortening velocity was faster for normal (1.42 \(\mu\)m/s) compared to mutant cTnT cells (0.67 \(\mu\)m/s). Acute treatment (1 dose with a 10 min activation dwell time) with omeprazole micellar (200nm), a cardiac myosin activator, increased the shortening and shortening velocity of normal (0.78 \(\mu\)m at 1.85 \(\mu\)m/s) but not cTnT mutant (0.32 \(\mu\)m at 0.70 \(\mu\)m/s) hiPSC-CM cells. Although more studies are necessary, these results suggest that cTnT may affect development of sarcomeres and the regulation of contractility. Furthermore, the myosin activator omeprazole micellar may not be sufficient to rescue dysfunction in the facilitation of rapid Ca\(^{2+}\) release.

Parvalbumin (Parv), an EF-hand Ca\(^{2+}\) buffer, facilitates rapid relaxation in fast-twitch muscle. Parv gene delivery to the heart has been studied as a therapeutic strategy for diastolic heart failure, in which slow Ca\(^{2+}\) reuptake is an important contributor. A limitation of WT-Parv in this context is the significant decrease of PLB-PLB interaction with and inhibit SERCA. To address the role of the phospholamban and sarcolipin pentamers, we have studied their interaction with SERCA using electron cryo-microscopy of two-dimensional co-crystals. In our previous studies, phospholamban oligomers were found interspersed between SERCA dimers and we constructed a three-dimensional model of the complex. We also addressed the molecular characteristics of phospholamban through its ability to its interaction with SERCA and effects of phosphorylation and mutation of phospholamban on the structure of the complex with SERCA. In our recent work, we compared two crystal forms of SERCA in the absence and presence of phospholamban by electron cryo-microscopy - namely, small helical crystals and large two-dimensional crystals. The SERCA dimer ribbons that are found in both crystal forms consist of a rigid assembly of calcium-free SERCA molecules. While the lattice formed by the SERCA dimer ribbons is different in the helical and two-dimensional crystals, we show that a phospholamban oligomer interacts with SERCA in a similar manner in both crystal types. With this information, we next undertook a structural investigation of SERCA and sarcolipin in the two-dimensional crystals. A projection map was determined for SERCA in the presence of sarcolipin to a resolution of 8.5 A\(^\circ\) and was consistent with a pentameric state for sarcolipin.

Contractile Structure-Function of Parvalbumin’s Ef-Hand Metal Ion Binding Loop in Isolated Adult Cardiac Myocytes
Michelle L. Asp, Joseph M. Metzger

Parvalbumin (Parv), an Ef-hand Ca\(^{2+}\) buffer, facilitates rapid relaxation in fast-twitch muscle. Parv gene delivery to the heart has been studied as a therapeutic strategy for diastolic heart failure, in which slow Ca\(^{2+}\) reuptake is an important contributor. A limitation of WT-Parv in this context is the significant decrease of PLB-PLB interaction with and inhibit SERCA. To address the role of the phospholamban and sarcolipin pentamers, we have studied their interaction with SERCA using electron cryo-microscopy of two-dimensional co-crystals. In our previous studies, phospholamban oligomers were found interspersed between SERCA dimers and we constructed a three-dimensional model of the complex. We also addressed the molecular characteristics of phospholamban through its ability to its interaction with SERCA and effects of phosphorylation and mutation of phospholamban on the structure of the complex with SERCA. In our recent work, we compared two crystal forms of SERCA in the absence and presence of phospholamban by electron cryo-microscopy - namely, small helical crystals and large two-dimensional crystals. The SERCA dimer ribbons that are found in both crystal forms consist of a rigid assembly of calcium-free SERCA molecules. While the lattice formed by the SERCA dimer ribbons is different in the helical and two-dimensional crystals, we show that a phospholamban oligomer interacts with SERCA in a similar manner in both crystal types. With this information, we next undertook a structural investigation of SERCA and sarcolipin in the two-dimensional crystals. A projection map was determined for SERCA in the presence of sarcolipin to a resolution of 8.5 A\(^\circ\) and was consistent with a pentameric state for sarcolipin.

Phospholamban and Sarcolipin Pentamers Naturally Associate with the Sarcoplasmic Reticulum Calcium Pump
John Paul Clavres, Przemek Gorski, Joseph Primeau, Catharine Triebel, Howard S. Young

Biochemistry, University of Alberta, Edmonton, AB, Canada. Phospholamban and sarcolipin interact with the sarcoplasmic reticulum calcium pump (SERCA) and regulate contractility in smooth, cardiac and skeletal muscle. While both proteins can form oligomers, it is thought that only the monomers interact with and inhibit SERCA. To address the role of the phospholamban and sarcolipin pentamers, we have studied their interaction with SERCA using electron cryo-microscopy of two-dimensional co-crystals. In our previous studies, phospholamban oligomers were found interspersed between SERCA dimers and we constructed a three-dimensional model of the complex. We also addressed the molecular characteristics of phospholamban through its ability to its interaction with SERCA and effects of phosphorylation and mutation of phospholamban on the structure of the complex with SERCA. In our recent work, we compared two crystal forms of SERCA in the absence and presence of phospholamban by electron cryo-microscopy - namely, small helical crystals and large two-dimensional crystals. The SERCA dimer ribbons that are found in both crystal forms consist of a rigid assembly of calcium-free SERCA molecules. While both phospholamban and sarcolipin interacted with transmembrane segment M3 of SERCA, the interaction of the sarcolipin pentamer was mediated by an additional density consistent with a SLN monomer. We conclude that pentameric forms of both phospholamban and sarcolipin naturally associate with SERCA.

Phospholamban C-Terminal Truncations Including Heart Failure Mutation L93Stop Decrease Membrane Localization and Oligomerization and Alter the Structure of the PLB-Serca Complex
Neha Abrol, Nikolai Smolin, Delaine K. Ceholski, Howard S. Young, Seth L. Robia

1Cell and Molecular Physiology, Loyola University Chicago, Maywood, IL, USA, 2Department of Biochemistry, University of Alberta, Edmonton, AB, Canada.

A naturally occurring missense Leu-39stop (L39X) mutation in phospholamban (PLB) results in truncation of the C-terminal transmembrane domain, leading to cardiomyopathy and premature death. In this study, we fused PLB and SERCA to fluorescent protein tags to determine the structural and thermodynamic consequences of progressive truncations of the C-terminal residues of PLB in the membranes of living cells. We found that deletion of only a few C-terminal residues resulted in significant loss of PLB membrane anchoring and mislocalization to the cytoplasm and nucleus. Selective permeabilization of the plasma membrane by saponin resulted in diffusion of fluorescently labeled PLB out of the cells, consistent with solubilization of truncated proteins. Western blot analysis showed the expected mobilities for truncation mutants relative to full length PLB-WT, indicating that the observed solubilization of PLB truncation mutants is not due to proteolysis. Moreover, molecular dynamics simulations recapitulated the observed loss of stable bilayer anchoring for truncated PLB. Fluorescence resonance energy transfer (FRET) analysis revealed that C-terminal truncations resulted in progressive loss of PLB-PLB FRET, indicating a decrease in affinity of PLB oligomerization. We quantified a similar decrease in the SERCA-PLB binding affinity. Despite this decrease in affinity, SERCA-PLB FRET was paradoxically increased by deletion of up to 4 C-terminal residues as a result of a 14 angstrom decrease.
We have used quantitative immunoblot, enzyme-linked immunosorbant assay (USA). Biochem/Molec Biol/Biophys, Univ Minnesota Med Sch, Minneapolis, MN, USA. David D. Thomas.

Clinical Trials of Gene Therapy for Heart Failure: Quantitation of the proximity to PLB, while the other is too far away to participate in FRET homo-dimer; in this regulatory complex one SERCA protomer is in close proximity to PLB, while the other is too far away to participate in FRET homo-dimer. In contrast, a control experiment with phospholamban (PLB) oligomer showed a non-linear YFP/Cer relationship, consistent with the observed FRET. Interestingly, average maximal FRET was 28\% between SERCA and PLB, fluorescence lifetime measurements revealed two different lifetimes, consistent with two different populations of FRET donors. One population showed very low FRET, while the other population exhibited high FRET- approximately double the measured average maximal FRET efficiency. The data are consistent with a single PLB bound to each SERCA homo-dimer; in this regulatory complex one SERCA protomer is in close proximity to PLB, while the other is too far away to participate in FRET with PLB.

SERCA2a protein content in KO hearts is less than 5\% of control. Despite severely impaired ex vivo function of isolated hearts and cardiomyocytes at this time, in vivo heart function is mildly impaired and KO mice survive until 7-10 weeks post-knockout, indicating that loss of SERCA2a is temporarily compensated. This finding of preserved cardiac function in vivo in the context of severe SERCA2a depletion is surprising and warrants a detailed dissection of potential compensatory mechanisms. Published studies have found that sarcosomal Ca\(^{2+}\) transport mechanisms such as L-type Ca\(^{2+}\) current, Na-Ca exchange, and plasma membrane Ca-ATPase activity are increased following this loss of SERCA2a protein. These observations prompt the hypothesis that sarcosomal Ca\(^{2+}\) transport remodeling may partially compensate for diminished SR Ca\(^{2+}\) handling in KO hearts. We have begun to investigate this hypothesis by perfusing isolated Serca2 KO hearts with caffeine to evaluate SR-independent contractile function. During caffeine perfusion, left ventricular (LV) end-diastolic pressures were lower in KO hearts than controls consistent with the reduced SR Ca\(^{2+}\) stores in KO myocytes. Interestingly, during prolonged caffeine perfusion, systolic performance was similar in control and KO hearts. This is functional evidence that trans-sarcosomal transient adaptations in KO hearts are not of sufficient magnitude to differentiate KO from WT when SR function is disabled by caffeine.

**2865-Pos Board B557** Simultaneous Imaging of Local Calcium and Single Sarcomere Length in Rat Neonatal Cardiomyocytes via Expression of Cameleon-Nano in Z-Discs

Seichi Tsukamoto\(^1\), Kotaro Oyama\(^2\), Seine A. Shintani\(^3\), Norio Fukuda\(^1\), Shin'ichi Ishiwata\(^1,\)\(^2\)

\(^1\)The Jikei University School of Medicine, Tokyo, Japan, \(^2\)Waseda University, Tokyo, Japan, \(^3\)Waseda Bioscience Research Institute in Singapore, Waseda University, Singapore, Singapore.

In cardiac muscle, contraction is regulated by micromolar concentrations of Ca\(^{2+}\) released from the sarcoplasmic reticulum (SR) (i.e., Ca\(^{2+}\)-induced Ca\(^{2+}\) release), resulting in the binding of Ca\(^{2+}\) to troponin C and subsequent formation of cross-bridges. In order to enhance our understanding of cardiac excitation-contraction coupling, we present the present study developed a novel experimental system for simultaneous measurement of local intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) and single sarcomere length via expression of yellow cameleon-Nano (i.e., FRET-based ultrasensitive Ca\(^{2+}\) indicator) fused to the C-terminus of \(\alpha\)-actinin in Z-discs in primary-cultured rat neonatal cardiomyocytes. In a given sarcomere, the fluorescence emission ratio (YFP/CFP) of the fluorescent protein tags, with the highest FRET efficiency achieved when the respective fluorescent proteins were fused to SERCA N-termini. Progress ive photobleaching of YFP showed that Cer intensity increased linearly with decreasing YFP intensity, suggesting that the stoichiometry of the SERCA complex is a dimer. In contrast, a control experiment with phospholamban (PLB) oligomer showed a non-linear YFP/Cer relationship, consistent with its well-known pentameric stoichiometry. We also investigated whether SERCA dimers could interact with PLB, the regulatory binding partner of SERCA. Interestingly, while average maximal FRET was 28\% between SERCA and PLB, fluorescence lifetime measurements revealed two different lifetimes, consistent with two different populations of FRET donors. One population showed very low FRET, while the other population exhibited high FRET- approximately double the measured average maximal FRET efficiency. 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