well. We are currently examining the effect of salt species, non-ionic compounds and environmental temperature on the luminescence response.

594-Pos Board B394
Interaction and Regulation of Calcium Homeostasis by TRPV Channels and SPCA2 in Breast Cancer
Brandie M. Cross, Mingye Feng, Jose P. Llongueras, Rajini Rao.
Breast cancer is one of the most common forms of cancer in women, causing over 40,000 deaths per year. The major concern with breast cancer is its tendency to metastasize, preferentially, to the brain, bone and other areas of high physiological calcium. Calcium is a vital component of the breast, as mammary tissue has the ability to sequester and compartmentalize such calcium. During lactation, mammary cells are required to concentrate the 2mM blood concentration of milk into the 40-80mM concentration of calcium found in milk. This action requires a highly coordinated group of calcium channels and pumps to seize the calcium into intracellular storage compartments. We have shown in previous studies that SPCA2 is highly upregulated during lactation and tumorigenesis. Additional studies from others have shown that TRP channels are also upregulated in lactation and tumorigenesis. Here we show that this induction is due to the interaction of these two molecular components of calcium homeostasis in breast tissue.

Bacteria & Motile Cells: Signal Transduction

595-Pos Board B395
New Features of Chemotactic Behavior in a Marine Bacterium
Tuba Altindal.
Using optical trapping we investigated chemotactic response of marine bacterium Vibrio alginolyticus to a brief stimulation by serine. Although this bacterial strain possesses only a single polar flagellum and its motility pattern is different from that of enteric bacteria, such as Escherichia coli (E. coli), the chemotactic response appears suprisingly similar, i.e. both are biphasic. However, in details, the two response functions are different in that the relevant time scales, the short activation and the long adaption times, are shorter in V. alginolyticus than in E. coli, reflecting perhaps different ecosystems these bacteria live. In addition to the overall biphasic response, the response function appears to have fine structures that are sensitive to the exposure time αt of the cell to serine, i.e., the amplitude of the fine structures increases with αt and reaches a maximum in about 1.5 s, and then the amplitude decreases with longer αt. We also investigated V. alginolyticus response to different serine concentrations. Such measurements allow us to conclude that this marine strain is at least a factor of ten more sensitive to low concentration of serine than E. coli. It remains a challenge in future studies to understand how the above measured attributes allow the cells of V. alginolyticus to thrive in an ocean environment.

596-Pos Board B396
Explore the Physical Limits of Eukaryotic Gradient Sensing in a Statistical Mechanical Approach
Bo Hu, Wen Chen, Wouter-Jan Rappel, Herbert Levine.
Many eukaryotic cells are able to detect chemical gradients by directly measuring spatial concentration differences. The precision of such gradient sensing is limited by fluctuations in the binding of diffusing particles to specific receptors on the cell surface. Here, we explore the physical limits of the spatial sensing mechanism by modeling the chemotactic cell as an Ising spin chain subject to a spatially varying field. This allows us to derive the $\therefore$emph{maximum likelihood estimators} of the gradient parameters as well as explicit expressions for their asymptotic uncertainties. Our analytical results demonstrate that the accuracy to sense the gradient direction increases dramatically with the cell size. Also, we show that this accuracy can be improved significantly by introducing neighboring receptor cooperativity. Thus, receptor coupling may open the possibility for small bacteria to perform spatial measurements of gradients, as supported by a recent experimental finding.

597-Pos Board B397
Breaking of Time-Reversal Symmetry in Bacterial Chemotaxis
Li Xie, Xiao-Lun Wu.
Vibrio alginolyticus is a marine bacterium with a single polar flagellum when growing in low viscous liquid media. Recently, we found that they carry out a novel cyclic 3-step motility pattern. In one cycle they swim forward, backward and at the end of the backward swimming they are able to use their flagellum to flick and actively change their cell body orientation. To understand how this novel motility pattern is regulated by *V. alginolyticus*, we studied their chemotactic response to a sudden increase of the attractant serine concentration. We made use of caged serine, which can be released by UV light in a fraction of second to achieve a sudden change in the serine concentration. We compared their swimming behavior before and after the UV flash. We found that in short time, they treat forward and backward swimming equally, i.e. they extend their current swimming interval regardless of the swimming direction. However, in a long time but before adaptation, cells become gradually forwardly biased. This chemotactic strategy enables the bacteria to swim up the attractant gradient quickly as well as to localize around the top of the gradient to get a maximum nutrient exposure. This perhaps is important in an ocean environment where nutrient are distributed intermittently in space and time.

598-Pos Board B398
On the Role of Src Family Kinases in Cyclic Strain Induced Cell Reorientation
Verena Niediek, Simone Born, Norbert Kirchgessner, Bernd Hoffmann, **Rudolf Merkel**.
Many cells recognize mechanical signals from their environment which is of vital importance for multicellular organisms. Cyclic stretch is a prominent mechanical signal experienced by various cell types around blood vessels, within the lung epithelia or around the intestine. In response to this signal, some cells reorient their actin cytoskeleton and main cell axis almost perpendicular to the direction of stretch. Despite the vital necessity of cellular adaptation to cyclic stretch, only incomplete ideas exist about the underlying mechanosensoric signal cascade. In this project we cultivated mouse embryonic fibroblasts in flexible cell culture chambers fabricated from silicone where they adhered via focal adhesions (FAs). Cyclic stretching of these chambers during culture provided well-defined mechanical stimuli to the cells. After straining, cells were fixed, stained for F-actin and adjacent micrographs of large areas of the sample were taken. For all cells imaged (some hundreds per chamber) we determined the preferential orientation of the actin fibers by digital image processing. This results in data sets with high statistical significance. Because FAs have been shown to be strongly influenced by the Src-family kinases and have been furthermore implied in mechanosensing we examined the influence of Src-family kinase knockout on cellular reorientation upon cyclic stretch. We found that lacking phosphorylation leads to strongly diminished cellular reorientation.

599-Pos Board B399
Molecular Sieving Properties of the Cytoplasm of Escherichia Coli and Consequences of Osmotic Stress
Jacek T. Mika.
We determined the diffusion coefficients (D) of (macro)molecules of different sizes (from approximately 0.5 to 600 kDa) in the cytoplasm of live Escherichia coli cells under normal osmotic conditions and osmotic upshift. D values decreased with increasing molecular weight of the molecules. Upon osmotic upshift, the decrease in D of NBD-glucose was much smaller than that of macromolecules. Barriers for diffusion were found in osmotically challenged cells only for GFP and larger proteins. These barriers are likely formed by the nucleoid and crowding of the cytoplasm. The cytoplasm of E. coli appears as a meshwork allowing the free passage of small molecules while restricting the diffusion of bigger ones.

Muscle Regulation I

600-Pos Board B400
FHC-Linked Myosin Regulatory Light Chain Mutations A13T and K104E Do Not Generate Major Structural Changes Sufficient to Affect Binding of Myosin to Actin
Wenrui Huang, Priya Muthu, Katarzyna Kazmierczak, Danuta Szczesna-Cordary.
A sarcomere, the essential unit of cardiac muscle, consists of myosin, actin, troponin and tropomyosin. The interaction among these proteins generates muscle contraction. The myosin Regulatory Light Chain (RLC) attaches to the myosin heavy chain (MHC) IQ motif and structurally supports the lever arm domain, known to propagate minute conformational changes between the actin and myosin filaments. Mutations in the RLC may therefore cause alterations in the lever arm structure, thus leading to cardiac dysfunction. Here, we investigated two Familial Hypertrophic Cardiomyopathy (FHC) mutations in RLC, A13T (alanine to threonine) and K104E (lysine to glutamate) and hypothesized that these RLC mutants may lead to cardiomyopathy by changing the lever arm structure, the interaction with the MHC and ultimately impairment of the binding of myosin to actin. To test our hypothesis, I performed the following experiments: 1) Titration of the RLC-depleted porcine myosin with different concentrations of recombinant human cardiac RLC wild-type (WT) and A13T and K104E mutants to study the RLC-MHC binding properties (affinity, kinetics); 2) Study of the actin-myosin interaction by fluorescence and light scattering measurements. My preliminary data shows that the WT-RLC displays a clearly cooperative binding to the MHC, however, a reduction in cooperativity was observed in both mutants. In parallel, the binding affinity of A13T

and K104E was slightly decreased (1.3 fold and 1.4-fold increase in Kd, respectively). Fluorescence and light scattering measurements monitoring the binding of WT or mutant-myosin to pyrene-labeled actin demonstrated a very strong binding with no mutant dependent changes in Kd. These results suggest that any structural changes that may be caused by these two FHC-RLC mutations are not sufficient to affect the myosin-actin binding. Supported by NIH-HL071778 (DSC).

601-Pos Board B401

The HCM-Linked Ala13thr Mutation in the Cardiac Myosin Regulatory Light Chain Increases Isometric Force Production

Katarzyna Kazmierczak, Priya Muthu, Wenrui Huang, Ana Rojas, Michelle Jones, Yingcai Wang, **Danuta Szczesna-Cordary**.

The myosin regulatory light chain (RLC) is attached to the α-helical neck region of the myosin head, the so called lever arm, which connects the catalytic and actin binding domains with the thick filament backbone thus participating in the transmission of external forces to the myosin active site. It is understandable that mutations in the RLC associated with hypertrophic cardiomyopathy (HCM) may lead to alterations in force generation affecting cardiac muscle performance. Here, we studied the physiological consequences of an Alanine to Threonine (A13T) mutation in the N-domain of myosin RLC, found in population studies to cause HCM with a specific disease phenotype characterized by mid-ventricular obstruction. We observed an A13T-induced 30-50% increase in maximal force measured in skinned cardiac muscle fibers from transgenic Tg-A13T mice compared to control, Tg-WT and non-Tg littermates. Furthermore, a mutation-mediated 1.3-fold decrease in V_{max} and a 1.5-fold increase in K_m were observed in the actin-activated myosin ATPase activity compared with myosin from the healthy controls. The binding of Tg-myosin to pyrene-actin was similar for all groups of mice. No changes in the maximal myofibrillar ATPase or in the Ca^{2+}-sensitivity were noted. The same was true for the force-pCa relationship and the mutation did not introduce any alterations in the Ca^{2+}-sensitivity of force development. Gross morphological evaluation revealed enlarged inter-ventricular septa and left ventricles in the hearts from Tg-A13T mice, a phenotype observed in patients harboring the A13T mutation. Our results indicate that the A13T mutation may result in a hypertrophic response through abnormally increased force that may exceed the tolerance of a healthy myocardium. A decreased rate of cross-bridge turnover further demonstrates inadequate energy generation in Tg-A13T mice adding to impaired sarcomeric function. Supported by NIH-HL071778 (DSC).

602-Pos Board B402

Role of the Tail in the Regulated State of Myosin 2

HyunSuk Jung, Neil Billington, Kavitha Thirumurugan, Bridget Salzameda, Hitesh Patel, Christine R Cremo, Joseph M Chalovich, Peter D Chantler, Peter J Knight.

Myosin 2 from vertebrate smooth muscle and non-muscle sources is in equilibrium between compact, inactive monomers and thick filaments under physiological conditions, provided its regulatory light chains (RLCs) are not phosphorylated. In the inactive monomer, not only are the two heads compactly packed together, but the long tail is folded into three closely-packed segments that are associated chiefly with one of the heads. The molecular basis of the folding of the tail remains unexplained. Here, we show that compact monomers of smooth muscle myosin 2 have the same structure in both the native state and following cross-linking between Cys108 on the RLC and segment 3 of the tail. Non-specific cross-linking of the folded monomer by glutaraldehyde does not affect the compact conformation, and stabilises it against unfolding at high ionic strength. Sequence comparisons among both the RLCs and segment 3 of the tail suggest that folding of the tail is stabilised by ionic interactions between the N-terminal sequence of the RLC and the tail, and that phosphorylation of the RLC could upset these interactions. Close packing of the three tail segments may use the same ionic interactions between segments that stabilise interactions between extended tails in thick filaments. Our results support the view that interactions between the heads and the distal tail perform a critical role in reducing basal ATPase activity of myosin 2 molecules in compact monomers.

603-Pos Board B403

Two Mechanisms For Increasing Muscle Calcium Sensitivity by ROS

Sean M. Gross, Steven L. Lehman.

Cardiac muscle is sensitive to reactive oxygen species (ROS). Most measurements of calcium sensitivity following ROS exposure have shown a decrease, but others have measured no change or even an increase. One difference between studies was the activation state when ROS was applied. We therefore sought to characterize ROS-induced changes in myofilament proteins and their functional effects under different conditions of [Ca] and [ATP]. First, we identified all reactive cysteines in myofilament proteins by exposing myofibrils to a fluorescent maleimide probe under varying calcium concentrations, followed by SDS-PAGE. Only cysteines in troponin C had reactivity modulated by [Ca]. To find functional effects, we measured myofibril ATPase after exposure to 100uM DTDP in solutions at pCa 4.0 or 9.0. Ca sensitivity increased only when DTDP had been added at pCa 4.0. Next, we compared the reactivity of cysteines in myofibrils exposed to DTDP under rigor or relaxing conditions. Rigor conditions decreased reactivity of myosin cysteines but increased reactivity of actin cysteines, compared to relaxing conditions. We then measured ATPase rates in myofibrils exposed to DTDP under rigor or relaxing solutions. Exposure to DTDP in relaxing solution decreased both the maximum ATPase rate and the calcium sensitivity compared to myofibrils not exposed to DTDP (control). In contrast, when DTDP was exposed in rigor solution the minimum ATPase rate was increased from control, and there was an increase in calcium sensitivity. In conclusion, we found two activation dependent mechanisms to increase calcium sensitivity: the first calcium-dependent and specific to troponin C, the second dependent on whether myosin was bound to actin.

604-Pos Board B404

Cardiac Sarcomeric Proteins Reveal Differential Susceptibilities to Oxidative-Stress

Laura Harvey, Amelia Sumandea, Gail Sievert, **Marius P. Sumandea**.

Many models of oxidative stress lead to heart failure syndromes that are not associated with changes in Ca^{2+}-homeostasis, and are likely attributable to oxidative stress-dependent modifications of sarcomeric proteins. Yet, the possible sarcomeric targets and specific modifications are poorly understood. In the present study, we evaluate whether cardiac sarcomeric proteins manifest different sensitivities to metal (iron) catalyzed oxidative stress. Exposure of rat myocytes to H_2O_2 lead to the production of: i) myofilament-protein aggregates resistant to reducing (DTT) and denaturing (urea/thiourea) conditions; and ii) myofilament breakdown, even in the presence of cell permeable protease inhibitors. Pre-incubation of myocytes with cell-permeable metal chelators (like desferoxamine) completely abolished myofilament breakdown and aggregation. Similar myofilament-protein aggregates were detected in failing rat and human myocardium. Isolated rat ventricular myofibrils exposed to H_2O_2 and iron (Fe^{2+}) closely recapitulate cellular results. Dose dependent experiments reveal that most sarcomeric phosphoproteins undergo dephosphorylation and breakdown at lower oxidative stress levels compared with non-phosphoproteins.

605-Pos Board B405

Expression of Slow Tropomyosin (TM-) in Fast Fibers of Extraocular Muscles

Peter J. Reiser, Sabahttin Bicer.

We previously reported marked differences in the myosin heavy and light chain (MHC and MLC) isoform composition of fast and slow fibers between the global and orbital layers of domestic dog extraocular muscles (EOM) (Bicer & Reiser, Invest. Ophthalmol. Vis. Sci. 45:138-143, 2004 and 50:157-167, 2009). In addition, many dog extraocular fibers, especially from the muscle orbital layer, have MHC and MLC isoform patterns that are distinct from those in limb skeletal muscles. The results of these studies also suggested possible differences in the tropomyosin (TM) isoform composition of fast fibers between the two EOM layers, based upon gel electrophoretic mobility patterns. The objective of this study was to determine whether differences in TM isoform expression exist between fast global and fast orbital fibers in dog EOMs. TM isoforms in global and orbital single fibers were identified by SDS-PAGE and immunoblotting. Fast and slow fibers in the global layer express fast TM-α and slow TM-γ, respectively, plus TM-β, identical to fast and slow fibers in limb muscles. Slow orbital fibers express TM-β and TM-γ, as expected. Fast orbital fibers, on the other hand, have an unusual pattern of TM-γ, along with TM-β and TM-α. The same TM isoform expression patterns were found in Sprague Dawley rat EOM fibers. Co-expression of all three TM isoforms was not observed in single fibers in limb muscles of either species. These results contribute to the understanding of the elaborate diversity in contractile protein isoform expression among mammalian EOM fibers. Supported by the National Science Foundation.

606-Pos Board B406

Actin Binding Sites of Tropomyosin: An Evolutionary Structural Bioinformatics Analysis

Bipasha Barua, Sarah E. Hitchcock-DeGregori.

Tropomyosin (Tm) is a two-chained, α-helical coiled coil protein that associates end-to-end to form a continuous strand along actin filaments and regulates the functions and stability of actin. Mutations in Tm cause skeletal and cardiac muscle myopathies. We carried out a phylogenetic analysis of tropomyosin to