### Title

Vinculin Remodeling of the Sarcomere Lattice Regulates Contractile Function




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measurements from lizards that explore the dynamics of SOAE activity in response to transient external stimuli (e.g., clicks and tone bursts). While the large parameter space and the complicated nonlinear dynamics both present challenges, preliminary findings indicate that the model captures some features of the data (e.g., the generation of distinct SOAE spectral peaks) but not others (e.g., SOAE bandwidths and the dynamic range of their response to stimuli). We also found that oscillators within a ''frequency cluster'' exhibit complicated motions and poor phase coherence, indicating that clusters have significant internal dynamics.

**484-Pos Board B264**

**Chaotic Behavior of Oscillatory Hair Cells**

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The inner ear is capable of detecting sounds that elicit motions below the stochastic noise levels. Hair cells are the specialized sensory cells essential for the hearing process. They convert mechanical energy from incoming sound into currents by opening and closing mechanically sensitive ion channels, in response to the induced deflections. Hair cells of certain species are also known to oscillate without external stimulation. The role of these spontaneous oscillations is not understood, but they are believed to be a signature of an underlying active mechanism. As this active process constitutes one of the most important open topics in auditory research, a deeper understanding of spontaneous motility could have important implications on understanding the extreme sensitivity of hearing. The motion of spontaneously oscillating hair cell bundles has been described with a number of theoretical models. Hair cells have been shown to flicker between the oscillatory and quiescent states; this phenomenon was modeled with dynamic feedback acting on an internal control parameter that determines the dynamic state of the cell. This simulation was also able to predict a range of values for the Lyapunov exponent, which quantifies the level of chaos in a dynamical system. A positive Lyapunov exponent was predicted, indicating chaotic motion. We will present on experimental measurements of spontaneous hair bundle oscillations, which were obtained from the sacculus of the American bullfrog. Using the delay-coordinate technique, the phase space of the oscillator was reconstructed, allowing for estimation of the Lyapunov exponent. These estimates were consistent with predictions based of the feedback model. A lower Lyapunov exponent was found during mechanical stimulation, indicating that the detection of sound reduces the level of chaos in the hair cell.

**485-Pos Board B265**

**Visualization of Mechanical Forces within the Immunological Synapse**

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The interaction between the T-cell receptor (TCR) and the antigen-presenting cell (APC) has a central role in the adaptive immune system. It is of the utmost importance that this process is fast, specific, and highly sensitive. The T-cell is able to effectively screen through a sea of self- and harmless antigens in order to rapidly find those that are harmful. Dynamic signaling processes and membrane reorganization events such as the creation of the immunological synapse support this screening procedure. However, preliminary experiments indicate the involvement of mechanical forces in antigen discrimination. Therefore, we seek to characterize the TCR-imposed forces on the APC by directly introducing a force sensor within the immunological synapse. Well defined and calibrated digital, as well as analog, fluorescent molecular sensors will be attached to the peptide-presenting MHC complex (pMHCs). The distance between two introduced fluorophores can be precisely determined via Förster resonance energy transfer microscopy, and will serve as an exact measure for the involved pulling forces. By characterizing a statistically meaningful amount of the aforementioned events, a force map of the immunological synapse can be created. Downstream signaling will be measured via Calcium imaging, and correlated with the stimulatory potency of the involved pMHCs. The aim of this project is to develop a reliable tool to measure molecular forces between two adjacent cells, and to use the topological information to create force maps.

**486-Pos Board B266**

**Vinculin Remodeling of the Sarcomere Lattice Regulates Contractile Function**

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The human heart is capable of functioning for decades despite minimal cell turnover or regeneration, suggesting that molecular alterations help sustain heart function with age. However, identification of compensatory remodeling events in the aging heart remains elusive. We present the cardiac proteomes of young and old rhesus monkeys and rats, from which we show that certain age-associated remodeling events within the cardiomyocyte cytoskeleton are highly conserved and beneficial rather than deleterious. Targeted transcriptomic analysis in Drosophila confirmed conservation and implicated vinculin as a unique molecular regulator of cardiac function during aging. Cardiac-restricted vinculin overexpression reinforced the cortical cytoskeleton and enhanced myofilament organization, leading to improved contractility and hemodynamic stress tolerance in healthy and myosin deficient fly hearts. Moreover, cardiac-specific vinculin overexpression increased median life span by more than 150% in flies. A broad array of potential therapeutic targets and regulators of age-associated modifications, specifically for vinculin, are presented. These findings suggest that the heart has molecular mechanisms to sustain performance and promote longevity, which may be assisted by therapeutic intervention to ameliorate the decline of function in aging patient hearts.

**487-Pos Board B267**

**Ultrasensitivity of Cell Adhesion to the Differential Mechanical Cues and Requirement of Reversibility**

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Cell adhesion regulates critical cellular functions in adherent cells. Yet, the fundamental mechanism during the early events in cell adhesion remains unclear. At the most elementary level, the sensing of the mechanical environment must be performed by single molecules in mechanical contact with the environment, and the cell then must be able to process the single molecular events for its decision making. Herein, we utilized our recently developed DNA tether called tension gauge tether (TGT) to study the mechanical requirements of integrin-mediated cell adhesion. Previously, we showed that cells need to experience a threshold force of 40 pN through single integrin-ligand bonds to initiate adhesion and spreading and that just a few copies of strong (~ 54 pN) TGTs per cell are enough elicit to cell adhesion and spreading as long as there is a high density of weak tethers. Here, we show that 23 pN and 12 pN tethers, which are unable to induce cell adhesion individually, can induce cell adhesion if they are presented together to the cell. Therefore, the cells appear to be able to perform differential force measurements instead of absolute force measurements. Furthermore, we show, by direct single molecule imaging, a cell needs only two copies of 23 pN tethers for such differential force measurements. We also show that such ultrasensitivity to differential mechanical cues requires reversible formation of the weak mechanical tugs through integrins, suggesting that the presence of relatively stronger tethers as nuclei may keep the cell membrane close to the surface. How the cells can make an important decision of adhesion and spreading in response to the presence of just two integrins that experience stronger tension than other integrin molecules do without amplifying noise is current investigation.

**488-Pos Board B268**

**Live Quantification of Changes to Membrane Cytoskeleton due to Restricted Access to Laminin or Substrate Stiffness**

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Physical (stiffness and dimensionality) and chemical (cell adhesion promoting molecules) properties of cellular environment are thought to take key roles in tumorigenesis. Mammary epithelial cells undergo mesenchymal transition during invasive progression of breast cancer. We modeled basal membrane with different gel stiffness and altering laminin concentrations. Our results indicate carcinoma cells become invasive in stiff tissues in response of limited access to laminin at basement membrane. Better understanding of the transition dynamics requires single cell level live quantification of membrane cytoskeleton changes for different sizes of observation areas simultaneously, in relevant timescales. We facilitate bimFCS technique, a non-perturbing optical method giving opportunity to acquire data over tens of minutes with less than a minute temporal resolution in analysis in a time-course manner. Our method lets quantitative analysis of (1) the diffusion speed of these proteins in live cell membranes while freely moving between meshwork fences, (2) their strength of interaction with the cytoskeleton and (3) the average separation of fences.