myosin subfragment 1 (M2B-S1) was achieved through the generation of recombinant adenovirus with subsequent infection in the C2C12 embryonic muscle cell line. A tetracysteine site, which enables site-specific labeling of the fluorescein biarscenical hairpin-binding dye (FlAsH), was introduced in the upper 50 kDa (U50) region (residues 316-321) corresponding to the previously utilized site in myosin V. Key conformational changes in the myosin motor, including those associated with nucleotide and actin binding, can be examined by fluorescence resonance energy transfer (FRET) using M2B-S1 FlAsH and fluorescent nucleotides or IAEDANs-labeled actin. We demonstrate the enzymatic properties of M2B-S1 FlAsH are similar to unlabeled M2B-S1, and both are capable of moving actin filaments in the *in vitro* motility assay. We utilized FRET between M2B-S1 FlAsH and Cy3ATP to examine structural changes in the U50 domain. The kinetics of structural changes were examined during the ATP-binding and actin-activated product release steps. We also designed a system to study structural changes in the force-generating lever arm using FlAsH labeling at the N-terminus of a human β-cardiac myosin construct containing a C-terminal GFP (M2B-GFP). The spectroscopic approach can be used as a tool to study the effects of hypertrophic cardiomyopathy (HCM) mutations on myosin structure or to study the impact of drugs that alter myosin motor activity.

2840-Pos Board B532

Differences in Activation and Relaxation Kinetics of Human Fetal Skeletal and Cardiac Myofibrils

Alice Ward Racca[1], Anita E. Beck[2], Michael J. Bamshad[2], Michael Regnier[1].
[1]Bioengineering, University of Washiington, Seattle, WA, USA, [2]Pediatrics, University of Washiington, Seattle, WA, USA.

Embryonic (*MYH3*), perinatal (*MYH8*), and alpha (*MYH6*) myosin heavy chains are predominantly expressed during prenatal development of human skeletal and cardiac muscle and may be upregulated following injury. To determine how mutations in these genes affect performance, development, and injury recovery, it is important to understand the contractile properties of the human fetal muscle in which these isoforms are dominant. However, information on developing human skeletal and cardiac muscle is limited. We previously reported that human fetal skeletal muscle from a single fetus of ~16 weeks gestation had slower crossbridge cycling myosin compared to adult rabbit psoas myosin (*in vitro* motility measurements) and slower myofibril activation & relaxation kinetics accompanying weaker force production than adult human skeletal myofibrils (Racca *et al*. (2013) *J.Phys* 591:3049-3061). Here we characterized and compared the contraction and relaxation properties of human fetal skeletal (HFS) and cardiac (HFC) muscle from fetuses of ~16 weeks gestation using myofibril mechanics techniques. HFC muscle was isolated and used fresh, while HFS muscle was flash-frozen in liquid nitrogen prior to use. The HFC myosin propelled unregulated F-actin filaments 49% slower than HFS myosin (HFC:$1.66 \pm 0.13 \mu m \cdot s^{-1}$; HFS:$2.64 \pm 0.02 \mu m \cdot s^{-1}$) (2mM ATP, 30°C). During maximal calcium activation (15°C), HFC myofibrils produced more force (F_{MAX}= $10.5 \pm 3.6 mN/mm^2$) than HFS ($5.9 \pm 1.2 mN/mm^2$), at slightly slower rates (HFC:k_{ACT}=$0.46 \pm 0.09 s^{-1}$; HFS:$0.66 \pm 0.10 s^{-1}$). The initial (slow) phase of relaxation was slightly longer in duration (HFC:$t_{REL,slow}$=203 ± 27ms; HFS:174 ± 13ms), and slower in slope (HFC:$k_{REL,slow}$=$0.26 \pm 0.03 s^{-1}$; HFS:$0.59 \pm 0.15 s^{-1}$), indicating slower crossbridge release which may also be influenced by troponin regulation. The secondary (fast) phase of relaxation was similar (HFC:$k_{REL,fast}$=$1.8 \pm 0.2 s^{-1}$; HFS:$1.5 \pm 0.2 s^{-1}$). Research to characterize the contractile properties of early development muscle and myosin heavy chain isoforms is ongoing, and may elucidate their role in development and muscle repair. Supported by F31AR06300(A.R.), 5K23HD057331(A.B.), HD048895(M.B.,M.R.).

2841-Pos Board B533

Improved Loaded In Vitro Motility Assay and Actin Filament Tracking Software Delineates the Effect of Hypertrophic and Dilated Cardiomyopathy Mutations on the Power Output of Cardiac Myosin

Tural Aksel, Masataka Kawana, Arjun Adhikari, Shirley Sutton, Kathleen Ruppel, James Spudich.
Biochemistry, Stanford University, Stanford, CA, USA.

Hypertrophic (HCM) and dilated (DCM) cardiomyopathies are important causes of heart failure, arrhythmia, and sudden cardiac death. HCM is the most common heritable cardiovascular disorder, affecting 1 in 500 individuals, and is caused primarily by mutations that alter proteins of the cardiac sarcomere. Sarcomeric protein mutations are an increasingly recognized cause of familial DCM as well. HCM is associated with severe thickening of the left ventricular wall, preserved/increased systolic (contractile) and impaired diastolic (relaxation) function of the heart. DCM hearts have dilated left ventricular chambers and suffer from inadequate systolic activity. More than 300 point mutations in beta-cardiac myosin are associated with HCM or DCM. It is believed that HCM and DCM mutations increase and decrease respectively the power output of cardiac myosin which leads to a cascade of downstream signaling that gradually leads to the disease phenotype. To test this hypothesis it is essential to measure the power output of all HCM/DCM mutants in a high throughput manner. To quantitatively measure the load dependent myosin power output, we first improved the loaded in vitro motility assay (LIMA) by replacing load generating molecule alpha-actinin with alpha-catenin, which was generously provided by James Nelson at Stanford University. For accurate-and-fast data analysis, we developed a software called FAST that runs over parallel CPUs. Our initial results indicate that two HCM mutants (R719W, R403Q) have higher power output than the wildtype cardiac myosin. FAST-LIMA will be an essential tool for screening drugs that revert the effects of HCM/DCM mutations on the power output of beta-cardiac myosin.

2842-Pos Board B534

Modulation of the Cardiac Sarcomere by a Small Molecule Agent MYK0000461: A Potential Therapeutic for the Treatment of Genetic Hypertrophic Cardiomyopathies

Hector M. Rodriguez[1], Stephanie Whitman-Cox[1], Raja Kawas[1], Yonghong Song[2], Arvinder Sran[2], Johan Oslob[2].
[1]Biology, MyoKardia, Inc., South San Francisco, CA, USA, [2]Chemistry, MyoKardia, Inc., South San Francisco, CA, USA.

We have identified a small molecule inhibitor, MYK0000461, of the cardiac myosin ATPase. This agent was characterized in steady state and transient kinetic assays to understand its mechanism of action. MYK0000461 decreases the steady-state rate of the ATPase activity of purified bovine β-cardiac myosin subfragment-1 (S1) as well as that of bovine cardiac myofibrils, wild type and the mutant R453C of recombinant human β-cardiac myosin S1. We also find that MYK0000461 inhibits cardiac myosin selectively as compared to systems containing rabbit skeletal or chicken smooth muscle myosins Analysis of the individual steps of the chemo-mechanical cycle of cardiac myosin suggests that MYK0000461 exerts its effect by inhibiting the actin-stimulated release of phosphate, presumably by stabilizing the detached state of cardiac myosin prior to the release of phosphate. We find no evidence to suggest that MYK000461 inhibits cardiac myosin in a strongly bound state and no other steps in the chemo-mechanical cycle are affected by MYK0000461. Thus, the enzymatic step governing the weak to strong transition of S1 binding to actin is inhibited without affecting the release from the strongly bound states. This decrease in the rate of transition from the weak to strongly bound state should decrease force production and may underlie its ability to decrease cardiac contractility in cellular and *in vivo* models of cardiac function. An agent such as MYK0000461 could potentially be used to treat cardiac disorders that stem from hyper contractility such as the genetic hypertrophic cardiomyopathies (HCM). By decreasing the net force of contraction and restoring it back to normal level could potentially be useful in treating patients that suffer from this disease.

2843-Pos Board B535

Biophysical Analysis of the Putative Heart Failure Drug Omecamtiv Mecarbil

Joseph M. Muretta, Ewa Prochniewicz, David D. Thomas.
University of Minnesota, Minneapolis, MN, USA.

The small molecule omecamtiv mecarbil (OM), currently in late phase-2 clinical trials for treatment of heart failure, activates force generation by cardiac myosin. How OM increases force generation is not known. Crosslinking and molecular docking studies suggest that the compound binds to the relay/SH1/converter region of myosin, hypothesized to control the actin-activated pre to post power-stroke structural transition in the myosin catalytic and light-chain domains. These transitions drive force production by myosin. We are currently using time-resolved FRET and EPR distance measurements to detect the pre- and post-power-stroke structural states of myosin. We have performed these studies on tissue-purified β-cardiac and α-skeletal myosin, and on a recombinant cys-lite myosin II catalytic domain model from Dictyostelium discoideum. In each of the myosin isoforms, we find that OM binding increases the mole fraction of the myosin post power-stroke structural state in the presence of ATP analogs. Transient time-resolved FRET experiments, mixing FRET-labeled myosin with ATP, show that drug binding also alters the structural kinetics of the myosin recovery-stroke. Ongoing studies are investigating the