spacing and a height of 4-12 μm. In this range forces of 1-50 nN per pillar are measured. The PDMS pillars were stamped with partly fluorescently labeled fibronectin that allowed us to accurately determine the pillar deflections. Subsequently, 3T3 mouse fibroblasts were seeded onto the pillars. Immunostaining was employed using standard procedures to visualize the actin cytoskeleton and focal adhesion complexes. The actin cytoskeleton, focal adhesions and pillar deflections were imaged with a confocal spinning disk setup. From these results, we quantified the degree of co-orientation of focal adhesion elongation with force direction and the increase in stress fiber- and focal adhesion size with forces in the range of 1-15 nN.

1766-Pos Board B536

Mechanical Strain in Actin Networks Regulates FilGAP and Integrin Binding to Filamin A

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Mechanical stresses elicit cellular reactions mediated by chemical signals. Defective responses to forces underlie human medical disorders, such as cardiac failure and pulmonary injury. Despite detailed knowledge of the cytoskeleton's structure, the specific molecular switches that convert mechanical stimuli into chemical signals have remained elusive. Here we identify the actin-binding protein, filamin A (FLNa) as a central mechanotransduction element of the cytoskeleton by using Fluorescence Loss After photo Conversion (FLAC), a novel high-speed alternative to FRAP. We reconstituted a minimal system consisting of actin filaments, FLNa and two FLNa-binding partners: the cytoplasmic tail of ß-integrin, and FilGAP. Integrins form an essential mechanical linkage between extracellular and intracellular environments, with ß integrin tails connecting to the actin cytoskeleton by binding directly to filamin. FilGAP is a FLNa-binding GTPase-activating protein specific for Rac, which *in vivo* regulates cell spreading and bleb formation. We demonstrate that both externally-imposed bulk shear and myosin II driven forces differentially regulate the binding of integrin and FilGAP to FLNa. Consistent with structural predictions, strain increases ß-integrin binding to FLNa, whereas it causes FilGAP to dissociate from FLNa, providing a direct and specific molecular basis for cellular mechanotransduction. These results identify the first molecular mechanotransduction element within the actin cytoskeleton, revealing that mechanical strain of key proteins regulates the binding of signaling molecules.

"Mechanical strain in actin networks regulates FilGAP and integrin binding to filamin A"
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Insights into the Importance of Mechanosensing through the Focal Adhesion Protein p130Cas

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The Src substrate p130Cas has previously been identified as one of several focal adhesion proteins implicated in mechanosensing, the process by which cells feel changes in their physical environment. As a protein that has also been implicated in enhanced cell migration, actin dynamics, and focal adhesion turnover, the manner by which p130Cas orchestrates such sensing is becoming of increasing interest. In vitro studies have suggested that the central substrate domain may relay mechanosensing information via spring-like stretching of this domain. However, in vivo detection of such a mechanism has not been possible to date. To gain greater insight into this problem, we used polyacrylamide substrates of varying rigidities and observed the abilities of mouse embryonic fibroblasts (MEFs) to adhere, spread, and form focal adhesions by spinning disk confocal microscopy. By comparing cells lacking and stably re-expressing p130Cas, we addressed the following questions: 1) How sensitive is p130Cas to these mechanical changes (i.e. at what rigidity does p130Cas first become tyrosine phosphorylated)? 2) Is p130Cas critical to the ability of the cell to sense these changes (analyzed by tracking changes in focal adhesion characteristics as well as cell spread)? and 3) What is the mechanism underlying the ability of p130Cas to transduce mechanical signals (i.e. is there a spring-like domain within p130Cas)? Our multi-parameter experimental strategy allowed us to analyze increases in cell spread and focal adhesion organization as substrate rigidity was altered from ~100 Pa to 69 GPa. Interestingly, we observed that MEFs deficient for p130Cas displayed changes in cell spread and focal adhesion rearrangement at lower rigidity scales than was observed in wild-type counterparts, illustrating a need for p130Cas to sensitize cells to substrate rigidity.

1768-Pos Board B538

The Non-Equilibrium Thermodynamics and Kinetics Governing Coupled Stress Fiber and Focal Adhesion Dynamics

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We consider the coupled mechano-chemistry governing the cytoskeletal force in cells with stress fibers and focal adhesions. Our studies include published experimental work in which we monitor the cytoskeletal forces in cells on elastomeric substrates of micron-sized posts. These experiments demonstrate complex dynamics involving substrate strain as well as the binding/unbinding of cytoskeletal and focal adhesion proteins. Our model explains these dynamics, which underlie force generation and motility of, especially, mesenchymal cells. The broader motivation for this research comes from our ongoing work on cancer cell motility and invasion. Guided by our experiments, our model considers a single stress fiber, the focal adhesion by which it is attached to a micropost, the cell reservoir of cytoskeletal and focal adhesion proteins, the deforming micropost and underlying substrate. The complex mechano-chemistry that controls these sub-systems' interaction is itself governed by non-equilibrium thermodynamics: The binding/unbinding of proteins is driven by free energy changes due to chemistry, elasticity and mechanical work done. The stress fibers' mechanical response has viscoelastic and active contributions, the latter due to myosin contractility. Our model, an extension of our recently published work, generates a very rich range of responses depending on the mechanical and chemical boundary conditions, and parameter values (which are obtained from our experiments, and well-established estimates from the literature). This range of model responses includes every case observed in our experiments. We find that while applied strain and acto-myosin contractility dictate the increase of force in stress fibers over the short to medium time scale (~600 sec.), the longer time scale response (~1000-10000 sec) is dominated by the growth and disassembly of focal adhesions. These findings have direct implications for published and ongoing work on cancer cell locomotion in our group.

1769-Pos Board B539

Exploring Feedbacks Between Small GTPases, Phosphoinositides, and the Actin Cytoskeleton

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I survey work done in my group on eukaryotic cell polarity and motility. The crosstalk and feedback between small GTPases, phosphoinositides (PIs), and F-actin has been probed both experimentally and computationally. In so doing, we have identified both likely connectivity of these signaling networks and their functional roles in motility. We found that GTPases can account for most essential polarity-motility function, but PIs sensitize the cells, and help to filter out conflicting spatial cues. This work is joint with Stan Maree, William R Holmes, Ben Lin, Andre Levchenko, and Veronica Grieneisen.

1770-Pos Board B540

The Geometry of Branching Actin Networks from Capping Branching and Filament Growth

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Branching actin network growth is the primary engine driving cell motility. At the front of a motile cell, inside the lamellipodium, is a dense mesh of actin filaments pushing the membrane forward. The structure of the mesh is believed to be largely regulated by three processes: branching of new filaments off of existing ones, capping of filament tips stopping filament growth, and filament growth. Filaments inside the lamellipodium have been observed to organize into a strict orientation pattern where filaments are angled approximately −35/35 degrees from the direction normal to the membrane. It has been previously hypothesized that the three processes above are sufficient to generate the unique orientation pattern.

We derive and analyze an integro-differential PDE for the angular density of branching actin network by incorporating the three constituent processes. Our analysis implies that there exist multiple equilibrium angular distributions, which strongly suggests additional process that regulates actin filament orientation.

1771-Pos Board B541

Unraveling the Role of the SCAR/WAVE Complex in Regulating the Traction Stresses during Amoeboid Motility

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Chemotaxis, or guided cell migration plays an essential role in many key physiological and disease processes. Chemotaxis requires a tightly regulated,