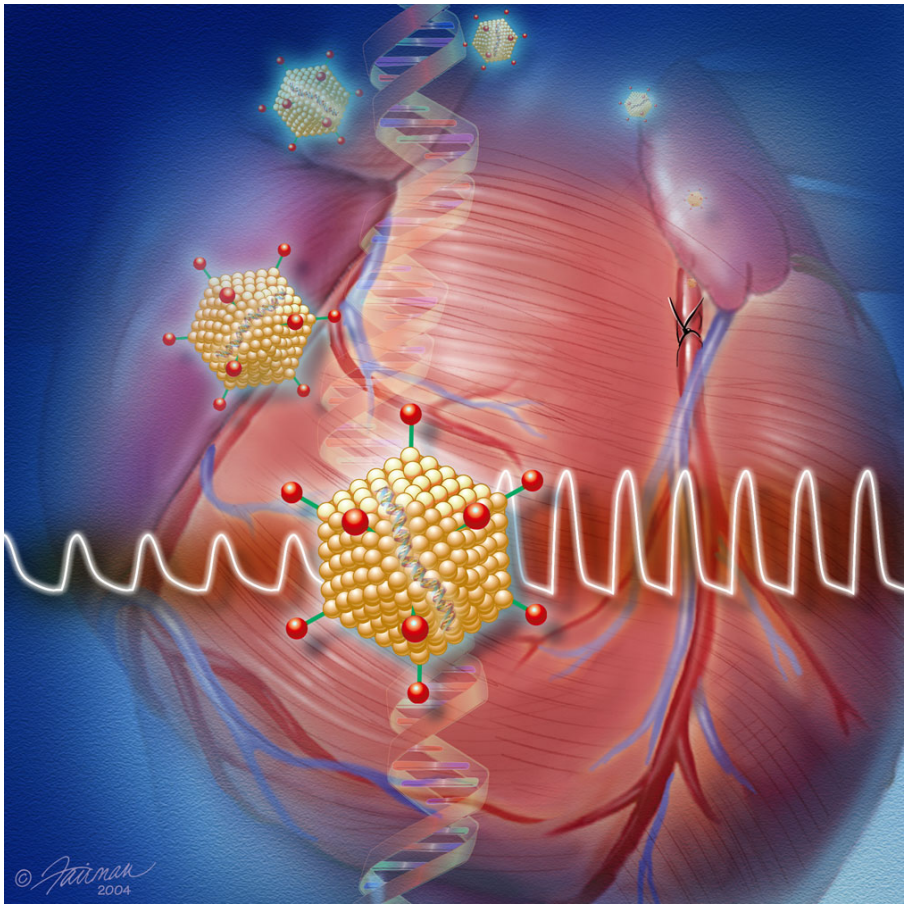
University of Minnesota

The Paul and Sheila Wellstone Muscular Dystrophy Center and NIH Training Program in Muscle Research present

The 13th Annual Minnesota Muscle Symposium



Guest lecturer

Roger Hajjar,

Professor of Cardiology, Gene and Cell Medicine
Mount Sinai School of Medicine

"Targeting calcium cycling in heart failure: from basic mechanisms to clinical trials."

Poster session and buffet lunch: 11:00 — 12:25
Lecture, q&a: 12:30 — 2:00

Contact: Bengt Svensson (svens005@umn.edu)

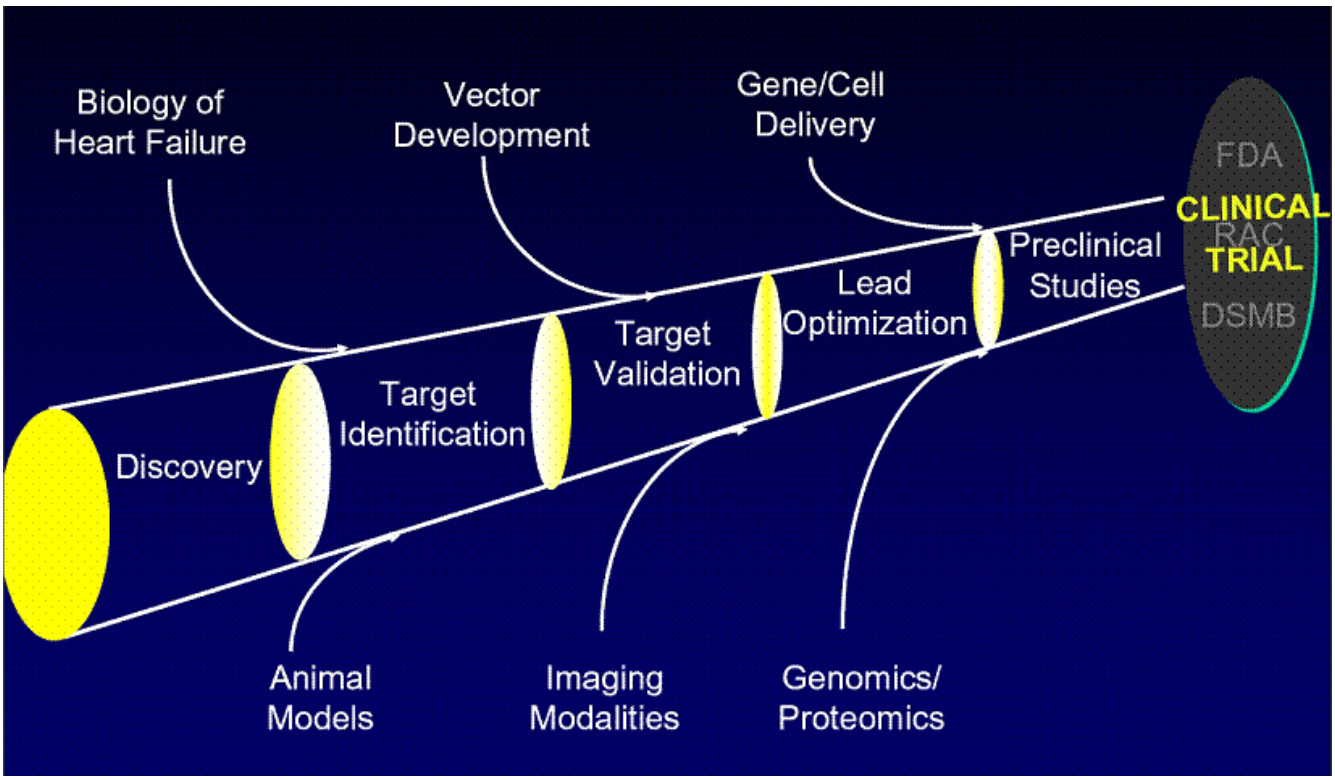
June 9, 2009
Nils Hasselmo Hall
312 Church St SE
2-101 & Atrium

Dr. Hajjar is Professor of Medicine, Division of Cardiology, Professor of Gene and Cell Medicine, and Director of the Cardiovascular Research Center, at Mt. Sinai School of Medicine, New York

Prior to coming to Mount Sinai, Dr. Hajjar was Associate Professor of Medicine at Harvard Medical School and Staff Cardiologist in the Heart Failure & Cardiac Transplantation Center at Massachusetts General Hospital. He was the director of the Cardiology Laboratory of Integrative Physiology & Imaging at Massachusetts General Hospital. He received his medical degree from Harvard Medical School and trained in Internal Medicine and Cardiology at Massachusetts General Hospital in Boston.

Dr. Hajjar leads a multidisciplinary research program in molecular cardiology, with the primary goal of developing gene therapy for treating heart failure. He has been the clear leader in this field for over a decade, most recently carrying out successfully the first human clinical trial, using an adeno-associated vector (AAV) to restore cardiac calcium pump function in human patients:

Jaski, B.E., M.L. Jessup, D.M. Mancini, T.P. Cappola, D.F. Pauly, B. Greenberg, K. Borow, H. Dittrich, K.M. Zsebo, and R.J. Hajjar, Calcium upregulation by percutaneous administration of gene therapy in cardiac disease (CUPID Trial), a first-in-human phase 1/2 clinical trial. *J Card Fail*, 2009. **15**:171-81



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Poster presentations:

Structural dynamics of the myosin relay helix resolved by DEER and time-resolved FRET
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Cerebellar dysfunction and glutamatergic deficits in murine models of spinocerebellar ataxia type 5 (SCA5)
Karen Armbrust, Xinming Wang, Takashi Obu, Timothy Ebner, and Laura Ranum

Functional Studies for MASH Gene Family in Muscle Stem Cells
Ronneil Aviles, Atsushi Asakura,

Contracture in the *Mdx* Mouse Model of Duchenne Muscular Dystrophy
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Altered Response to Mechanical Stress in Isolated Hearts of Mdx Mice
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MiR208 Prevents Phenylephrine Induced Hypertrophy in Adult Rat Cardiac Myocytes
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Development of a muscleblind-like 1 (Mbnl1) overexpression model to assess the use of MBNL1 as a potential therapeutic for myotonic dystrophy
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Role of Cytoplasmic β-actin in the Pathogenesis of Spinal Muscular Atrophy
Tom Cheever, Kevin Sonnemann and James Ervasti

The Mechanism of Cardiac Failure: Does it result from a primarily contractile problem or decreasing ATP levels?
John Foker, Nicholas Befera, Erik Strungs, James Berry, Richard Bianco

Structural characterization of FKBP interactions with RyR channels using site-directed fluorescent labeling and FRET
Razvan L. Cornea, Florentin Nitu, David D. Thomas, and Bradley R. Fruen

FRET-based mapping of calmodulin binding to the RyR1 channel
Razvan L. Cornea, Florentin R. Nitu, Katherine Kohler, David D. Thomas, and Bradley R. Fruen

Molecular Mechanisms of SCA8
Brian Gibbens, Mindy Moseley, Tao Zu

Hormone Therapy And Skeletal Muscle: A Meta-Analysis
Sarah M. Greising, Kristen A. Baltgalvis, Gordon L. Warren, and Dawn A. Lowe

Allosteric Changes in Phospholamban Structure and Dynamics Regulate Sarcoplasmic Reticulum Calcium-ATPase Function
Martin Gustavsson, Nathaniel Traaseth, Lei Shi, Kim Ha, Gianluigi Veglia

Controlling the Inhibition of the Sarcoplasmic Ca^{2+}-ATPase by Tuning Phospholamban Structural Dynamics
Kim N. Ha, Nathaniel J. Traaseth, Raffaello Verardi, Jamillah Zamoon, Alessandro Cembran, Christine B. Karim, David D. Thomas, Gianluigi Veglia

Structural Dynamics of SERCA and Phospholamban by Fluorescence Microscopy
Suzanne Haydon, Ji Li, Deborah Winters, Elizabeth Lockamy, J. Michael Autry, Seth L. Robia and David D. Thomas

Structure Function Analysis of Disease-Causing Missense Mutations in Dystrophin
Davin M. Henderson, Ann Lee, and James M. Ervasti

Transgenic over-expression of gamma-cytoplasmic actin protects against eccentric contraction-induced injury in *mdx* mice
Michele Jaeger, Kristin Baltgalvis, Daniel Fitzsimons, Dawn Lowe, James Ervasti

Molecular pathophysiology and CNS effects in mouse models of myotonic dystrophy types 1 & 2
Y.-L. Kang, X.Wang, G. Chen, J. M. Margolis, M. S. Swanson, T. J. Ebner, L. P. W. Ranum

The effects of estrogen depletion on skeletal muscle metabolism
Allison M. Kosir and Dawn A. Lowe

Stoichiometry of Dystrophin, Dystroglycan and Laminin in Skeletal Muscle
Bin Li, and James M. Ervasti

Dystrophin and utrophin have distinct effects on the microsecond dynamics of actin
Ewa Prochniewicz, Davin Henderson, Ava Yun Lin, James Ervasti and David D. Thomas

Biophysical Engineering of the PLB-SERCA Interaction for Treatment of Heart Failure
Elizabeth L. Lockamy, Bonnie M. Fedor, Christine B. Karim, Joshua Palmer, Razvan Cornea, and David D. Thomas

CCUG RNA gain-of-function effects in a conditional mouse model of myotonic dystrophy type 2 (DM2).
Jamie M. Margolis, Yuan-Lin Kang, Maurice S. Swanson and Laura P.W. Ranum

Dominant effect of histidine modified troponin to normalize SR Ca^{2+} load and rescue ischemia/reperfusion deficits in phosholamban deficient hearts.
Joshua J. Martindale, Todd J. Herron, Sharlene M. Day, Nathan J. Palpant, Joseph M. Metzger,

MR spectroscopic and kinetic investigations of the interaction of Protein Kinase A with phospholamban and phospholamban mutants
Larry R. Masterson and Gianluigi Veglia

Compensatory adaptation of protein expression in resistance-trained dystrophic mouse muscle
Jim McKeehen, Jarrod Call, Kristen Baltgalvis, and Dawn Lowe

EPR Analysis of Myosin Structural Dynamics in a Pre-power Stroke Conformation
Ryan N. Mello, Leanne J. Anderson, Andrew Thompson, Jason W. Sidabras, James S. Hyde and David D. Thomas

Generation of induced pluripotent stem cells from patient-specific FSHD myoblasts
Ramiro Nandez, Darko Bosnakovski, Mathew Struck, Nathan Zaidman and Michael Kyba

Bone Deterioration in Duchenne's Muscular Dystrophy
Susan A. Novotny, Kristen A. Baltgalvis, Gordon L. Warren and Dawn A. Lowe

Single Histidine Button in Cardiac Troponin I Sustains Heart Performance in Response to Severe Acidosis In Vivo
Nathan J. Palpant, Louis G. D'Alecy, and Joseph M. Metzger

Dystrophin is a Microtubule-Associated Protein
Kurt Prins, Jill Humston, Amisha Mehta, Evelyn Ralston and James Ervasti

Cardioprotective pretreatment for global ischemia of isolated swine hearts: assessed using Visible Heart® methodologies
Christopher D. Rolfes, Michael D. Eggen, Eric S. Richardson and Paul A. Iaizzo

Cardiac Myosin Isoform Remodeling in Duchenne Muscular Dystrophy Cardiomyopathy
Evelyne M. Houang Brent M. Berry, Mihir Pendurkar, and Osha Roopnarine

Analysis Of The Sarcolipin:Serca Regulatory Complex Using Fluorescence Resonance Energy Transfer Microscopy
John E. Rubin, J. Michael Autry, and David D. Thomas

The Effects of Tissue Bath pH During Hypoxia on an In Vitro Ischemia / Reperfusion Injury Model
Vidur Sharma, Charles L. Soule, and Paul A. Iaizzo

TAT-Utrophin crosses cell barriers to combat dystrophin deficiency
Kevin J. Sonnemann, Hanke Heun-Johnson, Amy J. Turner, Kristen Baltgalvis, Dawn Lowe, and James M. Ervasti

Structural dynamics of sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) studied by molecular simulations of site-specific labeled protein
Bengt Svensson, Deborah L. Winters, Joseph M. Autry, L. Michel Espinoza-Fonseca, Elizabeth L. Lockamy and David D. Thomas

MR Imaging of Pacing Induced Ventricular Dyssynchrony in an Isolated Human Heart
Michael D. Eggen, Michael G. Bateman, Christopher D. Rolfes, Stephen A. Howard, Cory M. Swingen, Paul A. Iaizzo

GADD45γ is a Novel Downstream Target of MyoD and Negatively Regulates Survival and Self-Renewal of Muscle Stem Cells.
Christopher Tastad, Hiroyuki Hirai, and Atsushi Asakura

Structural Dynamics of Cardiac Calcium Regulation
Kurt D. Torgersen, Christine B. Karim, Edmund Howard and David D. Thomas

Prevention of dilated cardiomyopathy by chronic infusion of the membrane sealant Poloxamer 188 in canines with muscular dystrophy
DeWayne Townsend, Immanuel Turner, Joshua Martindale, Joseph Kornegay and Joseph Metzger

Ca^{2+}-ATPase Drives a Topological Rearrangement in the Transmembrane Domain of Phospholamban as Measured by Solid-State NMR Spectroscopy
Nathaniel J Traaseth, Raffaello Verardi, Lei Shi, Martin Gustavsson and Gianluigi Veglia

Improvement of muscular dystrophy phenotypes through an increase in vasculature in *mdx* mice
Mayank Verma, Hiroyuki Hirai, Yoko Asakura and Atsushi Asakura

Designer Calcium Buffers for Enhancing Contractile and Relaxation Performance of Normal and Failing Adult Cardiac Myocytes
Wang Wang, Erik Arden, Terri Edwards, Todd J. Herron, Eric Devaney, Immanuel Turner, Jonathan Davis, James Potter and Joseph Metzger

Gene transfer of engineered cardiac troponin C for modulation of myofilament Ca^{2+} sensitivity
Lin Yang, Jen Davis and Joseph M. Metzger

Structural dynamics of the myosin relay helix resolved by DEER and time-resolved FRET

Roman Agafonov, Sarah Blakely, Igor V. Negrashov, Margaret A. Titus, David D. Thomas, Yuri E. Nesmelov
University of Minnesota, Minneapolis, Minnesota, USA

We have used DEER (double electron-electron resonance) and TR-FRET (time-resolved fluorescence resonance energy transfer) to detect structural transitions within the myosin II relay helix. Major structural changes during the myosin II ATPase cycle take place in the force-generating domain. Crystal structures show that the converter domain, the relay helix, and SH1 helix have different conformations in the proposed pre- and post- powerstroke structural states of myosin. In the present study, we focus on the relay helix as a crucial structural element involved in coupling between the force-generating domain and the nucleotide-binding pocket. Cysteine mutations were introduced into a Cys-lite construct of *Dictyostelium discoideum* (*Dicty*) myosin in the lower 50k domain and the C-terminal end of the relay helix. These constructs were selectively modified with MSL/MSL or IAEDANS/DABCYL pairs, and the distance between probes was measured (a) in different myosin conformations trapped with nucleotides or nucleotide analogs and (b) after rapid mixing of myosin with ATP. In the latter experiment, designated transient time-resolved FRET, **$(TR)^2$FRET**, we use a novel instrument that can **acquire a complete subnanosecond time-resolved fluorescence decay 10,000 times per second**, during the transient phase of the ATPase reaction. Two conformations of the relay helix (with distinct probe-to-probe distances, presumably corresponding to the **straight** and **bent** states of the helix) were resolved. Observed distances were in good agreement with existing crystal structures, but at least two distinct structural states were present in certain biochemical states. The mole fraction of the "bent" conformation was higher with post-hydrolysis analogs (ADP.V_i, ADP.AlF_4) bound at the active site. Kinetic experiments identified the same conformational states and showed a transition from the straight state of the helix to the bent state upon addition of ATP, allowing us to determine the rate of the conformational change within the helix. Our results reveal time-resolved rearrangements within a single subdomain of myosin and provide insights into the coupling between binding of ATP and structural changes in the force-generating region.

Cerebellar dysfunction and glutamatergic deficits in murine models of spinocerebellar ataxia type 5 (SCA5)

Karen Armbrust[1,2], Xinming Wang[3], Takashi Obu[1,2], Timothy Ebner[3], Laura Ranum[1,2]
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(2) Institute of Human Genetics, University of Minnesota, Minneapolis, MN.
(3) Neuroscience, University of Minnesota, Minneapolis, MN.

The spectrin repeat is a characteristic triple-alpha-helical bundle found in cytoskeletal proteins such as dystrophin, utrophin, and spectrin. Two mutations that produce in-frame deletions within the third spectrin repeat of beta-III spectrin cause spinocerebellar ataxia type 5 (SCA5), a slowly progressive, autosomal dominant neurodegenerative disease that primarily affects the cerebellum. Analysis of human SCA5 cerebellar autopsy tissue shows that the glutamate transporter EAAT4 and the glutamate receptor GluRδ2 fractionate abnormally, which suggests that SCA5 pathogenesis may involve abnormal glutamatergic neurotransmission and excitotoxic changes. To test this hypothesis and to elucidate other potential mechanisms of disease pathogenesis we have developed two transgenic murine models of SCA5 which express mutant beta-III spectrin in cerebellar Purkinje cells. Behavioral studies with a 3xFLAG-tagged SCA5 murine model and a second conditional murine model that drives expression of untagged beta-III spectrin show that overexpressing mutant beta-III spectrin in cerebellar Purkinje cells causes cerebellar dysfunction. Histologic analysis of the 3xFLAG-tagged SCA5 murine model shows that the SCA5 mutation alters the Purkinje cell distribution of the mutant β-III spectrin protein itself. Further studies with the conditional tet-regulated mice show that overexpression of untagged mutant beta-III spectrin alters the localization of the glutamate transporter EAAT4 and the metabotropic glutamate receptor mGluR1α and produces a concomitant deficit in mGluR1 function. These findings suggest that abnormalities in perisynaptic glutamatergic transmission may contribute to SCA5 pathogenesis.

Functional Studies for MASH Gene Family in Muscle Stem Cells

Ronneil Aviles, Atsushi Asakura,
Department of Neurology

Duchenne Muscular Dystrophy (DMD) is a disorder in which absence of the protein dystrophin results in decreasing muscle mass and progressive loss of muscle function. It is possible that muscle satellite cells, stem cells for skeletal muscle, transplanted into DMD patients will have the potential to replace these damaged tissues. The satellite cells are found in skeletal muscle and are characterized by the expression of muscle specific basic-helix-loop-helix (bHLH) transcription factors MyoD and Myf5. In normal adult muscle, satellite cells are mitotically quiescent but activated by muscle damage to expand their progeny of myogenic precursor cells prior to formation of muscle fibers. Importantly, a continuous supply of satellite cells for the damaged muscle fibers is essential for successful long term therapy. Recently, Dr. Asakura lab cloned new type of bHLH transcription factors from satellite cell cDNA, termed Mouse Achaete Scute Complex Homolog 3, 4 and 5 (MASH3, MASH4 and MASH5). MASH4 is expressed in quiescent satellite cells while MASH3 and MASH5 are expressed in differentiating satellite cells. The functions for these three genes on satellite cells remain to be found. Since bHLH genes play essential roles in many different cell-type determination and differentiation, MASH3, MASH4 and MASH5 genes must possess important functions on satellite cells. The luciferase reporter gene assay demonstrates that MASH3, MASH4 and MASH5 cannot execute E-box mediated transcriptional activation but rather suppress other bHLH transactivation. This result suggests MASH3, MASH4 and MASH5 may play an important role in suppression of myogenic differentiation and in maintenance of quiescent status of satellite cells through suppression of MyoD and Myf5. Furthermore, over-expression for MASH3, MASH4 or MASH5 may convert myoblasts to more primitive stem cells which would be beneficial to cell therapy for muscular dystrophy.

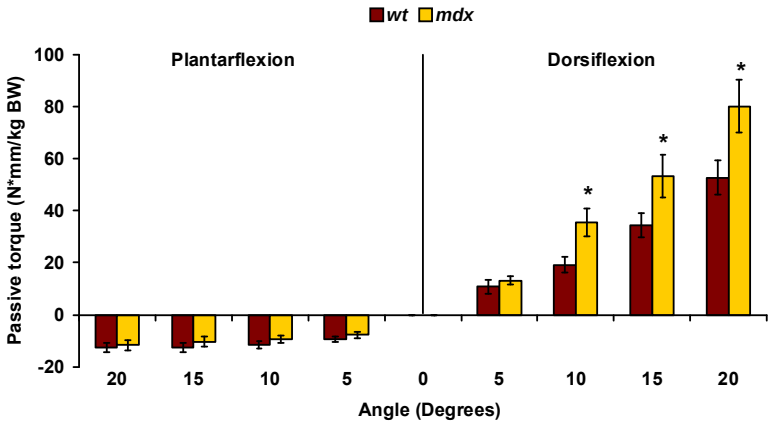
Contracture in the *Mdx* Mouse Model of Duchenne Muscular Dystrophy

Kristen A. Baltgalvis[1], Michael Garlich[2], Jarrod A. Call[2], Lisa Dorsey[2], and Dawn Lowe[2]
[1] Department of Biochemistry, Molecular Biology, and Biophysics, and [2] Department of Physical Medicine and Rehabilitation, University of Minnesota

Muscle contractures are one of the most debilitating aspects of Duchenne Muscular Dystrophy (DMD) for children, because it makes walking increasingly difficult as the disease progresses. A gradual decrease in ankle range of motion (ROM) concurrent with false hypertrophy of the gastrocnemius muscle typifies the progression of contractures in boys with DMD as they lose the ability to walk efficiently. A mouse model of DMD, the *mdx* mouse, has been used extensively in disease research, but the presence of contractures has not been investigated. The primary purpose of this study was to develop measurements in order to determine the presence or absence of contractures in the hindlimb of the *mdx* mouse. Range of motion, *in vivo* passive torque (Figure 1), and *in vivo* active torque about the ankle were measured on 8 wildtype (*wt*) and 6 *mdx* mice over the course of 12 wk. *Mdx* mice had ~10^{o} less dorsiflexion compared to wildtype mice. Passive torque was also about 50% greater in *mdx* mice compared to wild-type mice as the ankle was pushed into 20° of dorsiflexion (Figure 2). The passive-to-active torque ratio was also higher in *mdx* mice relative to *wt* mice. Together, these results indicate that *mdx* mice have plantarflexion contractures similar to those seen in children with DMD. Future studies can be designed to investigate treatments preventing the development of contractures in the *mdx* mouse and to delineate the relationship between contractures and muscle weakness.

Figure 1. Examples of passive range of motion about the ankle in the *mdx* mouse. A. 20° of plantarflexion. B. Neutral starting position at 0°. C. 20° of dorsiflexion.

Figure 2. Passive torque of the *mdx* mouse increases as the ankle is moved into dorsiflexion.



Altered Response to Mechanical Stress in Isolated Hearts of Mdx Mice

Matthew Barnabei[1, 2], Joseph Metzger[2]

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[2]Department of Integrative Biology and Physiology, University of Minnesota, Minneapolis

Duchenne muscular dystrophy is a lethal inherited disease affecting 1 in 3500 males. Although historically thought of as a skeletal muscle disease, 20% of Duchenne deaths are due to heart failure. Previously, our lab has shown that isolated mdx myocytes show enhanced susceptibility to stretch-induced injury. Specifically, mdx myocytes are abnormally stiff and show increased membrane permeability, unregulated calcium influx and contracture following stretch within a normal physiological range. To study the effects of dystrophin in the response to mechanical stress in an intact, beating heart, isolated mouse hearts were subjected to mechanical stress by incrementally increasing the volume, and subsequently, the pressure, within the left ventricle. Surprisingly, as left ventricular volume was increased, the resultant increase in left ventricular end diastolic pressure was blunted in mdx mice compared to wild-type controls. This difference was not affected by perfusate calcium concentration and mdx mice showed no difference in systolic function through the volume challenge compared to wild-type mice. These findings support dystrophin's role in stabilizing the sarcolemma in response to the mechanical stress associated with contraction and relaxation and shed light on how dystrophin deficiency affects the mechanical function of the heart.

MiR208 Prevents Phenylephrine Induced Hypertrophy in Adult Rat Cardiac Myocytes

Fikru B Bedada, Erik Arden, Joseph M. Metzger

Department of Integrative Biology and Physiology, University of Minnesota, Minneapolis, MN 55455, USA

MicroRNAs (miRNAs) are short endogenous RNAs that act as post-transcriptional regulators of gene expression by base-pairing with their target mRNAs. Recent study has described miR-208 as cardiac-specific regulator of ßMHC expression in response to stress. Deletion of the miR-208 in mice caused inhibition of ßMHC expression and protection against hypertrophy in response to TAB and calcineurin over-expression. Our study examines the effect of exogenously administered miR208 in normal and alpha adrenergic agonist stimulated adult rat ventricular cardiac myocytes. We show that Phenylephrine (PE) caused a significant change in myocyte structure, namely sending out lamelipodia, increase in size with mean ± SEM (2638 ± 96.66, 2561 ± 169.3, 3391 ± 132.5 and 2440 ± 103.3, $p<0.05$) respectively for, miR-208, miR-208+PE, PE and wild type myocytes. Typically, there were areas of deposition of new myofilaments detected by alpha actinin staining in the lamelipodia of the myocyte which is also co-localized with the Non-Muscle Myosin IIB (NMMIIB). Interestingly, miR208 fully blocked this cellular remodeling and abrogated the hypertrophic effect observed with PE. Consistent with this, miR-208 negatively regulated expression of GATA4 and calcineurin which are key players of cardiac hyperthrophy. Further, miR-208 prevented cardiac remodeling induced by GATA4 and calcineurinAß expressing viruses. Functionally, PE treated cardiac myocytes showed poor mechanical contraction and relaxation which is corrected by miR-208. Together, miR-208 prevents myocardial hypertrophy when expressed in rat ventricular cardiac myocyte prior to the application of PE. These observations suggest that miR208 is involved in a complex pathway regulating hypertrophy. Thus miR208 may hold promise for the design of therapies that prevent hypertrophy and consequent heart failure. [This work was supported by NIH]

Development of a muscleblind-like 1 (Mbnl1) overexpression model to assess the use of MBNL1 as a potential therapeutic for myotonic dystrophy

Christopher M. Chamberlain and Laura P.W. Ranum

Department of Genetics, Cell Biology and Development and Institute of Human Genetics, University of Minnesota, Minneapolis, MN, USA

Myotonic Dystrophy (DM) is the most common form of adult muscular dystrophy. The multisystemic clinical features of DM include myotonia, iridescent cataracts, muscle weakness, cardiac arrhythmia, testicular failure, insulin insensitivity, and cognitive impairment. This autosomal dominant disorder is caused by either a CTG trinucleotide repeat expansion in the 3' UTR of dystrophia myotonica protein kinase (DMPK) in DM type 1 (DM1) or a CCTG tetranucleotide repeat expansion in the first intron of zinc-finger 9 (ZNF9) in DM type 2 (DM2). Several lines of evidence support a novel RNA gain of function mechanism in which CUG or CCUG transcripts aggregate in the nuclei and bind with RNA binding proteins: 1) DM1 and DM2 share clinical features, yet the genes involved are functionally distinct, 2) expression of DMPK and ZNF9 proteins is not affected by the expansion, 3) CUG and CCUG transcripts aggregate as nuclear foci and co-localize with RNA binding proteins such as muscleblind-like 1 (MBNL1), 4) mice expressing long CUG repeats share features of DM, and 5) mice lacking MBNL1 share features of DM1. MBNL1 is involved in developmental regulation of alternative splicing. DM patients and mouse models show significant alternative splicing of MBNL1 targets. These results are explained by a model where MBNL1 is sequestered by CUG or CCUG RNA foci and is not capable of binding its endogenous splice targets. Adeno-associated viral delivery of MBNL1 to DM mouse tibialis anterior muscle results in decreased myotonia, improved splicing, and diffusion of MBNL1 throughout the nuclei. To validate MBNL1 as a potential therapy for DM, we must show that MBNL1 overexpression has little/no toxic effects. ***The hypotheses of this project are: 1) Long-term systemic overexpression of MBNL in mice will not have toxic effects, and 2) long-term, systemic MBNL overexpression in a DM mouse model will alleviate the DM-like phenotype.*** We have engineered transgenic mice with MBNL1 under the regulation of a chicken beta-actin promoter with a CMV (cytomegalovirus) enhancer. Transgenic lines displaying MBNL1 upregulation will be tested for viability, muscle histology, RNA splicing, as well as potential CNS defects. These mice will be bred with a DM mouse model to generate doubly transgenic DM/MBNL-OE mice. Doubly transgenic mice will be tested for histological improvements, splicing changes, RNA foci, and muscle force production to determine the extent of DM phenotype rescue. These experiments will test the central hypotheses of the project. An essential component of establishing a potential therapy for DM or any disorder is to analyze the potential toxicity of the therapy. This will be the first test of ubiquitous overexpression of MBNL1 as a potential therapy for myotonic dystrophy. **The goal of this project is to determine the toxicity of MBNL1 in order to further elucidate its potential as a therapy for myotonic dystrophy.**

Role of Cytoplasmic β-actin in the Pathogenesis of Spinal Muscular Atrophy

Tom Cheever, Kevin Sonnemann and James Ervasti
Biochemistry, Molecular Biology and Biophysics Department

Spinal muscular atrophy (SMA) is an autosomal recessive disorder that results in the loss of lower α-motor neurons and skeletal muscle atrophy. Although the gene responsible for this disease has been identified as Survival of Motor Neuron 1 (*Smn1*), how loss of the ubiquitously expressed SMN protein leads to selective motor neuron and muscle pathology is unknown. SMN predominantly localizes to cell bodies where it participates in snRNP biogenesis. However, recent studies have identified SMN throughout neurites in neurons both *in vitro* and in *vivo*, raising the possibility that SMN may have additional, neuron specific functions. It has been hypothesized that in neurons, SMN also participates in the transport and localization of specific mRNAs to the growth cones that cap developing neurites. One such mRNA trafficked by SMN was recently identified as cytoplasmic β -actin, whose localization and local translation is essential for neurite elongation and growth cone guidance *in vitro*. Interestingly, cultured motor neurons from a mouse model of SMA have decreased β-actin at growth cones, resulting in defects in axon elongation and growth cone structure, as well as delocalized voltage-gated calcium channels. The mislocalization and misregulation of β-actin could thus hinder motor neuron development and muscle innervation, leading to motor neuron death and disease. To test the hypothesis that β-actin is a downstream mediator of SMA pathogenesis, we are generating motor-neuron specific β-actin knock-out mice and characterizing motor neuron development and function through physiological and histological analysis. These studies will elucidate the role of β-actin in the pathogenesis of SMA.

The Mechanism of Cardiac Failure
Does it result from a primarily contractile problem or decreasing ATP levels?

John Foker, Nicholas Befera, Erik Strungs, James Berry, Richard Bianco

Divisions of Cardiothoracic Surgery and Experimental Surgical Sciences at U of M

Background

Many abnormalities have been described once heart failure is established, however, the basic mechanism producing its onset and progression is unknown. The rat myocardial infarction (MI) model allows failure to be studied in normal muscle. Following the MI, the remaining (remote) myocardium (RM) must take over the entire load and maintain an adequate cardiac output. Unfortunately, the RM (in rats and humans) often goes on to fail, with apoptosis and remodeling. The two most general causes would be a primary failure of the contractile apparatus, or the energy supply is not able to keep up with demand and [ATP] slowly falls. Because the resulting AMP is catabolized, ATP levels have little chance to recover. We tested which mechanism was operative by giving ribose, which is not used as a fuel, and whose only action is to greatly stimulate nucleotide synthesis, especially AMP (ATP). If decreased [ATP] were the primary problem, ribose should slow the development of failure.

Methods

Lewis rats (N=7 for each group) had central venous infusion pumps placed and 1 day later, the left anterior descending artery was ligated just distal to the circumflex artery, resulting in infarction of 35% of the LV. Ribose was infused for 2 weeks at 100mg/k/d. Echo analysis was carried out at 0, 2, and 4 weeks (2 weeks beyond the infusion).

Results

	Weeks			
		0	**2**	**4**
Ejection Fraction (%)	Ribose	60±14	**56±13***	**46±8.0***
	Control	65±14	**29±3**	**26±4.6**
LV-SD (cm)	Ribose	0.39±0.06	**0.47±0.22***	**0.64±0.09***
	Control	0.40±0.03	**0.73±0.10**	**0.95±0.07**
RM-WT (cm)	Ribose	0.13±0.02	0.18±0.12	**0.13±0.03***
	Control	0.13±0.03	0.10±0.01	**0.10±0.01**
LV-DV (mL)	Ribose	0.22±0.03	**0.38±0.08***	0.57±0.24
	Control	0.22±0.06	**0.61±0.11**	0.71±0.25

LV-SD=systolic diam, LV-DV=diastolic vol, WT=wall thickness; *p<0.05 vs. control

In this model, MI produced LV dysfunction and dilation with thinning of the RM. Ribose reduced RM and LV dysfunction following MI as shown.

Conclusions

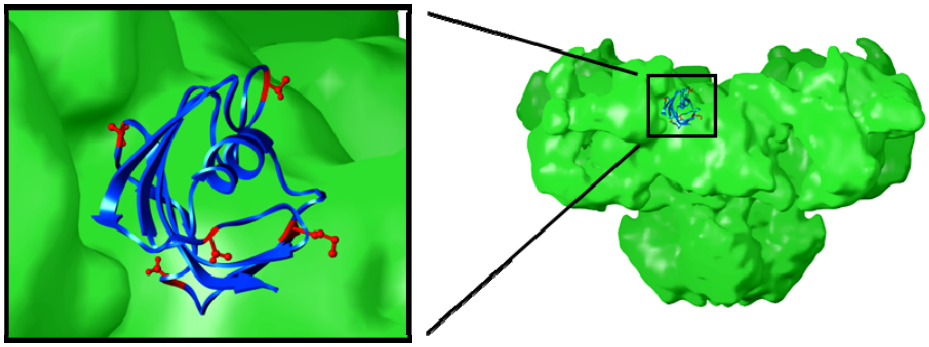
1) Ribose significantly reduced the RM dysfunction (higher EF, less dilation, and reduced RM wall thinning) following a MI.

2) Ribose was continued for only 2 weeks and some functional deterioration occurred by 4 weeks suggesting the energy supply/demand imbalance remained.

3) These data suggest that the mechanism of failure is a decrease in ATP levels, not a primary breakdown of the contractile apparatus.

Structural characterization of FKBP interactions with RyR channels using site-directed fluorescent labeling and FRET

Razvan L. Cornea, Florentin Nitu, David D. Thomas, and Bradley R. Fruen*
Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota



The 12 kDa FK506-binding proteins (FKBP12 and FKBP12.6) are regulatory subunits of ryanodine receptor (RyR) Ca^{2+} release channels. To investigate the structural basis of FKBP interactions with the RyR1 and RyR2 channel isoforms, we used site-directed fluorescent labeling, direct binding measurements, and fluorescence resonance energy transfer (FRET). Single cysteines were introduced at five positions distributed over the surface of FKBP12.6. Fluorophore attachment at four positions (14, 32, 49, and 85) did not affect high-affinity binding of FKBP12.6 to the RyR1, suggesting that these positions are removed from the major RyR1 binding interface. By comparison, fluorophore attachment at one position (41) resulted in decreased FKBP12.6 binding affinity. The orientation of FKBP12.6 bound to the RyR1 and RyR2 was examined by measuring FRET from the different positions on FKBP12.6 to an acceptor attached within the RyR CaM subunit. FRET was dependent on the position of fluorophore attachment on FKBP12.6, and the rank order of FRET efficiency from the different positions was the same whether FKBPs were bound to the RyR1 or RyR2 isoform (position 49 > 85 ≥ 14 > 32). These results demonstrate that in binding either the RyR1 or RyR2 channel, FKBP12.6 is oriented such that position 49 is nearest to the RyR CaM subunit. These results are consistent with a recent structural model of FKBP12 binding based on RyR1 cryo-EM, and point to FKBP loop 39-46 as a key component of the RyR binding interface. Newly-characterized fluorescent FKBPs offer a powerful approach for targeting spectroscopic probes to RyR channels.

FRET-based mapping of calmodulin binding to the RyR1 channel

Razvan L. Cornea, Florentin R. Nitu, Katherine Kohler, David D. Thomas, and Bradley R. Fruen*
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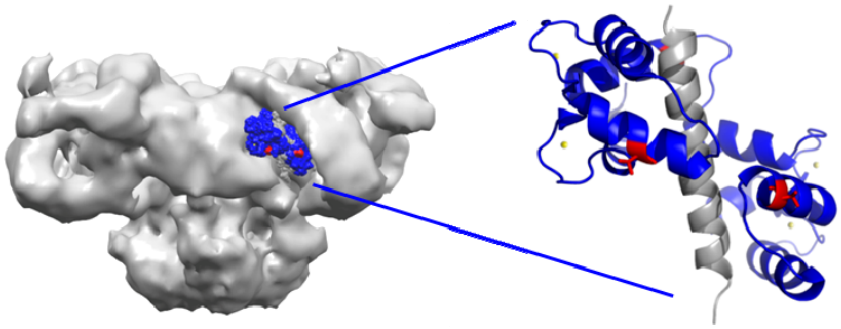

Calmodulin (CaM) functions as a regulatory subunit of ryanodine receptor (RyR) channels, modulating channel activity in response to changing [Ca^{2+}]. To investigate the structural basis of CaM regulation of the RyR1 isoform, we used site-directed labeling of channel regulatory subunits and fluorescence resonance energy transfer (FRET). Donor fluorophore was targeted to the RyR1 cytoplasmic assembly by preincubating sarcoplasmic reticulum membranes with a fluorescent FK506-binding protein (FKBP), and FRET was determined following incubations in the presence of fluorescent CaMs in which acceptor fluorophore was attached within the N-lobe, central linker, or C-lobe. Results demonstrated strong FRET to acceptors attached within CaM's N-lobe, whereas substantially weaker FRET was observed when acceptor was attached within CaM's central linker or C-lobe. Surprisingly, Ca^{2+} evoked little change in FRET to any of the three CaM domains. Donor-acceptor distances derived from our FRET measurements provide insights into CaM's location and orientation within the RyR1 3D architecture and the conformational switching that underlies CaM regulation of the channel. These results establish a powerful new approach to resolving the structure and function of RyR channels.

Molecular Mechanisms of SCA8

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Myotonic Dystrophy type 1 (DM1) is a multisystemic disease caused by a CTG•CAG expansion in the 3' untranslated region (UTR) of the DMPK (myotonic dystrophy protein kinase) gene. Patients with DM1 have a multitude of clinical features including myotonia, myopathy, iridescent cataracts, cardiac arrhythmias, and a specific set of serological changes. Interestingly, the DM1 CTG•CAG repeat expansion mutation has recently been shown to be transcribed in both the CTG and CAG directions. Attempts to determine the mechanism of DM1 have generated strong support for an RNA-toxicity model in which CUG expansion transcripts cause misregulation of splicing factors such as muscleblind (MBNL1) and CUG binding protein 1 (CUGBP1). Dysregulation of these splicing-factors, and in particular the loss of MBNL1, results in the aberrant splicing of several pre-mRNAs including the skeletal muscle chloride channel (CLCN1) and insulin receptor (IR). These splicing abnormalities are believed to underlie many of the multisystemic disease features.

While studying another CTG•CAG repeat disorder, Spinocerebellar Ataxia Type 8 (SCA8), our lab has shown that the SCA8 gene locus is expressed bi-directionally resulting in the production of two different repeat containing transcripts, ATXN8OS and ATXN8 (Nature Genetics 38:758-769). The ATXN8OS transcript contains a large CUG repeat tract. Research on DM1 has demonstrated that long CUG repeat transcripts can cause disease via an RNA gain-of-function mechanism involving the sequestration of splicing factors. The ATXN8 transcript contains an ORF encoding a nearly pure CAG repeat tract. Our lab has shown that this CAG repeat tract is translated into a polyglutamine (polyQ) protein in SCA8 BAC mice and human patients. Proteins containing abnormally long polyglutamine tracts are responsible for a number of diseases including spinobulbar muscular atrophy, dentatorubral-pallidoluysian atrophy, SCA1, 2, 3, 6, 7, 17, and Huntington's disease. Polyglutamine proteins are believed to cause disease through protein gain-of-function mechanisms which involve the sequestration of cellular factors, the inhibition of cellular processes, and caspase mediated apoptosis.

The SCA8 locus therefore makes two potentially pathogenic transcripts. Either or both of these transcripts could be responsible for the disease but the relative contribution of each of these transcripts to disease phenotypes is currently unknown. The goal of this project is to elucidate the effects of each transcript to determine their relevance to disease pathogenesis. Transcripts will be knocked down using a shRNA strategy (Chung, 2006). Current attempts to specifically knock down the ATXN8 (CAG) transcript in 293 cells have resulted in 70% reduction in polyQ protein. Future studies will focus on refining the shRNA constructs in 293 cells, and testing them in primary neuronal cell lines. Successful constructs will be used to generate shRNA mice which can subsequently be crossed to SCA8 mouse models. Crosses will be used to determine if ATXN8 knock down can rescue the histological and behavioral abnormalities of the SCA8 BAC mice. The relative abundance and distribution of polyQ foci in the cerebellums of these mice will be scored by immunohistochemistry and coordination defects will be tested with rotarod analysis.

Hormone Therapy And Skeletal Muscle: A Meta-Analysis

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There is not a consensus whether or not estrogen affects muscle strength. Therefore, our objective was to perform a systematic review and meta-analysis of the research literature that compared muscle strength in post-menopausal women who were and were not on estrogen-based hormone therapy (HT). To meet this objective, 24 relevant studies were found. Effect sizes (ES) were calculated as the standardized mean difference and the meta-analyses were completed using a random effects model. HT was found to result in a small, beneficial effect on muscle strength in post-menopausal women (ES=0.23; P=0.002) that equated to a ~5% greater strength for women on HT. A large variation among the 24 studies was seen, with effect sizes ranging from -0.56 to 1.15. To address this variability, possible moderator variables were explored. Among the 24 studies, various muscle groups were assessed for strength and those that benefitted the most were the thumb adductors (ES=1.14; P<0.001). This equated to a 17% greater adductor pollicis muscle strength for women taking HT compared with those not. Nine studies that compared muscle strength in rodents that were and were not estradiol deficient were also analyzed. The ES for absolute strength was moderate but not statistically significant (ES=0.52; P=0.08), while estradiol had a large effect on strength normalized to muscle size (ES=0.76; P=0.02). Overall, HT was found to beneficially affect strength. This result justifies the need to consider skeletal muscle as a target tissue in response to HT. In addition, there is a need to further investigate the role of estrogen in mediating HT's effects on skeletal muscle. Supported by NIH Grants AG-25861 and T32-AR-07612.

Allosteric Changes in Phospholamban Structure and Dynamics Regulate Sarcoplasmic Reticulum Calcium-ATPase Function

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The main mechanism of relaxation in cardiac muscle is pumping of Ca^{2+} ions from the cytosol into the sarcoplasmic reticulum (SR). This pumping is performed by Sarcoplasmic Reticulum Ca^{2+}-ATPase (SERCA). Phospholamban (PLN) inhibits SERCA and is thereby a crucial regulator of cardiac muscle function. A previous study showed how changing the dynamics of PLN by mutations can tune the function of SERCA. In this study structural and dynamic NMR data is used to explain the difference in SERCA affinity and inhibition between different mutants of PLN. We show how these differences between mutants can be explained from different rigidity of the membrane-spanning helix.

Controlling the Inhibition of the Sarcoplasmic Ca^{2+}-ATPase by Tuning Phospholamban Structural Dynamics

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Cardiac contraction and relaxation are regulated by conformational transitions of protein complexes that are responsible for calcium trafficking through cell membranes. Central to the muscle relaxation phase is a dynamic membrane protein complex formed by Ca(2+)-ATPase (SERCA) and phospholamban (PLN), which in humans is responsible for approximately 70% of the calcium re-uptake in the sarcoplasmic reticulum. Dysfunction in this regulatory mechanism causes severe pathophysiologies. Here, we used a combination of nuclear magnetic resonance, electron paramagnetic resonance, and coupled enzyme assays to investigate how single mtuations at position 21 of PLN affects its structural dynamics and in turn, its interaction with SERCA. We found that it is possible to control the activity of SERCA by tuning PLN structural dynamics. Both increased rigidity and mobility of the PLN backbone cause a reduction of SERCA inhibition, affecting calcium transport. Although the more rigid, loss-of-function (LOF) mutants have lower binding affinities for SERCA, the more dynamic LOF mutants have binding affinities similar to that of PLN. Here, we demonstrate that it is possible to harness this knowledge to design new LOF mutants with activity similar to S16E (a mutant already used in gene therapy) for possible application in recombinant gene therapy. As proof of concept, we show a new mutant of PLN, P21G, with improved LOF characteristics in vitro.

Structural Dynamics of SERCA and Phospholamban by Fluorescence Microscopy

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We have investigated the structure of phospholamban (PLB) and its regulation of the sarcoplasmic reticulum Ca-ATPase (SERCA) using fluorescence resonance energy transfer (FRET) on fluorescent fusion proteins expressed in living cells, and using total internal reflection fluorescence (TIRF) on the labeled cytoplasmic domain of PLB. Fusion proteins were created with either the donor fluorophore, cyan fluorescent protein (CFP), or the acceptor fluorophore, yellow fluorescent protein (YFP), attached to one terminus of the protein of interest. Both N-terminal and C-terminal fusions of CFP and YFP were made to SERCA and N-terminal fusions were made to PLB. These proteins were expressed and co-expressed in either SF21 insect cells or HEK-293 cells. In fluorescence transfer recovery (FTR) experiments, FRET was calculated from the recovery of CFP fluorescence due to photobleaching of YFP. The dependence of donor fluorescence on acceptor photobleaching showed that PLB exists primarily as oligomers in cells but binds to SERCA exclusively as a monomer. Polarized TIRF of PLB, labeled in the cytoplasmic domain helix with bifunctional rhodamine (BFR), shows that this domain lies parallel to the membrane surface. The structural and functional effects of PLB phosphorylation and mutation are under investigation.
This work was supported by NIH (GM27906) and the Minnesota Supercomputing Institute.

Structure Function Analysis of Disease-Causing Missense Mutations in Dystrophin

Davin M. Henderson, Ann Lee, and James M. Ervasti

Duchenne muscular dystrophy (DMD) affects 1 of every 3500 males and results in death during the mid to late twenties. Mutations in the dystrophin gene leading to DMD commonly result in loss of protein expression or expression of a truncated protein lacking essential ligand binding domains. In some cases, point mutations leading to a single amino acid change in the dystrophin protein cause DMD, Becker muscular dystrophy or X-linked cardiomyopathy. Of the known disease causing mutations, 9 are located in the N-terminal actin-binding domain of dystrophin. Examining the effects of these mutations on actin binding activity will lead to a better understanding of key residues for dystrophin function *in vivo*. With this in mind, we engineered all 9 N-terminal disease-causing mutations into the full-length dystrophin cDNA and have begun to characterize the biochemical properties of each mutant protein expressed in the baculovirus system. We have found that the R82P and A172P mutants did not express well enough to enable further biochemical characterization. We have analyzed four mutants K18N, L54R, D165V and L172H for their ability to bind F-actin and found that K18N and L54R decrease the affinity for F-actin by 3-4 fold. The L172H mutation affects the solubility but not the actin binding properties of the full-length dystrophin protein. In fact, all mutations generated in the tandem calponin homology domain of dystrophin were consistently less soluble and more aggregated than WT dystrophin. These data suggest that the disease phenotypes associated with miss-sense dystrophin mutations are caused by either loss of solubility or a combination of insolubility and decreased F-actin affinity. We also found that mutations that cause a more severe disease phenotype (K18N and L54R) bound actin with a lower affinity and were less soluble than WT dystrophin.

Transgenic over-expression of gamma-cytoplasmic actin protects against eccentric contraction-induced injury in *mdx* mice

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Alpha-skeletal actin is the predominant actin species present in adult skeletal muscle, with small amounts of cytoplasmic actins also expressed. Unfortunately, the role of cytoplasmic actins in skeletal muscle is not well understood. We previously reported that levels of gamma-cytoplasmic actin are elevated in dystrophin-deficient *mdx* mouse skeletal muscle (Hanft, *PNAS*, 2006; Prins, *PLoS ONE*, 2008). To investigate how elevated levels of gamma-cytoplasmic actin may affect the dystrophic phenotype, we transgenically over-expresssed gamma-cytoplasmic actin specifically in *mdx* skeletal muscle (*mdx*-TG mice). Levels of gamma-cytoplasmic actin in *mdx*-TG skeletal muscle were elevated 200-fold compared to *mdx* skeletal muscle and showed incorporation into thin-filaments. Gross over-expression of gamma-cytoplasmic actin did not improve or exacerbate *mdx* muscle pathology. However, *mdx*-TG skeletal muscle had significantly increased twitch force and specific tetanic force during eccentric contractions. Moreover, gamma-cytoplasmic actin over-expression was able to protect against force loss after initial eccentric contractions. Further investigation into gamma-cytoplasmic actin's ability to protect *mdx* muscle during lengthening contractions may lead to insight into the mechanism of eccentric contraction-induced injury in dystrophin-deficient muscle.

Molecular pathophysiology and CNS effects in mouse models of myotonic dystrophy types 1 & 2

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Myotonic dystrophy (DM) is a multisystemic disease that significantly affects the CNS causing abnormal cognition, behavioral abnormalities and progressive memory problems. DM is caused by non-coding CTG or CCTG expansions in the DMPK (DM1) or ZNF9 (DM2) genes, respectively. Although in skeletal and cardiac muscle, an RNA gain-of-function mechanism involving the accumulation of CUG/CCUG expansion transcripts, the sequestration of the RNA binding protein muscleblind (MBNL1), and downstream alternative splicing changes is well established, almost nothing is known about pathophysiology and molecular CNS effects. To investigate CNS RNA gain-of-function effects in DM1 and DM2, functional cerebellar optical imaging studies were performed on newly developed DM1 and DM2 mouse models and a MbnlΔE3/ΔE3 loss-of-function model of DM.

We developed multisystemic transgenic mouse models of DM1 and DM2. TRE-(CCTG)300 expansion mice were crossed to BAC mice expressing the tet-inducible transactivator (tTA) under the control of either the endogenous human DMPK or ZNF9 promoter. Doubly transgenic DM1-CCUG [TRE-CCTG(300):DMPK-tTA] and DM2-CCUG [TRE-CCTG(300):ZNF9-tTA] mice show broad expression and multisystemic phenotypes in skeletal muscle and brain.Flavoprotein optical imaging studies of DM2-CCUG and MbnlΔE3/ΔE3 mice in vivo show a marked reduction in mGluR1 dependent parallel fiber-Purkinje cell long-term potentiation (PF-PC LTP).

The effects of estrogen depletion on skeletal muscle metabolism

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Department of Physical Medicine and Rehabilitation

Skeletal muscle plays a significant role in altering metabolic activity of the body by influencing blood lipid profiles and insulin sensitivity through enzymatic processes. Hormones such as estrogen have been shown to affect skeletal muscle function. Thus, the metabolic influence of skeletal muscle function may be altered by the depletion of estrogen. Through its role in energy metabolism and expenditure, skeletal muscle likely influences the development of cardiovascular risk factors, including, diabetes, dyslipidemia, and obesity.

To study the effects of estrogen depletion on skeletal muscle metabolic activity, 17 female C57BL/6 mice were randomly divided into two groups for the duration of 60 days, ovariectomized (OVX) and non-OVX. Circulating concentrations of total cholesterol, high–density lipoprotein (HDL), and glucose were measured prior to ovariectomy surgery and at 20, 40, and 60 days post-surgery. Pre and post trial measurements of body composition were measured on an EchoMRI. Body weight was measured weekly and food intake was calculated during a one week period. Twenty-four hour cage activity was monitored in activity chambers at 50 days post-surgery. Grip strength and whole body tension evaluation of voluntary muscle performance was also assessed. Additionally, *in vivo* measurements of maximal muscle strength were made. After 60 days, mice were euthanized and muscle tissues, para-ovarian fat pads, and uteri were removed and weighed. To evaluate lipid oxidation and glycolytic enzymatic activity, two assays were performed on the soleus muscles and tibialis anterior (TA) muscles: β-hydroxy acyl-CoA dehydrogenase (β-HAD) and citrate synthase (CS), respectively. Protein content was measured using the bicinchoninic acid protein assay.

After 60 days, OVX mice tended to have higher total cholesterol, lower HDL-to-total cholesterol ratios, and higher glucose serum levels. The EchoMRI measurements revealed no difference in body fat between surgical groups, however a change in lean mass and total body water mass in both OVX and non-OVX mice was observed over time. Body mass increased more in the estrogen-deficient mice over time but there was no difference in food intake between OVX and non-OVX mice. OVX mice tended to exhibit less cage activity. Grip test measurements were not different between surgical groups but OVX tended to have less whole body tension. *In vivo* muscle strength measurements revealed no significant differences. When comparing tissue masses, OVX had lower uterine masses per gram of body weight and higher fat pad masses compared to non-OVX. Total protein content and enzymatic activities were not different between muscles from OVX and non-OVX mice.

Collectively, we found that 8 wk of ovarian hormone depletion had minor effects on cholesterol and glucose blood levels, body mass and composition, physical activity and muscle strength, uterine and fat pad masses, and muscle protein content and enzymatic activity. These subtle changes occurred over a relatively short period of time following ovariectomy, and perhaps long-term effects of estrogen-depletion had not yet been established and subsequently translated to whole-body effects. If this holds true, then longer-term studies will be warranted to determine whether the cellular adaptations eventually translate to whole-body effects such as the development of increased total cholesterol with lower levels of HDL, obesity, and diabetes, which are known risk factors for cardiovascular disease.

Stoichiometry of Dystrophin, Dystroglycan and Laminin in Skeletal Muscle

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Muscular dystrophy is a category of hereditary diseases that are characterized by progressive muscle weakness, muscle degeneration, muscle fibrosis and other associate symptoms such as cardiopulmonary complications and nervous system malfunction. Studies on the genetic and molecular causes of muscular dystrophies reveal that deficiency in dystrophin-glycoprotein complex (DGC) is the key of most types of muscular dystrophy. The DGC consists of two core proteins – a cytoplasmic protein dystrophin (DYS) and a transmembrane protein dystroglycan (DG). On the cytoplasmic side of sarcolemma, DYS binds to F-actin and DG. On the extracellular side, DG binds to laminin (LAM), an extracellular matrix protein. Together, these proteins form an axis of F-actin-DYS-DG-LAM from cytoplasm across sarcolemma to extracellular space, linking cytoskeleton to extracellular matrix, which has been proven to play an important structural role in stabilizing sarcolemma and transmitting force across sarcolemma during muscle fiber contraction. Further studies reveal that DG also plays an important role in basal membrane assembly, cell signaling and neuromuscular junction formation. Interestingly, these functions are less or not affected by disruption of other components of the DYS-DG-LAM axis then by disruption of DG, which indicates that there might be “free DG” that works without being incorporated into DGC complex. To test this hypothesis, we used quantitative western blot to quantify the absolute concentration of DYS, DG and LAM in skeletal muscles and found out that the molar stoichiometry of DYS:DG:LAM in skeletal muscle was 1:40:1, indicating a large fraction of DG might not be incorporated in DGC. We also used dystrophin antibody to coimmunoprecipitate DGC. The result showed only a small fraction of DG associated with DYS, further confirm the existence of “free DG”.

Dystrophin and utrophin have distinct effects on the microsecond dynamics of actin

Ewa Prochniewicz, Davin Henderson, Ava Yun Lin*, James Ervasti and David D. Thomas

This study addresses the molecular mechanisms of actin's interaction with dystrophin and utrophin, in relationship to the pathology of Duchenne muscular dystrophy (DMD) and X-linked cardiomyopathy. Dystrophin and utrophin bind actin *in vitro* with similar affinities, but with different molecular contacts. It has been proposed that these differences alter the elasticity of actin-dystrophin and actin-utrophin linkages to the sarcolemma, affecting the cell's response to muscle stretches. To test this hypothesis, we have determined the effects of dystrophin and utrophin on the microsecond dynamics of erythrosin iodoacetamide-labeled actin using transient phosphorescence anisotropy (TPA). Binding of dystrophin or utrophin to actin resulted in significant changes in the TPA decay, revealing similarities as well as differences in the structural effects of each protein on actin. At a low level of actin saturation (≤20%) both proteins induced similar changes in actin dynamics, but at higher levels of saturation, utrophin was more effective than dystrophin and induced more pronounced changes in the final anisotropy, correlation time, and initial anisotropy of actin. The simplest interpretation of these changes is that utrophin restricted the amplitude and increased the rates of motion of the probe to a substantially larger extent than dystrophin. Further analysis indicated that the actin-utrophin complex is much more torsionally flexible than the actin-dystrophin complex. We propose that these differences between dystrophin and utrophin in their effects on actin dynamics affect elastic properties of actin-mediated linkages with the sarcolemma. Preliminary data on fragments containing all the proposed actin binding domains (DN-R17/UN-R10) show less effect on regulating rotational amplitude and nearly no effect on rotational rate. Future experiments looking at other fragments of dystrophin and utrophin and their functionally relevant mutants will determine which structural elements of these proteins are critical in determining the flexibility of actin filaments and what level of actin flexibility is physiologically optimal. This will greatly aid in both the therapeutic designs to treat dystrophin deficiency in both the skeletal and cardiac muscles.

Biophysical Engineering of the PLB-SERCA Interaction for Treatment of Heart Failure

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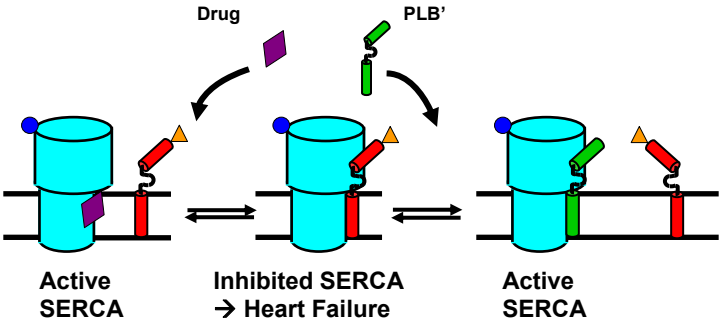
We are using **solid-phase peptide synthesis** of phospholamban (PLB), **functional membrane reconstitution** of PLB with its regulatory target (SERCA, the cardiac calcium pump), and **fluorescence resonance energy transfer (FRET)**, with the goal of designing drugs or peptides for **heart failure therapy**. It has been proposed that heart failure can be treated by decreasing, but not eliminating, the inhibitory effect of PLB on SERCA. We have developed a **quantitative *in vitro* approach**, involving a microplate-based FRET assay, to detect the **physical interactions of PLB and SERCA** in reconstituted membranes. The idea is that an unlabeled drug or peptide that displaces labeled PLB from labeled SERCA (and thus relieves SERCA inhibition) will decrease FRET. The present study focuses on the synthesis of a series of **loss-of-function PLB variants**, which we have probed for their ability to displace labeled wild-type PLB from SERCA, as detected by a decrease in FRET. Second, we have engineered **truncated PLB variants**, in order to generate a soluble reagent, which would be more useful for therapeutic delivery. The results are promising on both fronts: (1) We have identified two loss-of-function PLB variants that displace ST-PLB from SERCA and relieve its inhibition. (2) We have created a truncated PLB variant that is water-soluble and interacts functionally with SERCA.



CCUG RNA gain-of-function effects in a conditional mouse model of myotonic dystrophy type 2 (DM2).

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We developed a tetracycline inducible murine model of DM2 to test the hypotheses that $CCUG_{300}$ expansion transcripts expressed in the absence of the endogenous gene context, are sufficient to replicate the skeletal muscle features of myotonic dystrophy and that many of these features are reversible. Skeletal muscle from transgenic animals expressing transcripts with 300 but not 5 CCUGs show a number of phenotypic changes characteristic of myotonic dystrophy including: 1) variation in fiber size and central nuclei; 2) electrical myotonia; 3) ribonuclear inclusions; and 4) aberrant splicing of *Capzb*, *Insr*, and *Clc1*. RT-PCR assays show that the administration of doxycycline to 10 month old animals for 10 weeks turns off transgene expression and results in the loss of ribonuclear inclusions as well as a significant reduction in the number of central nuclei. These studies show that expression of $(CCUG)_{300}$ expansion transcripts as part of an exogenous non-coding transcript is sufficient to cause a number of phenotypic characteristics of myotonic dystrophy and that many of the pathogenic changes are reversed when expression of the transgene is turned off.

Additional studies show variable expression of Cugbp1 in $(CCUG)_{300}$ mice but no statistically significant increase in the mutant vs. control groups ($p= 0.11$). Combined FISH/IF studies in skeletal muscle show an increase of Cugbp1 levels in some nuclei, but no correlation with the presence or absence of CCUG foci. Unexpectedly, a dramatic increase in Cugbp1 protein ($p = 0.0085$), but not RNA, was found in a chloride channel (ADR) knock-out mouse but not in other mouse models of muscle disease ($mdx^{-/-}$ and $mdx^{-/-}/utr^{-/-}$). Additionally, Mbnl1 and CUG-BP2 were also elevated in the ADR but not the $(CCUG)_{300}$ mice. In summary, overexpression of CUGBP1 is not a consistent feature in our $(CCUG)_{300}$ model and upregulation of CUGBP1 is not unique to DM.

Dominant effect of histidine modified troponin to normalize SR Ca^{2+} load and rescue ischemia/reperfusion deficits in phosholamban deficient hearts.

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Genetic modification of cardiac Ca^{2+} handling has been proposed to improve functional deficits in the failing heart. For example, ablation of phospholamban (PLN), a key inhibitor of the sarcoplasmic reticulum Ca^{2+} - ATPase (SERCA2a), causes enhanced cardiac contractility and faster relaxation. However, phospholamban deficient hearts (PLN KO) are highly susceptible to increased ischemia/reperfusion (I/R) injury. We hypothesized that histidine-modified cardiac troponin I, cTnI (A164H), which has increased Ca^{2+} sensitivity especially during ischemia/acidosis, could rescue PLN KO hearts from I/R mediated functional deficits. Isolated PLN KO hearts exposed to I/R exhibited dramatically reduced recovery after 1 hr of reperfusion compared to controls (2.5% +/- 1.4% recovery vs 32.5% +/- 9.3%, $P<0.05$), highlighting a major deficiency in PLN KO hearts. Interestingly, expression of cTnIA164H had nearly a 14-fold improvement and restored functionality to PLN KO hearts (34.7% +/- 8.5% recovery). Echocardiography of mice exposed to 10 minutes acidosis (40% CO2) showed that cTnIA164H improved ejection fraction, stroke volume, and cardiac output in PLN KO mice in vivo. Immunoblot analysis of the calcium handling proteins SERCA and calsequestrin did not show any differences between PLN KO hearts and PLN KO hearts expressing cTnIA164H. As PLN KO hearts have been shown to have increased SR Ca2+ load, we sought to determine whether cTnIA164H has an effect on Ca^{2+} dynamics. PLN KO myocytes have a nearly two-fold increase in caffeine-induced SR Ca^{2+} release compared to control, consistent with previous results. Surprisingly, PLN KO myocytes expressing the cTnI A164H transgene had normalized caffeine-releasable SR Ca2+ load, yet still maintained the rapid SR Ca^{2+} uptake characteristic of PLN KO myocytes. These findings suggest a new paradigm of Ca^{2+} handling in the heart by indicating a dynamic interplay between SR and myofilaments, with cTnIA164H myofilaments having a dominant effect to normalize SR Ca^{2+} load and rescue ischemia/reperfusion deficits in hearts with dysregulated Ca^{2+} pumps.

MR spectroscopic and kinetic investigations of the interaction of Protein Kinase A with phospholamban and phospholamban mutants

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The Catalytic-subunit of Protein Kinase A (PKAc) mediates the phosphorylation of a number of proteins in cardiomyocytes which, in turn, governs myocardial contraction and relaxation. Although a wealth of kinetic and atomic-level structural data is available for the interactions of PKAc with standard, largely non-physiologically relevant substrates, these data are nearly absent for the interactions with substrates found in cardiomyocytes. Phospholamban (PLN) is a substrate of PKAc in cardiomyocytes, where it regulates the sarcoplasmic reticulum Ca^{2+}-ATPase. Phosphorylation of PLN allows the relief of its inhibitory affects on Ca^{2+} transport into the sarcoplasmic reticulum. Here, we investigate the interactions of PKAc with PLN using a variety of biophysical techniques which include NMR spectroscopy, isothermal calorimetry (ITC), and steady-state kinetic assays. Kinetic assays were used to define the steady-state kinetic parameters for the catalytic efficiency of phosphorylating PLN and two mutants of PLN, R9C and R14-delete. The ability of PKAc to bind these proteins was also measured using ITC to investigate any differences in binding affinity. Finally, TROSY-based NMR spectroscopy was used to observe and map the residue specific differences in the amide fingerprint of PKA-C when bound to each of these substrates. These data will be presented to model the effects of PLN mutations on the interactions with PKAc.

Compensatory adaptation of protein expression in resistance-trained dystrophic mouse muscle

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The purpose of this pilot study was to determine if muscles of exercise-trained *mdx* mice upregulate structural proteins α 7 β 1 integrin, utrophin, and β-dystroglycan as a compensatory adaptation to their absence of dystrophin. We modeled resistance exercise training using resisted training wheels. At 25 days of age, WT mice were separated into 3 groups: sedentary (n=4), resistance training (n=5), and no resistance training (n=4). 25 day-old *mdx* mice were separated similarly: sedentary (n=5), resistance training (n=2), and no resistance (n=1). The resistance wheel mice were introduced to the wheel with only 1 g of resistance for 1 wk, which was then increased to 4 g at Wk 2 and 7 g at Wk 3&4. The no resistance wheels had <1 g resistance throughout the study. After a total of 4 weeks all mice were sacrificed and triceps muscles were dissected for protein analysis using Western Blotting techniques. Free wheel mice ran more than8. km/day while resistance wheel mice ran ~ 50% less, however resistance mice did approximately 3-fold more work than free wheel mice. As a result, preliminary western blot results show that resistance-trained mice had ~17% more β-dytroglycan protein than sedentary mice. The upreguation of specific structural proteins in *mdx* mice as a compensatory adaptation to resistance wheel training may be even better realized after we complete our current 12 Wk study using 34 *mdx* mice.

EPR Analysis of Myosin Structural Dynamics in a Pre-power Stroke Conformation

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Cross-linking the two most reactive Cys of the myosin catalytic domain (CD) (SH1 and SH2) inhibits force production and ATP hydrolysis and locks myosin in a weak actin-binding conformation. Recent work using a bifunctional spin label (BSL) to crosslink SH1 and SH2 has shown that the CD is immobilized and orientationally disordered, suggesting that cross-linking traps myosin in a an intermediate state that primes the myosin head to generate force (Thompson et al., 2008). Fig. 2 illustrates this intermediate state in the traditional actomyosin ATPase cycle. In the present study, we measured light chain domain (LCD) structural dynamics in muscle fibers as a function of SH crosslinking. If the CD really is orientationally disordered by crosslinking and the two domains are structurally coupled, some of this disorder should be propagated to the LCD. To measure LCD structural dynamics, we used site-directed spin labeling to label chicken gizzard regulatory light chain (RLC), and then exchanged this labeled RLC for the native RLC in rabbit psoas fibers. Prior to crosslinking, EPR spectra acquired with the fiber axis parallel and perpendicular to the external field were very different, but cross-linking decreases this difference, indicating increased disorder. Saturation transfer EPR on these fibers showed that the heads remained immobile and thus attached to actin. These results support our hypothesis that SH1-SH2 crosslinking traps an actomyosin complex, possibly the first force-generating state in the power stroke, in which the CD is highly disordered and LCD is partially disordered, indicating a partially flexible linkage. A secondary goal of this research is to improve the technology for EPR on muscle fibers by developing a novel high-sensitivity EPR resonator for analysis of spin-label mobility, orientation, and force on labeled fibers.

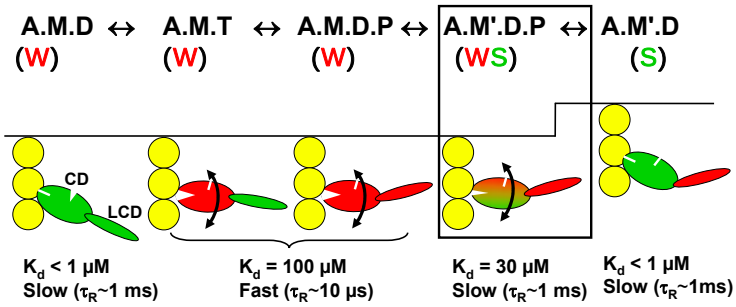

Fig. 2. Actomyosin ATPase cycle adapted from Thompson et al., 2009. Red = Weak binding. Green = Strong binding. Arrow indicates orientational disorder.

Generation of induced pluripotent stem cells from patient-specific FSHD myoblasts

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Facioscapulohumeral muscular dystrophy (FSHD) is a dominant inherited neuromuscular disease. The underlying molecular mechanism of the disease is still unknown but 95% of patients carry a subtelomeric deletion on chromosome 4q35.2 thought to affect chromatin and gene expression in the region. The recent discovery of methods to reprogram somatic cells to the embryonic state allows the creation of patient- and disease-specific cells, which can serve as a model for the disease, and the possibility of autologous cell therapy with genenetically corrected cells. In this study we have generated 3 induced pluripotent stem (iPS) cell lines from myoblasts obtained from FSHD patient biopsies as well as a 4^{th} line derived from unaffected myoblasts. To induce reprogramming we delivered the classical transcription factor quartet (Oct4, Sox2, Klf4, and Myc) by retroviral transduction and also included SV40 Large-T-antigen to increase the efficiency of reprogramming. These cell lines appear indistinguishable from human embryonic stem (hES) cells and express hES markers. We are in the process of characterizing each of the clones for gene expression, teratoma formation and *in vitro* differentiation with the intention of understanding the chromatin changes that occur at 4q35.2 in FSHD-affected myogenic progenitors.

Bone Deterioration in Duchenne's Muscular Dystrophy

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The incidence of bone fracture is 18-44% higher in boys with Duchenne's Muscular Dystrophy (DMD). This incidence becomes further elevated after prednisone treatment, which is thought to strengthen skeletal muscle while thwarting the progression of DMD. To date, the cause of bone fracture in boys with DMD has received little attention; however, it has been well established that bone's functional and geometric properties are tightly regulated by muscular contractions. The combined effect of diminished frequency and magnitude of muscular contractions, hallmark features of DMD, is likely to compromise bone's functional and geometric properties. Additionally, prednisone treatment alone has a catabolic effect on bone reducing bone volume and strength. However, little is know about how prednisone treatment and diminished physical activity affect both tissues simultaneously, particularly in muscular dystrophy. Therefore, the purpose of this study was to investigate the effects of prednisolone treatment on bone and muscle functions in *mdx* mice with typical physical activity and mechanical loading of bone compared to *mdx* mice with restricted physical activity and presumably reduced loading. **Methods:** Male *mdx* mice were given prednisolone (n=8 with typical activity (*mdx*+Pred) or n=8 restricted cage activity (*mdx*+Pred+RA)) or placebo (n=8 with typical activity (*mdx*)). After 8 wk of treatment, muscle strength was assessed *in vivo* by stimulation of the anterior crural muscles (peak eccentric and maximal isometric torque) and *in vitro* stimulation of the EDL muscle (maximal isometric force). Tibial bone's functional capacity was assessed by three point bending (ultimate load and stiffness) and micro CT (cortical and trabecular bone volume fraction and trabecular density). One-way ANOVA was used to determine group differences in bone and muscle functional parameters. **Results:** Following 8 wks of prednisolone treatment, muscle strength remained unaltered for each of the muscle functional parameters ($p>0.0.381$). In bone, both ultimate load and stiffness were 10-14% lower in the *mdx*+Pred+RA group compared to the *mdx* and *mdx*+Pred groups ($p<0.02$ for all), showing that the combination of prednisolone and restricted activity resulted in structurally weakened bone. Furthermore, bone volume fractions of both cortical and trabecular bone were 20-61% lower in the *mdx*+Pred+RA group compared to the other two groups (Figure 1, $p<0.001$ for all). **Conclusion:** Despite the ability of the lower leg muscles of all *mdx* mice in this study to produce similar magnitudes of muscular contractions and torques, the condition of reduced physical activity drastically altered bone's functional and geometric properties making the bone substantially weaker. Thus in DMD, the combination of lowered physical activity in addition to disease and drug treatment likely increases the risk of bone fracture.

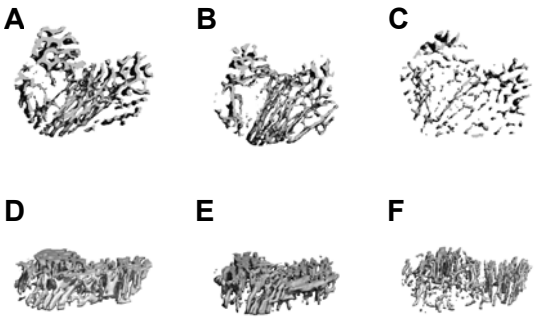

Figure 1. Micro CT images of trabecular bone of the proximal tibia in *mdx* mice. A-C are superior views of 50 stacked images, and D-F are the same 50 slices rotated to better represent the cross-section of bone used in the analyses. A and D are *mdx*, B and E are *mdx*+Pred, and C and F are *mdx*+Pred+RA.

Single Histidine Button in Cardiac Troponin I Sustains Heart Performance in Response to Severe Acidosis In Vivo

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Intracellular acidosis is a profound negative regulator of myocardial performance. The aim of this study was to test the effect of a unique bio-sensor in modulating cardiac performance in a pH-dependent manner. We hypothesized that titrating myofilament calcium sensitivity by a single histidine substituted cardiac troponin I (cTnI A164H) would act as a molecular rheostat protecting the whole animal physiological response to acidosis *in vivo*. **Methods/Results:** Severe acidosis was induced in mice by ventilation with 40% CO_2. Echocardiographic analysis showed that systolic function and ventricular geometry were maintained in Tg mice. By contrast, nontransgenic (Ntg) littermates experienced marked deterioration in global cardiac performance and significant LV dilation during this same challenge. For detailed hemodymanic assessment, cardiac conductance micromanometry analysis was performed. To specifically isolate intrinsic cardiac performance during acidosis, animals were treated with a beta blocker during ventilation with 40% CO_2. Hemodynamic analysis during beta blockade and acidosis showed that Ntg mice underwent marked cardiac decompensation with 100% mortality within five minutes. In contrast, Tg mice maintained systolic *and* diastolic function and had 100% survival during the twenty minute time course of the challenge. At the end of the challenge and severe acidemia (pH = 6.5) numerous measures of cardiac performance in Tg mice were not significantly different from baseline based on a Dunnets test. **Conclusion:** This study shows that, independent of any beta adrenergic compensation, histidine-modified cardiac troponin I is a unique myofilament-based molecular bio-sensor that maintains contractile function and markedly improves survival during severe acidosis *in vivo*.

Dystrophin is a Microtubule-Associated Protein

Kurt Prins, Jill Humston, Amisha Mehta, Evelyn Ralston, and James Ervasti.

Cytolinkers are giant proteins that stabilize cells by linking actin filaments, intermediate filaments, and microtubules to transmembrane complexes. Dystrophin, the protein absent in Duchenne muscular dystrophin, is functionally homologous to cytolinkers as it links actin and intermediate filaments to a transmembrane complex. Although no direct link between dystrophin and microtubules has been documented, costamere-associated microtubules are disrupted when dystrophin is absent. Using tissue-based cosedimentation assays on mice expressing endogenous dystrophin or truncated transgene products, we found constructs encoding spectrin-like repeat 24 through the first third of the WW domain cosedimented with microtubules. Purified Dp260, a truncated isoform of dystrophin, bound microtubules with a K_d of 0.66 μm, a stoichiometry of 1 Dp260/1.4 α-β tubulin heterodimer at saturation, and stabilized microtubules from cold-induced depolymerization. Finally, α-and β-tubulin expression was increased approximately 2.5 fold in *mdx* skeletal muscle without altering the tubulin: microtubule equilibrium. Collectively, these data suggest dystrophin directly organizes and/or stabilizes costameric microtubules, and classifies dystrophin as a cytolinker in skeletal muscle.

Cardioprotective pretreatment for global ischemia of isolated swine hearts: assessed using Visible Heart® methodologies

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Background: The total number of heart transplanted in the US could be greatly increased by increasing viable time outside the body. Drug delivery into the pericardial space is a method that could not only potentially improve function in the transplanted heart, but also prolong viability.

Methods: Docosahexaenoic acid or an equal amount of carrier (a small amount of ethanol) was delivered into the swine pericardial space 30 minutes before cardioplegic arrest. The heart was explanted and after cannulation of the great vessels, the heart was reperfused using Visible Heart® methodologies for 60 minutes alternating between right sided working and full working modes. Hemodynamic data was taken to assess cardiac function and circulating buffer was sampled for protein quantification.

Initial results: With the current protocol treated hearts take a longer time to fibrillate and flatline than controls ($p=0.054$). Upon reanimation, the control hearts obtain better pressures and elute fewer proteins than the treated hearts, though statistical significance has not been achieved.

Cardiac Myosin Isoform Remodeling in Duchenne Muscular Dystrophy Cardiomyopathy

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Duchenne muscular dystrophy (DMD) is an inherited neuromuscular disease in humans that is caused by a deficiency in the dystrophin protein, which leads to deterioration of skeletal muscle and cardiomyopathy. The finding that cardiomyopathy is not reversed in a gene rescue of skeletal muscular dystrophy in a mouse model of the MDC suggests that the mechanism for cardiomyopathy is different from skeletal myopathy, so it is important to delineate these differences. The skeletal and cardiac muscle fibers from a mouse model lacking the dystrophin gene, (called *mdx* mouse) show decreased force generation indicating that contractile proteins are affected by the absence of dystrophin, suggesting a downstream effect of the DMD disease. Also, this further implies that a force-determining muscle protein in the muscle (e.g. myosin) is affected by the absence of dystrophin. Previous studies show that in the skeletal muscle of DMD mice, myosin undergoes an isoform shift from the fast to the slow isoform; therefore it is plausible that cardiac myosin undergoes similar transitions. It is unknown if cardiac myosin is remodeled in DMD cardiomyopathy patients or mice, as is observed during diabetes, failing hearts, and aging. The **first specific aim** of the proposed research is to determine whether cardiac myosin undergoes isoform remodeling in dystrophic mice hearts from two mouse models; one lacking dystrophin (*mdx*) and another lacking both dystrophin and utrophin (*mdx/utrn*$^{-/-}$). Our results show that cardiac α-myosin is remodeled to β-myosin to a higher extent in the LV papillary muscle of *mdx:utrn*$^{-/-}$ mice than in the LV or RV muscles. We also observed β-myosin in the *mdx* muscle at lower levels compared to the *mdx:utrn*$^{-/-}$ muscle, but not in the WT samples. The **second specific aim** of our study is to determine whether other sarcomeric muscle proteins undergo alterations in their expression levels. Our proposed research is designed to clarify and give insight into the role of cardiac myosin in DMD cardiomyopathy. *Supported by funds from a Nash Avery Award to OR and Gregory Marzolf Fellowships to EMH and MP from the Paul and Sheila Wellstone Muscular Dystrophy Center at the Univ. of Mn, and by funds from the NIH-NIAMS R01 grant (AR052360) to OR.*

Analysis Of The Sarcolipin:Serca Regulatory Complex Using Fluorescence Resonance Energy Transfer Microscopy

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We have used fluorescence resonance energy transfer (FRET) microscopy to identify physical interactions between the sarcoplasmic reticulum Ca-ATPase (SERCA) and its regulatory subunit sarcolipin (SLN) from fast-twitch skeletal muscle. SLN is a phospholamban-like protein that inhibits calcium cycling by decreasing the calcium affinity of SERCA. It is not known whether SLN forms pentamers like phospholamban, nor is it known if SLN monomers or oligomers bind to SERCA. Here we express fluorescent fusion proteins of SLN and SERCA to monitor molecular interactions between the two proteins. FRET microscopy in live cells has demonstrated that SLN monomers self-associate to form dimers and that SLN monomers interact with SERCA monomers to form 1:1 binary complexes. FRET results further demonstrate that the binding affinity of SLN:SLN homo-dimers is about equal to the binding affinity of SLN:SERCA hetero-dimers. Site directed mutagenesis demonstrated that Isoleucine-17 of SLN decreased FRET in half for both SLN:SLN and SLN:SERCA interactions. We conclude that (1) SLN monomers compete in equilibrium between SLN oligomerization and SERCA binding and (2) Isoleucine-17 of SLN participates in both types of interactions.

The Effects of Tissue Bath pH During Hypoxia on an In Vitro Ischemia / Reperfusion Injury Model

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Muscle recovery from ischemic conditions is inhibited by factors such as increased metabolic waste, increased levels of CO_2, and low pH environment. Interestingly, many *in vitro* ischemia / reoxygenation models, utilize a buffering system that maintains a normal pH of about 7.4 during the ischemia phase(1-2). The goal of this research was to modify the incubation conditions of our *in vitro* hypoxia muscle model to better simulate *in vivo* ischemia / reperfusion injury.

Rectus abdominis skeletal muscle bundles from Yorkshire crossbreed swine were maintained in a modified Krebs buffer in tissue baths between two platinum electrodes which delivered a supramaximal,15 volt, 1 msec stimulus pulse every 10 seconds(1). Peak forces were measured as an indicator of the muscle's viability. The Krebs was continuously gassed with either a mixture of 95% O_2 and 5% CO_2 during normoxia periods or 95% N_2 and 5% CO_2 during hypoxia; maintaining a pH of 7.4. In another group the gassing during hypoxia was supplemented with CO_2 lowering the pH to 6.5. After hypoxia, the bundles were reoxygenated for 2 hours. All forces were normalized to their prehypoxia values and reported as percent changes over time ± StDev.

After 2 hours reoxygenation bundles exposed to pH 7.4 hypoxia recovered to 35.73%±14.92% of their prehypoxia value compared to the pH 6.5 hypoxia force recovery of 14.73%±6.81%. This indicates that the lower pH during hypoxia reduces the post hypoxia recovery of skeletal muscle. Furthermore, when the bundles were preconditioned before hypoxia with 10 uM of [D-Pen^2,D-Pen^5]-Enkephalin (DPDPE), bundles exposed to pH 7.4 hypoxia recovered to 35.21%±14.02% while the pH 6.5 hypoxia bundles recovery increased to 19.16%±12.89%, indicating that DPDPE preconditioning may only benefit the recovery of tissue that has been exposed to the more intense hypoxia with a lower pH.

In another series of experiments, pH 7.4 hypoxia bundles were exposed to the free radical H_2O_2 during reoxygenation. The muscle bundles exposed to 1250 uM H_2O_2 recovered 32.08%±10.47% of its original peak force while those exposed to 125 uM H_2O_2 recovery of 35.79%±8.12% was similar to the pH 7.4 hypoxia control group at 35.73%±14.92%, suggesting that free radicals might play less of a role in the recovery of the muscle from hypoxia than the pH during hypoxia.

In summary, the results of these studies may be applied to identifying a better *in vitro* model of ischemia / reperfusiuon injury of striated muscle

1. Hong J, Sigg DC, Coles JA Jr, Oeltgen PR, Harlow HJ, Soule CL, Iaizzo PA: Hibernation induction trigger reduces hypoxic damage of swine skeletal muscle. Muscle Nerve. 2005; 32(2):200-7

2. Kilian JG, Nakhla S, Sievking DP, Celermajer DS: Adenosine prevents neutrophil adhesion to human endothelial cells after hypoxia/reoxygenation. Int J Cardiol. 2005; 105(3):322-6

TAT-Utrophin crosses cell barriers to combat dystrophin deficiency

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The loss of dystrophin compromises muscle cell membrane stability and causes Duchenne muscular dystrophy and/or various forms of cardiomyopathy. Increased expression of the dystrophin homolog utrophin by gene delivery or pharmacologic upregulation has been demonstrated to restore membrane integrity and improve the phenotype in the dystrophin-deficient *mdx* mouse. However, the lack of a viable therapy in humans predicates the need to explore alternative methods to combat dystrophin deficiency. We investigated whether systemic administration of recombinant full-length (Utr) or ΔR4-21 "micro" (μUtr) utrophin protein modified with the cell-penetrating TAT protein transduction domain could attenuate the phenotype of *mdx* mice. Recombinant TAT-Utr and TAT-μUtr proteins were expressed using the baculovirus system and purified using FLAG-affinity chromatography. Age-matched *mdx* mice received six twice-weekly intraperitoneal injections of either recombinant protein or PBS. Three days after the final injection, mice were analyzed for several phenotypic parameters of dystrophin deficiency. Injected TAT-μUtr transduced all tissues examined, integrated with members of the dystrophin complex, reduced serum levels of creatine kinase, the prevalence of muscle degeneration/regeneration, the susceptibility to eccentric contraction-induced force drop, and increased specific force production. These results establish the efficacy and feasibility of TAT-utrophin-based constructs as a novel direct protein-replacement therapy for the treatment of skeletal and cardiac muscle diseases caused by loss of dystrophin.

Structural dynamics of sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) studied by molecular simulations of site-specific labeled protein

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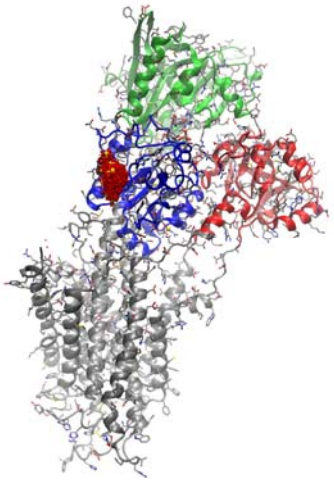
Structural dynamics of the proteins involved in Ca^{2+} transport and its regulation is studied in our laboratory by EPR and fluorescence spectroscopy. To interpret these experimental results and to generate new structural and mechanistic models, we have performed computational simulations of SERCA labeled with spectroscopic probes. Our approach provides information on the conformational landscape sampled by SERCA during its catalytic cycle.

X-ray crystal structures suggest that the nucleotide-binding and actuator domains of SERCA move apart by about 3 nm upon Ca^{2+} binding, undergoing a transition from open to closed conformations. To test this hypothesis, we constructed a fusion protein containing CFP linked to the N-terminus (the A-domain) of SERCA. CFP-SERCA was then specifically labeled with FITC in the N-domain. FRET was then used to monitor the A to N interdomain distance (Winters, Autry, Svensson and Thomas, 2008, *Biochemistry* 47, 4246–56). To interpret the FRET data, simulations of the CFP-SERCA fusion protein were conducted to generate a representative ensemble of conformations. FRET parameters were calculated using both distance and orientation information. Based on FRET data and simulations, we conclude that (a) the cytoplasmic headpiece maintains a compact structure throughout its catalytic cycle, rather than the open E1.Ca crystal structure, and/or (b) the Ca-bound E1 state is dynamically disordered and samples both open and closed conformations, with an average structure that is only slightly different from the closed E2 structure.

We have developed molecular mechanics force-field parameters for the fluorescence labels AEDANS in order to perform molecular dynamics simulations on SERCA with the label attached to Cys674. These conformational sampling simulations of the fluorescent probe and its protein environment will enable direct comparisons with fluorescence spectroscopy experiments. Time-resolved FRET was measured for the donor IAEDANS attached to Cys674 and the acceptor TNP-ADP. The high time resolution allows for determination of not only the mean distance, R_{DA}, but also the distance distribution (disorder). The combined approach of time-resolved fluorescence spectroscopy and simulations gives us insight into local structural dynamics of SERCA.

This work was supported by NIH (GM27906, AR007612) and the Minnesota Supercomputing Institute.



SERCA with the AEDANS probe at position 674. The region in space which the probe samples is highlighted.

MR Imaging of Pacing Induced Ventricular Dyssynchrony in an Isolated Human Heart

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Background: The right ventricular apex (RVA) has been the standard cardiac pacing lead implantation site for decades; but recently it has been shown via echocardiography, that pacing from this location can induce both ventricular dyssynchrony and deleterious ventricular remodeling. With advances in magnetic resonance imaging (MRI), such as myocardial tagging, it is now possible to study regional myocardial deformation and transmural cardiac strain. However, the clinical use of MRI in the assessment of ventricular function during cardiac pacing is nonexistent: as having an implanted pacing system is considered as a contraindication for cardiac MR (CMR). Here we have employed MR imaging to visualize RVA pacing induced dyssynchrony in an isolated human heart.

Methods and Results: A human donor heart deemed non-viable for transplantation, was reanimated using an MR compatible, four-chamber working perfusion system. The heart was imaged using a 1.5 tesla MR scanner while being paced at 80 beats/minute from the RVA via an epicardial pacing lead. Both four-chamber and tagged short-axis cines were acquired. The activation patterns of the LV during pacing demonstrated intraventricular dyssynchrony; as the LV mechanical activation proceeded from the septum and anterior wall to the lateral wall, with the posterior wall being activated the last. Likewise, interventricular dyssynchrony was demonstrated from the four-chamber cine as the time difference between the peak LV and RV free wall motion was 180 ms. Pacing induced dyssynchrony can be clearly seen in the supplemental videos.

Conclusions: This unique MRI characterization of RVA pacing induced dyssynchrony within an isolated human heart further emphasizes the value of more physiological pacing.

GADD45γ is a Novel Downstream Target of MyoD and Negatively Regulates Survival and Self-Renewal of Muscle Stem Cells.

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MyoD is a skeletal muscle-specific transcription factor that plays essential roles in muscle stem cell (muscle satellite cells) differentiation and regeneration. Satellite cells derived from mice lacking the *MyoD* gene display significantly delayed myogenic differentiation, compared to wild-type satellite cells. To understand molecular mechanisms for differentiation of satellite cells by MyoD, we investigated the pathways regulated by MyoD. We noticed that GADD45γ (growth arrest and DNA-damage-inducible protein 45 gamma) is a direct MyoD downstream gene. GADD45γ has been shown to play an active role in DNA demethylation process. Methylation is known to be regulating transcriptional activities and has also been found to be involved in muscle-specific gene regulation. Our preliminary results demonstrate that GADD45γ was highly expressed in wild-type but not in $MyoD^{-/-}$ satellite cells. Over expression of GADD45γ increased myogenic differentiation of wild-type satellite cells and $MyoD^{-/-}$ satellite cells. In addition, over expression of GADD45γ in $MyoD^{-/-}$ satellite cells resulted in increased cell death. These findings provide valuable insights into mechanisms by which *MyoD* deficiency impairs the role of muscle stem cells in transition from primitive stem cells to muscle precursor cells and, thus MyoD may negatively regulate self-renewal of muscle stem cells. GADD45γ is the effecter downstream target of MyoD and executes processes of cell differentiation and apoptotic cell death through DNA demethylation.

Structural Dynamics of Cardiac Calcium Regulation

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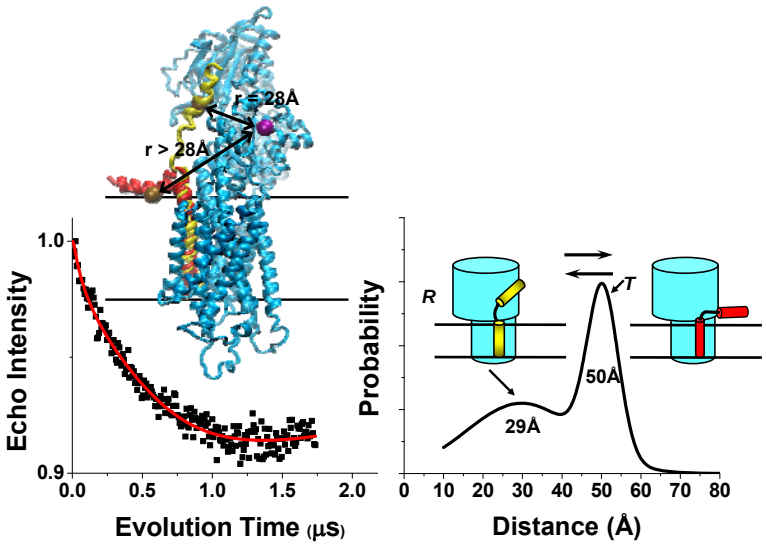
We are using solid-phase peptide synthesis (SPPS), site-directed spin labeling, and electron paramagnetic resonance (EPR) to investigate the structural dynamics and protein interactions of cardiac calcium regulation. SPPS has the unique advantage of incorporating the amino acid spin label TOAC into the backbone of peptides, which allows direct detection of PLB backbone dynamics. We used this system to measure PLB cytoplasmic domain dynamics as a function of S16 phosphorylation and interaction with SERCA. EPR shows that the cytoplasmic domain of PLB exists in a dynamic equilibrium between an **ordered (T) state** and a **dynamically disordered (R) state**. **Phosphorylation induces an order-to-disorder (T-to-R)** transition in the cytoplasmic domain of isolated PLB, but induces the opposite effect on SERCA-bound PLB, showing that **phosphorylated PLB remains bound to active SERCA** [1]. Based on this and other data, we hypothesize that phosphorylation-dependent inhibition relief is dependent on the vertical position of PLB in the lipid bilayer. We are testing this model by measuring the accessibility of TOAC-labeled PLB to paramagnetic relaxation enhancers in the lipid bilayer and aqueous phase as a function of SERCA binding and S16 phosphorylation.

We used DEER (double electron-electron resonance, a pulsed EPR technique) in conjunction with NMR, to solve the structure of the PLB pentamer in lipid bilayers. Both EPR and NMR data show that the PLB pentamer adopts primarily a **pinwheel conformation**, in which the cytoplasmic domains flare outward and interact with the bilayer surface [2].

We spin-labeled SERCA at Cys674 in the P-domain and used DEER to measure distances to selected sites on the PLB monomer, to determine the structure of the SERCA-PLB complex in both inhibitory and activating states. The distance distribution suggests that **PLB binds to SERCA in both the R and T states (see figure).**



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1. Karim, C. B., Zhang, Z., Howard, E. C., Torgersen, K. D. & Thomas, D. D. (2006). Phosphorylation-dependent Conformational Switch in Spin-labeled Phospholamban Bound to SERCA. *J Mol Biol* 358, 1032-40.
2. Traaseth, N. J., Verardi, R., Torgersen, K. D., Karim, C. B., Thomas, D. D. & Veglia, G. (2007). Spectroscopic validation of the pentameric structure of phospholamban. *Proc Natl Acad Sci U S A* 104, 14676-81.

Prevention of dilated cardiomyopathy by chronic infusion of the membrane sealant Poloxamer 188 in canines with muscular dystrophy

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Heart disease is rapidly emerging as a central component of the clinical management of patients with Duchenne Muscular Dystrophy (DMD). Previous studies have shown that acute intravascular administration of the chemical-based membrane sealant Poloxamer 188 (P188) can improve cardiac performance in a mouse model of DMD. The long-term effects of P188, however, have not been tested in any DMD model. To address this, we used Golden Retriever muscular dystrophy (GRMD) animals that have markedly greater cardiomyopathy at baseline than dystrophic mice. We instrumented GRMD animals for continuous intravascular infusion of P188 (60 mg/kg/hr) or saline (control) for 60 days. Prior to treatment, the dystrophic dogs had an end-diastolic volume (EDV) of 33±5 ml (n=7). In the chronic saline delivery group EDV dilated to 49±6 ml (n=4). In marked contrast, treatment with P188 prevented this change in ventricular dimension (EDV 32±4 ml, n=3, $P<0.05$). In addition, chronic infusion of P188 significantly improved diastolic function in GRMD animals (Tau: pre-treatment 25.9±0.8 msec vs. 18.9±0.7 msec post treatment n=4, $P<0.05$). Saline delivery did not alter diastolic parameters. These results provide the first evidence that chronic infusion of membrane sealant P188 can effectively block emergent LV dilation in a large animal with muscular dystrophy.

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Ca^{2+}-ATPase Drives a Topological Rearrangement in the Transmembrane Domain of Phospholamban as Measured by Solid-State NMR Spectroscopy

Nathaniel J Traaseth, Raffaello Verardi, Lei Shi, Martin Gustavsson, and Gianluigi Veglia

Calcium cycling in muscle cells drives the relaxation and contraction of both skeletal and heart tissue. The sarcoplasmic reticulum (SR) Ca^{2+}-ATPase is central in the relaxation of the heart, accounting for ~70% of calcium sequestration. Phospholamban (PLN) is a small integral membrane protein regulator of Ca^{2+}-ATPase. Its inhibition of the enzyme is shown in calcium dependent activity curves, resulting in decreased ATPase affinity for Ca^{2+}. While there have been several successful attempts to gain structural knowledge of the complex between PLN and Ca^{2+}-ATPase, no high-resolution structure exists.

One important feature that differentiates membrane proteins from soluble ones is topology. This is the specific *entanglement* between the membrane protein and the lipid bilayer. Probing the orientation of the protein has been best achieved using oriented solid-state NMR experiments such as PISEMA. These experiments correlate an anisotropic chemical shift (^{15}N) with a dipolar coupling (^{1}H-^{15}N), allowing for the resolution of backbone restraints with respect to the lipid bilayer normal.

To investigate whether topology plays a role in this membrane protein complex, we reconstituted PLN (using ^{15}N labels) in the presence and absence of Ca^{2+}-ATPase (purified from rabbit skeletal muscle) into mechanically oriented lipid bilayers. Our results unambiguously show that PLN's topology is substantially altered upon binding the ATPase. Specifically, we see the membrane embedded helix (residues 31-52) changes its tilt angle with respect to the bilayer normal from ~23° in the absence of the enzyme to ~40° in its presence. This substantial topology rearrangement might be a necessary attribute of PLN regulation and potentially a central difference between an active and inactive PLN bound state to Ca^{2+}-ATPase.

Improvement of muscular dystrophy phenotypes through an increase in vasculature in *mdx* mice

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Duchhene Muscular Dystrophy (DMD) is an X-linked recessive genetic disease in which the gene coding for the protein dystrophin is missing. Recent work demonstrates that the lack of dystrophin has been linked to vascular insufficiency. Consequently, the decreased circulation may induce a state of ischemia increasing the muscular dystrophy pathogenesis. For an effective form of therapy of DMD, both the muscle and the vasculature need to be repaired. For this reason, *mdx* mice with increased vasculature were created by crossing them with *Flt1* gene knockout mice. *mdx* mice are an animal homologue for DMD. Flt1 is a decoy receptor for vascular endothelial growth factor (VEGF) and therefore *Flt-1*$^{+/-}$ mice display increased vascular density. Interestingly, the *mdx:Flt1*$^{+/-}$ mice with increased angiogenesis display an improved muscle histology compared to the *mdx* mice, including decreased fibrosis, calcification and membrane permeability. Functionally, *mdx:Flt1*$^{+/-}$ mice are also shown to have a slightly increase in muscle force production, compared to the *mdx* mice. Consequently, *mdx:utrophin*$^{-/-}$*:Flt1*$^{+/-}$ mice display higher survival rates compared to *mdx:utrophin*$^{-/-}$ mice which show more severe muscle phenotypes than *mdx* mice and typically die within 5 months. These data strongly suggest that increasing the vasculature in DMD may be able to ameliorate the histological and functional phenotypes associated with this disease.

Designer Calcium Buffers for Enhancing Contractile and Relaxation Performance of Normal and Failing Adult Cardiac Myocytes

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The cytosolic calcium (Ca^{2+}) binding protein parvalbumin (Parv) is a unique delayed Ca^{2+} buffer that accelerates cardiac relaxation at base line and during pathophysiological challenges. Application of Parv in diastolic heart failure is limited by Parv's inhibition of contractility due to partial Ca^{2+} buffering during systole. Here we show that targeted genetic engineering of the EF hand metal binding sites of Parv produces Parv mutants with altered Ca^{2+} buffering capacities. Through adenovirus-mediated acute gene transfer in adult cardiac myocytes, the Parv mutants increased myocytes relaxation speed according to their metal binding properties. Surprisingly, in distinction from the wild type Parv and other Parv mutants, the Parv D51A/E101Q/F102W (ParvDEF), which has lowered Ca^{2+} binding affinity and increased Mg^{2+} binding affinity, not only significantly accelerated relaxation but also enhanced contractility of myocytes. ParvDEF buffered diastolic Ca^{2+} to speed up cell relaxation, while preserving systolic Ca^{2+} release and sarcoplasmic reticulum Ca^{2+} content. ParvDEF enhanced contractility by influencing myofilament Ca^{2+} binding, since a troponin C mutant (L29Q) blocked ParvDEF's effect. In a rabbit model of heart failure, ParvDEF rescued the impaired contractility and relaxation of cardiac myocytes. Therefore, ParvDEF specifically buffers diastolic Ca^{2+} to speed up relaxation and interacts with myofilaments to enhance contractility in normal and diseased myocytes. The positive lusitropic and inotropic effects of ParvDEF make this designer Ca^{2+} buffer a promising candidate for the treatment of cardiac dysfunction.

Gene transfer of engineered cardiac troponin C for modulation of myofilament Ca^{2+} sensitivity

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Cardiac troponin C (cTnC) is a myofilament Ca^{2+} sensor responsible for initiating striated muscle contraction and relaxation in response to cytosolic Ca^{2+} fluctuations. Modification of the myofilament Ca^{2+} sensitivity thus holds tremendous therapeutic promise for heart diseases like congestive heart failure and inherited cardiomyopathy (CM). We recently studied two cTnC mutants, M47A and E40A as Ca^{2+} sensitizer or desensitizer and for modulating sarcomere contraction in adult rat cardiac myocytes via adenovirus-mediated gene transfer. M47A cTnC significantly increased myofilament Ca^{2+} sensitivity and maximally activated tension as well as significantly slowed sarcomere relaxation and Ca^{2+} transient decay. By contrast, E40A cTnC decreased myofilament Ca^{2+} sensitivity and hastened relaxation time in intact cardiac myocytes. Based on these in vitro data, on-going studies detailed here will focus on exploring physiological roles and therapeutic effects of the two cTnC mutants in mouse heart. The M47A and E40A will be respectively delivered to myocardium of ischemic heart failure and the R193H cTnI transgenic mouse models. New AAV serotypes will be used to realize effective myocardial gene transfer and optimized regulation elements will be integrally applied for stable transgene expression. The left ventricle (LV) function will be followed by measurement of dP/dt values and haemodynamics by use of the Millar catheter. Echocardiography will be employed to record the indices including ejection fraction percentage and internal diastolic diameter of LV. All these in vivo data will provide the further information on direct physiological effects of M47A and E40A in diseased mouse heart. Collectively, gene transfer of designer cTnC Ca^{2+} sensors into in vitro and in vivo cardiac models creates great opportunity for defining their physiological roles in modulating myofilament contraction and exploring their therapeutic application for heart disease.