signaling", depends on NOX2 (NADPH oxidase) to generate reactive oxygen species (ROS), which appears to oxidize ryanodine receptors (SR Ca^{2+} release channels) and increases their sensitivity to $[Ca^{2+}]i$. Stretch mediates X-ROS signaling through microtubules, which appear to interact with NOX2 to enable it to generate ROS.
We evaluated X-ROS signaling in murine atrial myocytes (C57/B6). Ca^{2+} sparks were recorded before and after stretch (10-20% of cell length). Ca^{2+} spark frequency increased during stretch and returned to pre-stretch values during relaxation. Interestingly, microtubule density was higher and protein expression levels were increased in murine atria compared to ventricles (Fig 1 A). Similarly, protein expression levels of the catalytic NOX2 subunit gp91phox were higher in atria compared to ventricles (Fig 1 A). Inhibition of gp91phox with the inhibitory peptide gp91ds-tat prevented stretch-induced Ca^{2+} spark increases in atrial myocytes (Fig 1 B). Thus, X-ROS signaling is also operative in atrial myocytes.

900-Plat
Novel Genetically Encoded Ratiometric Calcium Indicators
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Genetically encoded calcium indicators (GECIs) have recently undergone dramatic improvements in brightness, dynamic range, kinetics, and calcium sensitivity and now are capable of reporting single neuronal action potentials - comparing favorably to synthetic calcium dyes. Nevertheless, GECIs still lag behind synthetic calcium dyes in applications requiring quantitation of absolute intracellular calcium concentration. Here, we describe a novel approach to achieve dual-color ratiometric calcium measurements.
When expressed individually, GCaMP and mCherry, respectively, exhibit calcium-dependent and -independent fluorescence intensities; and when present at the same level, their fluorescence intensity ratio should provide an estimate of absolute calcium concentration. Unfortunately, the two proteins do not always express equally in all locations in a cell. A simple tandem construct ensures equal levels everywhere of the two proteins, but non-radiative energy transfer from GCaMP to mCherry reduces the dynamic range of GCaMP signals and leads to apparent calcium-dependent fluorescence changes of mCherry. We introduced a rigid alpha helix, the ER/K helix, between the fluorophores, in order to prevent significant Förster resonance energy transfer (FRET). In parallel experiments, we demonstrated in vitro and in cellulo that the tandem GCaMP-ER/K-mCherry construct provides quantitative estimates of calcium concentration, without suffering reduction in dynamic range due to FRET activity.

Platform: Cell Mechanics, Mechanosensing, and Motility I

901-Plat
Coupling of Apical Contractions and Adherens Junction Maturation by Synaptopodin-Depedent Recruitment of A-Actinin-4
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Cell-cell adhesive contact is a central player in mechanotransduction, providing both mechanical support and a hub for signaling. Yet, the molecular mechanism is poorly defined, partly due to a lack of cell culture model to mechanically manipulate tension at cell-cell contacts. Here, using live cell imaging of α-actinin, we showed that MDCK cells exhibit many cellular behaviors of epithelial cells in vivo, including (1) oscillating contractility of the adherens junction, (2) pulsatile centripetal cellular contractions, and (3) ratchet constrictions of apical junctional domain. We hypothesized that pulsatile contractions participate in the maturation of junctional complexes. To test our hypothesis, we applied cyclic pulsatile tension to cell-cell contacts in confluent cell monolayers. We found that pulsatile intercellular tension induces recruitment of α-actinin-4 to the adherens junction in a time and tension-dependent manner without changing the localization of canonical adherens junction proteins, suggesting that a missing factor is involved. We have identified this missing factor as synaptopodin. Synaptopodin recruitment to the adherens junction is also tension-sensitive, which is necessary for generation of contractility at cell-cell adhesions. Recruitment of a-actinin-4 strengthens cell-cell adhesion and promote epithelial permeability barrier. Thus, by controlling the mechanical force generated through apical contractility, epithelial cells can adjust the strength of cell-cell adhesion and the maturation process of adherens junction maturation. Conversely, synaptopodin dictates the efficiency of mechanical-biochemical coupling between contractility and junction assembly through the recruitment of a-actinin-4. Our studies reveal a tunable molecular system with both mechanical and biochemical inputs and underscore the complexity of junction assembly in epithelial monolayers in vivo and in cultured cell systems.

902-Plat
Two Distinct Actin Networks Mediate Traction Oscillations to Confer Mechanosensitivity of Focal Adhesions
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Adherent cells actively sense the mechanical stiffness of their extracellular matrix (ECM) by exerting traction force through focal adhesions (FAs), which are integrin-based protein assemblies. Also, FAs control cell spreading, proliferation, survival, differentiation, and migration. FA-mediated mechanosensation underlies cell durotaxis - the tendency of most cell types to migrate toward stiffer microenvironment. Strikingly, FA-mediated traction forces oscillate in time and space, and this oscillation governs durotaxis. The interactions underlying this intriguing spatio-temporal pattern of FA traction force are unknown, as are the contributions of these interactions to this mechanosensation. To address these questions, we established the first coherent, experimentally validated model of FA formation. The model integrated the spatiotemporal coordination between a branched actin network and stress fibers during FA growth. Our model predicted that retrograde flux of branched actin network contributed to a traction peak near the FA distal tip and that stress fiber-mediated actomyosin contractility generated a second traction peak near the FA center. Furthermore, a negative feedback loop involving formin-mediated stress fiber elongation and actomyosin contractility developed and resulted in oscillation of the center traction peak. This oscillation competed with the distal traction peak, and the competition underpinned oscillation of the FA traction maximum in time and space. More importantly, this negative feedback loop broadened the substrate stiffness range, over which the FAs could accurately adapt with traction force generation. Our findings shed light on the fundamental mechanism of FA mechanosensation and durotaxis.

903-Plat
Mechanical Activation of α-Catenin and Vinculin
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Shaping, maintenance and repair of adult tissues require fine-tuning of cell-cell adhesion. α-catenin, a cytoplasmic adapter links the actin cytoskeleton to cell-cell junctions, plays a central role in regulation of the cell-cell adherence. This regulation requires binding of α-catenin to vinculin in a force dependent manner. By stretching single α-catenin construct using magnetic tweezers, we find that force in physiological range can expose the vinculin-binding sites buried in α-catenin, drastically promoting subsequent binding of the head domain of vinculin with a nanoMolar affinity. The bound vinculin head then irreversibly locks α-catenin in its unfolded conformations after force is released. The bound vinculin head can however be displaced at high forces > 30 pN, resulting in a biphasic force dependent binding of α-catenin to vinculin head. Further, we find that full-length vinculin also binds to mechanically unfolded α-catenin, implying release of the auto-inhibition conformation of full-length vinculin. Together, these results provide important insights into mechanosensing at cell-cell adherence.

904-Plat
Molecular Regulation of Actin Turnover at the Leading Edge of Migrating Cells
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Actin couples molecular motors to extracellular adhesions and its turnover regulates cell polarization and protrusion efficiency. Actin filament turnover is regulated by polymerization and depolymerization at the ends, which is increased by severing filaments internally. To determine how these reactions affect F-actin turnover rate we simulated the molecular mechanisms revealed by experiments and determined the ODEs for changes in the average filament length and number of filaments that determine total F-actin. These results