Mutations in MYBPC3, the gene encoding the muscle regulatory protein cardiac myosin binding protein-C (cMyBP-C), are among the most common causes of hypertrophic cardiomyopathy (HCM) in both people and cats. However, despite the high prevalence of mutations in MYBPC3, relatively little is understood regarding how mutations lead to disease. One possibility is that some point mutations alter cMyBP-C protein structure leading to enhanced degradation and elimination of the mutant protein. If levels of cMyBP-C protein expression are reduced, then haploinsufficiency (lack of sufficient protein) can trigger disease. Here we tested this idea by analyzing the impact of the A31P mutation, linked to HCM in Maine Coon cats, on 1) the in vitro protein structure of the C0 domain of cMyBP-C, and 2) the total protein expression of cMyBP-C in myocardium of aged cats heterozygous for the A31P mutation. In vitro results demonstrated that the A31P mutation disrupts folding of the C0 domain as shown by three independent methods: altered epitope recognition on Western blots; changes in sensitivity to proteolytic degradation; and reduced β-sheet content assessed by circular dichroism. Western blots of endogenous cMyBP-C obtained from myocardial samples also suggested that C0 structure is altered in vivo because an antibody that preferentially recognizes C0 reacted less with A31P cMyBP-C compared to wild-type cMyBP-C. However, despite these significant structural differences, the A31P cMyBP-C was incorporated into sarcomeres and total cMyBP-C protein (wild-type plus mutant) was similar in wild type and heterozygous A31P cats. These results suggest that despite protein folding abnormalities, the A31P mutation does not lead to haploinsufficiency in the population of older heterozygous cats studied here. Supported by NIH R21HL093603.

1007-Plat

Cell and Myofibril Contractile Properties of hiPSC-Derived Cardiomyocytes from a Patient with a MYH7 Mutation Associated with Familial Cardiomyopathy

Josè Manuel Pioner[1,2], Kai-Chun Yang[3], Lil Pabon[4], Alice Ward Racca[2], Mark Y. Jeong[5], Christian I. Childers[6], Jesse Macadangdang[2], Chiara Tesi[1], Corrado Poggesi[1], Deok-Ho Kim[2], Charles E. Murry[4], Michael Regnier[2].
[1]Experimental and Clinical Medicine, University of Florence, Florence, Italy, [2]Bioengineering, University of Washington, Seattle, WA, USA, [3]Medicine (Div. Cardiology), University of Washington, Seattle, WA, USA, [4]Pathology, University of Washington, Seattle, WA, USA, [5]Medicine (Div. Cardiology), University of Colorado, Denver, CO, USA, [6]University of Washington, Seattle, WA, USA.

Myosin heavy chain 7 (MYH7) mutations are associated with familial cardiomyopathies (FCM) and result in a high rate of sudden cardiac death. Human induced pluripotent stem cells derived cardiomyocytes (hiPSC-CMs) have recently shown promise as a model for studying FCM. We identified a cohort with familial cardiomyopathy (FCM) associated with a MYH7 mutation (E848G) and middle-age onset of systolic dysfunction and arrhythmias. hiPSC-CMs from patient affected (FCM-CMs) and non-affected (WT-CMs) individuals were generated from skin fibroblasts. Here we report, for the first time, contractile properties of isolated myofibrils from these cultured hiPSC-CMs for comparison using cultured cells and 3D engineered cardiac tissue (3D-ECT) constructs. Isolated myofibrils were obtained from differentiation day 20 hiPSC-CMs that were replated onto fibronectin-coated nanopatterned cover slides and matured in culture for an additional 60 days to obtain elongated and aligned myofibrils. This procedure produced hiPSC-CMs that were usually > 100+ μm in length. hiPSC- FCM-CMs and WT-CMs were harvested and skinned in a rigor solution containing 1% Triton and contractile properties of single or small bundles of myofibrils were measured in a custom built apparatus with rapid solution switching capabilities. During maximal calcium activation FCM-CM myofibrils produced approximately half the amount of force of WT-CM myofibrils, but preliminary data suggests no differences in the kinetics of force development or relaxation. This compares well with 50 day cardiomyocytes plated on nanopatterned surfaces or seeded into 3D-ECT constructs, where shortening and force (respectively) of FCM-CMs was much less than for WT-CMs, with no difference in calcium transient amplitudes. We speculate this early stage contractile deficit may contribute to disease development and conclude hiPSC-FCM-CMs can be a viable model for mechanical studies of cardiomyopathies in vitro.

1008-Plat

Modulation of Cardiac Twitch Dynamics by the Troponin I Inhibitory Region

Yasser Aboelkassem[1], Jordan Bonilla[2], **Stuart G. Campbell**[1].
[1]Biomedical Engineering, Yale University, New Haven, CT, USA, [2]Electrical Engineering, California Institute of Technology, Pasadena, CA, USA.

We have created a computational model of cardiac thin filament regulation that includes a representation of the troponin I inhibitory region (or inhibitory peptide, IP) and its binding interactions with actin. According to a canonical view of thin filament activation, IP-actin binding prevents movement of tropomyosin out of its blocked position under low Ca^{2+} conditions. Ca^{2+} binding to troponin C (TnC) causes dissociation of the IP from actin, and permits tropomyosin transition. Instead of assuming that IP-actin interactions are infinitely strong in the absence of Ca^{2+}, our model allows some spontaneous IP-actin dissociation. We have used the energetic cost of Ca^{2+}-free dissociation (ΔG) as a free parameter to determine whether the model can recapitulate changes to the IP. For instance, lowering ΔG while keeping all other model parameters constant increases the Ca^{2+} sensitivity of steady-state force in model simulations. These model results closely resemble experiments in which the IP is mutated (T144P; Tachampa et al., Circ Res 101:1081, 2007). We hypothesize that alterations to the IP in the form of cardiomyopathic mutations or phosphorylation have the ability to tune the dynamic Ca^{2+} sensitivity of cardiac muscle, altering the magnitude and time course of twitches. Twitch simulations demonstrate that lowering ΔG from infinity to 6.75 kJ/mol increases the magnitude and duration of contraction by 17 and 20%, respectively. These results suggest that twitch dynamics can be modified substantially by the energy of IP-actin binding. They further suggest that the model can be used to explore the effects of IP mutations and posttranslational modifications.

Platform: Protein Lipid Interactions II

1009-Plat

An Unusual Membrane-Protein Topology for Sensing Bilayer Thickness and Triggering Bacterial Biofilm Formation

Sandro Keller, Jana Broecker, Sebastian Fiedler, Katharina Gimpl, Martin Textor.
Molecular Biophysics, University of Kaiserslautern, Kaiserslautern, Germany.

We propose a new topology of a bacterial membrane protein that serves to sense changes in mechanical bilayer properties and, through interactions with a potassium ion channel, to trigger biofilm formation (1). The membrane-associated protein MstX (2) from Bacillus subtilis self-inserts into lipid bilayers in such a way that its four helices lie parallel to the bilayer plane, with two helices residing in each of the two apposing lipid headgroup regions. This topology suggests a functional role of the protein as a modular entity for sensing membrane properties such as bilayer thickness and hydration, as indicated by a combination of different optical-spectroscopic techniques probing protein structure and dynamics as a function of the effective hydrophobic diameter of the membrane core. Accordingly, increasing membrane thickness or decreasing membrane hydration results in a loosening of the helical-bundle structure of MstX, which, through physical but noncovalent contacts, affects the open probability or the single-channel conductance of YugO, a hitherto uncharacterised potassium ion channel essential for biofilm formation that is encoded in the same bicistronic operon as MstX (3). Leakage of potassium ions through YugO finally initiates known signal transduction cascades that result in the derepression of a set of genes required for biofilm formation.

References:
(1) Broecker et al. J. Am. Chem. Soc. 2014, accepted, DOI 10.1021/ja5064795
(2) Roosild et al. Science 2005, 307, 1317
(3) Lundberg et al. PLoS One 2013, 8, e60993

1010-Plat

Insights into the Specificity of Neisserial Opa Protein Interactions with Human Receptors

Jennifer N. Martin[1], Ryan H. Lo[1], Alison K. Criss[2], Linda Columbus[1].
[1]Chemistry, University of Virginia, Charlottesville, VA, USA, [2]Microbiology, Immunology and Cancer Biology, University of Virginia School of Medicine, Charlottesville, VA, USA.

Pathogenic *Neisseria gonorrhoeae*, the causative agent of gonorrhea, possess a family of outer membrane proteins referred to as opacity-associated (Opa) proteins. These Opa proteins are β-barrel outer membrane proteins that bind to human host cell receptors, inducing engulfment of the bacterium. To date, there have been over 300 distinct *opa* alleles sequenced. The differences in sequence have stemmed primarily from recombination events, and are most pronounced in two regions of the protein in the second and third extracellular loops (termed hypervariable regions, HV1 and HV2). These HV regions are responsible for determining receptor specificity. The most abundant Opa family engages human CEACAM receptors (carcino-embryonic antigen-like cellular adhesion molecules). While the Opa protein family has conserved structural elements, the molecular determinants of the receptor interactions are unknown. We