activated by phorbol 12-myristate 13-acetate (PMA), which also affects shape and adhesion. Different cell types express different subsets of isoforms. In tracheal epithelial cells, the activation of beta and epsilon isoforms by PMA was followed by their degradation. We sought to determine whether such activation-degradation patterns affected protrusion formation or turnover. Cells were treated by transcriptional knockdown (KD) and the prevalence of each protrusion class was analyzed by computerized morphometry. Latent factors for filopodia (#4) and nascent neurites (#7) were calculated based on geometric variables determined for known populations. At zero time, factor #7 was elevated by alpha KD. Control samples, into which a random KD sequence had been introduced, showed an increase in neurites throughout the PMA exposure. During the time course, all KD samples differed from controls at one or more times. Epsilon KD followed by PMA drastically downregulated the isoform by 15 h and decreased #7 values relative to control. Alpha KD was similar suggesting that the early KD effect (increasing #7) was reversed later on. Beta KD cells resembled control at 15 h but alpha KD at the 5-h time. Filopodia were eliminated by a 2-h PMA exposure regardless of the KD agent introduced. Control samples remained depressed, but epsilon and alpha KD samples partially recovered by 5 h. Alpha KD declined again dramatically by 15 h. The time courses suggest that the main effects were exerted by the actin-binding PKC epsilon, but alpha, which is not actin-binding, could have similar effects. The data suggest that competitive binding on RACKs may be occurring, complicating the picture of kinase regulation of adhesion and protrusive activities.

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In Vitro Reconstitution of Remodeling Actin Asters - Steps towards a Minimal Active Actomyosin Cortex

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A major aim of our work is to understand the mechanisms behind dynamic organization of the cellular plasma membrane, especially local heterogeneities such as nanometer sized lipid domains (Mayor and Rao, 2004). As reported previously, glycosyl-phosphatidylinositol-anchored protein (GPI-AP) organization in nano-clusters in the plasma membrane is driven by the activity of cortical actin (Goswami et al., 2008).A recent theoretical framework and its experimental verification suggests that the engagement of short actin filaments together with myosin-motor like activity at the inner leaflet is sufficient to explain all the unusual features of GPI-AP organization at the outer leaflet (Gowrishankar et al., 2012).

Here, we present a strategy to reconstitute cortical actin dynamics *in vitro* on supported lipid bi-layers. This allows us to explore the role of proteins thought to be involved in actin cluster formation and to test predictions of the theoretical model. In a first step, we investigate how the diffusion of membrane bound actin binding proteins is affected by actin filaments of varying lengths. Then, we increase the complexity of the system including myosin motors, and actin modifying proteins, and identify conditions under which actin remodeling, i.e. transient formation of actin asters, occurs. As suggested by observation in cells and by the theoretical framework, short actin filaments (< 1 μm) are the main source of fast remodeling events whereas longer filaments create a more static meshwork, which can confine membrane bound particles. In summary, we introduce a new kind of minimal dynamic actin cortex, and show how dynamic short actin filaments can drive the organization of membrane components.

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Role of CARP as a Bio-Mechanosensor

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Cardiomyocyte mechanical stretch induces hypertrophic gene expression, however, the mechanisms for this are poorly understood. Cardiac ankyrin repeat protein (CARP) is highly expressed in cardiomyocytes and interacts with the spring domain of sarcomeric titin and is also localized in the nucleus. This dual localization suggests that CARP may couple titin spring mechanics to muscle gene expression. CARP is also expressed in cardiac fibroblasts (CF) and that CARP interacts with the transcription factor GATA4. We hypothesize that CARP is a bio-mechanosensor that upon cell stretch, translocates to the nucleus and interacts with GATA4 to induce gene expression. Methods: Neonatal Rat Ventricular Myocytes (NRVMs) and CF were isolated from 1-2 day old rats and cultured on BioFlex plates. After 2 days, NRVMs were transfected with CARP siRNA (50nM) and/or a GATA4-luciferase vector. NRVMs and CF were stretched 5-10% for 60 min (S60) to 48hr at 1Hz using the Flexcell system. Cells were fixed or lysed for microscopy, western blotting, or luciferase assay. Results: Unstretched NRVMs have predominantly sarcomeric CARP immunostaining with low nuclear CARP. CARP translocates to the nucleus with S60 and remains nuclear up to 48hr stretch. ERK inhibition with U0126 prevented S-60-induced CARP nuclear translocation. NRVM S60 induced GATA4 phosphorylation and increased GATA4-luciferase expression, which are both inhibited with CARP siRNA. NRVM S60 followed by cessation of stretch for 60 min (SC60) resulted in depletion of nuclear CARP. CF show nuclear CARP, which becomes cytoplasmic with S60. CF subjected to S60 followed by SC60 showed re-localization of CARP to the nucleus. Conclusion: We conclude that CARP is involved in stretch-mediated signaling in cardiac myocytes and fibroblasts. Our data further suggest disparate mechano-sensing roles for CARP in these cells types.

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Primary Cilia Respond to Uniaxial Strain by Reorienting and Elongating along the Axis of Stretch

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The primary cilium is a ubiquitous, microtubule-based organelle that protrudes from the cell surface and acts as the cell's antenna to sense extracellular signals, such as growth factors, fluid flow and developmental morphogens. Ciliary defects have been implicated in a diverse spectrum of diseases including retinal degeneration, polycystic kidney disease, mental retardation, obesity and situs inversus. In order to sense extracellular signals appropriately, cells must have primary cilia of certain lengths in each organ or tissue. However, it is unclear how cells control ciliary length. We hypothesize that assembly and disassembly of primary cilia are affected by the cellular environment, including mechanical stress from the substrate and cell shape. Recent studies indicate a relationship between the dynamics of actin filaments and the length of cilia. To test whether cell environment is involved in cilia length and orientation, we investigated the effect of uniaxial strain on cilia in retinal pigment epithelial (RPE) cells using a polydimethylsiloxane (PDMS) stretching device. After uniaxial strain, cilia elongated and rotated along the axis of stretch. In addition, we found that this behavior is dependent on actin network integrity and myosin activity. These novel findings propose a potential link between primary cilia behavior, cellular mechanosensation and actomyosin contractility. This stretching system can be used for understanding other biological problems related with mechanical stress, such as organelle size and orientation of cell division.

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Measuring Sub-Cellular Rheology in Zebrafish Embryos

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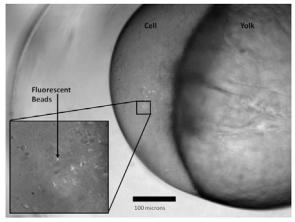
Cells are more than just bags of chemicals. The cell's mechanical properties, in addition to its chemical properties, play a large role in determining its motility, shape, differentiation, adhesion, and growth. Our goal is to measure these mechanical properties of the cell, specifically the frequency-dependent viscoelasticity of the cytoplasm at the sub-cellular level. Measurements on this scale allow us to link cytoskeletal rearrangements that occur during development with cellular viscoelastic evolution. Accordingly, we used particle tracking microrheology in the large (~500-micron-diameter) one-cell zebrafish embryo to accurately measure these parameters on a sub-cellular scale. In particle tracking microrheology, the Brownian motion of injected beads is tracked to determine both the viscous and elastic moduli of the cytoplasm. We injected fluorescent beads into the one-cell stage of the zebrafish embryo (see Figure) and assigned the beads to separate domains based on their radial, axial, and temporal positions within the cell. Viscoelastic values measured in each domain were compared with fluorescent data of the embryo's cytoskeleton to assess the current models of how cytoskeletal variations affect cellular rheology.



863-Pos Board B618

Mapping Internal Stress of in Vitro Cytoskeletal Networks with UV-Laser Ablation

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In vitro networks open up the possibility of investigating cytoskeletal components individually and in a very controlled environment. The process of