mechanisms. Identifying the factors that control molecular motor distribution is essential for understanding cell division. The contractile ring forms by myosin assembly in the middle and disassembly in the flanking regions. To better understand the mechanical and biochemical mechanisms of contractile ring assembly, we imaged HeLa cells expressing MRLC-GFP by confocal microscopy at high spatial and temporal resolution. MRLC-GFP also marks spindle poles; this allowed us to study the correlation between mitotic spindle movements and cortical myosin distribution. We confirmed that the onset of myosin assembly in the contractile ring occurs in anaphase. We observed that the mitotic spindle exhibited oscillations upon entry into anaphase, similar to oscillations observed during the asymmetric division of C. elegans embryos by other groups. We used particle tracking software to follow spindle position and JFiIum to track cortical myosin intensity distribution during these oscillations. We further exerted controlled and localized forces while following the cellular response using fluorescence imaging. Anti-integrin coated micro-beads were attached to HeLa cells expressing MRLC-GFP. We applied external forces to a bead by an optical tweezers and imaged the dynamics of myosin and mitotic spindle during cell division. Experimental results showed that the spindle appeared to move to an asymmetric post perturbation position. This observation is consistent with the expected coupling between force-dependent cortical flow and spindle position and will be useful in more detailed studies of the response of cytoskeleton to external or internal forces.

2421-Pos Board B407
Effect of Tensile Stress on Smooth Muscle Cells
Soon-Mi Lim, Jerome P. Trzciakowski, Andreea Trache.
Cellular responses to mechanical stresses play an important role in the physiology of many cell types in healthy and diseased states. In vascular smooth muscle cells (VSMC), as in any other cells anchored to extracellular matrix, external mechanical stresses are imposed on a preexisting force equilibrium generated by the cytoskeletal tension. The ability to measure real-time mechanosensitive events at sub-cellular level in response to discrete and physiologically relevant mechanical stimulation is the critical component in understanding mechanically-induced cellular remodeling. Mechanical perturbation of a VSMC by the atomic force microscope (AFM) tip mimics the tensile stress in the vessel wall. By integrating tensile stress stimulation with simultaneous optical imaging using total internal reflection fluorescence and fast spinning-disk confocal microscopy, we have the unique opportunity of investigating in real-time the mechanically-induced cross-talk between apical and basal cell surface. Thus, we were able to measure vinculin and actin recruitment at focal adhesions upon tensile stress stimulation, and stress fibers bundling and reassembly. Understanding the real-time contractile and adhesion events associated with live VSMC response to force, provides fundamental new information regarding the coordinated cellular responses involved in VSMC adaptation to the local extracellular environment in the vessel wall.

2422-Pos Board B408
Cell Structure and Morphology Alterations Through Controlling Localized Substrate Stiffness on in Fibroblasts and Neuroblasts
Szu-Yuan Chou, Chao-Min Cheng, Chih-Cheng Chen, Philip R. LeDuc.
At the interface of extracellular-substrate interactions, substrate elasticities strongly influence cell type specific function and morphology. This finding is important in diverse areas including neural function, metastasis, and heart diseases. To address the effects of localized elasticity, we developed a new method to control the microenvironment through generating a substrate with localized alternating stiffnesses interacting with cells. We first fabricated polymeric microchannels (30µm and 100µm wide, both 50µm deep) through using conventional soft lithography with poly(dimethylsiloxane) (PDMS) and a 3:1-base/curing agent. We then poured 30:1-base/curing PDMS into the channels to produce alternating surfaces with elasticities of 800kPa and 200kPa adjacent to one another. We used these substrates and independently seeded NIH 3T3 fibroblasts and Neuro2A neuroblasts onto the systems to analyze their morphologies and structure. We found that fibroblasts attached on the soft surface had smaller projected area, lower cell density, and multiple cells versus single cell differences. Also, the neuroblasts had distinct patterns of neural outgrowth that emerged as they extended processes to these alternating stiffness substrates, depending on the location of the soma and the surface stiffness. We believe further use of this approach will promote greater understanding of cellular responses as well as insights into a variety of diseases that can be investigated using controlled cell-material interactions and substrate elasticities.

2423-Pos Board B409
Mechanics and Biochemistry of Matrix Assembly: Pulling it all Together
Chris A. Lemmon, Christopher S. Chen, Lewis Romer.
The interface between eukaryotic cells and the extracellular matrix directs key cellular functions. An emerging theme in cell adhesion biology is that cell-matrix interactions are regulated by both mechanical and biochemical signaling. Further definition of the cellular mechanics and the molecular signaling that pattern matrix will provide a new generation of targets and tools for developmental biology. We have used a unique approach to the study of the cell-matrix interface that allows for the quantitative and simultaneous study of cell traction force and fibronectin fibroligenesis at individual discrete sites of cell-matrix contact. This is accomplished by growing fibroblasts on the tops of microfabricated silicon posts with known spring constants, and using quantitative fluorescence imaging of post deflections and fibronectin. These studies show that fibrologenesis involves the translation of cell traction force from the cell periphery toward the cell center and a transition from compressive strain to neutral strain at the cell periphery. Further studies indicate that the establishment of these different zones of traction force and strain is dependent upon spatially regulated effects of Rho-family GTPases on the actin cytoskeleton.

2424-Pos Board B410
Exploring Cellular Mechanotransduction at the Focal Adhesions, One Molecule at a Time
Mohammad R.K. Moftad.
It is now widely established that living cells sense mechanical signals and respond actively by changing their phenotype accordingly. Cellular mechanotransduction is mediated by a combination of biochemical and biophysical mechanisms via mechanically induced changes in the structure and function of specific molecules. These mechanically responsive molecules can be described as the cell’s mechanosensors and can function to initiate key biological processes, such as focal adhesion formation. A series of molecular dynamics investigations will be presented to explore the potential mechanosensing and mechanotransduction functions of key molecules involved in focal adhesion formation and cytoskeletal dynamics, namely talin, vinculin, alpha-actinin and filamin. Using state-of-the-art molecular dynamics modeling and simulation techniques, the molecular mechanics of these proteins, their force-induced activation, and changes in their molecular conformation and binding partnership are investigated.

2425-Pos Board B411
Single Microtubule Dynamics with Nanometer Resolution: Effects of End-Tracking Proteins and Force
Olga Svtina, Svenja-Marei Kalisch, Marileen Dogterom.
Microtubules (MTs) are one of the main cytoskeletal polymers in all eukaryotic cells. Their polymerization dynamics depends on the activity of regulatory factors as well as forces generated at dynamic MT ends. In our experiments, we study molecular mechanisms of microtubule (MT) self-assembly, force generation, and regulation by plus-end tracking proteins with nanometer spatial and nanometer temporal resolution. We will present our experimental technique which integrates regular optical tweezers with an optical line trap, coupled to high-resolution DIC microscopy, and microfluidic cells with incorporated microfabricated barriers. In the experiment a microbead is bound to an axoneme, which serves as a nucleation site for MT growth. This construct is trapped in the laser beam and oriented against a rigid barrier. MT polymerization from the axoneme is initiated by injection of a reaction mix (including tubulin and GTP). The displacement of the bead is monitored with nanometer resolution using MT growth and shrinkage. In our tweezers assay we compare single MT dynamics with and without the presence of plus-end tracking proteins such as Mal3, Tea2, and Tip1. We incorporate a force feedback mechanism to be able to monitor MT dynamics under constant load.

2426-Pos Board B412
A direct Force Measurement Reveals that Human Microtubule-Associated-Protein tau Modulates the Interactions Between Microtubules in an Isoform Dependent Manner
M.C. Choi, Peter J. Chung, Uri Raviv, Yousif Li, Erkan Kiris, Herb P. Miller, Leslie Wilson, Stuart C. Feinstein, Cyrus R. Safinya.
Microtubules (MTs), a major component of the eukaryotic cytoskeleton, are 25 nm protein nanotubes with walls comprised of assembled protofilaments
built from $\beta$ heterodimeric tubulin. A variety of microtubule-associated-proteins (MAPs) bind to tubulin subunits and regulate microtubule dynamics, although the process in which this occurs is not well understood. In mature neurons MAP tau promotes MT assembly while in developing neurons MAP tau also plays a critical role in axonogenesis [1]. Furthermore, aberrant tau function has been implicated in numerous neurodegenerative diseases, such as Alzheimer’s, Pick’s, and supranuclear palsy. Understanding the interactions between MAP tau and MTs will be critical in further elucidating the role that MAP tau plays in neurodegenerative diseases. We examined the effects of the human MAP tau on the assembled structure of taxol-stabilized MTs under osmotic pressure (mimicking the crowded environment of axonal neurons) using synchrotron small angle x-ray scattering (SAXS) and binding assays [2, 3]. Previous work had shown that MAP tau isoforms regulate the distribution of protofilament numbers in MTs resulting in an increase in the average MT radius with increasing tau [4]. Significantly, the pressure-tau concentration phase diagram (using the SAXS-osmotic pressure technique) reveal that tau does indeed modify the interactions between MTs in an isofrom dependent manner. Supported by DOE DE-FG02-06ER64314, NSF DMR-0803103, NIH NS35010, NIH NS13560.


2427-Pos Board B413

Single-Molecule Dynamics of FtsZ During Cell Division
Dongmyung Oh, Lili Niu, Ji Yu.

Escherichia coli cell division is initiated by the formation of FtsZ-ring structure at the middle of cell body while the internal mechanisms and Min system regulate the special and temporal localization of FtsZ protein. During division process the FtsZ protein interacts with many other division-involved or -associated molecules, which are located in cytoplasm, inner membrane and periplasm, expectating the variation in the dynamics of FtsZ-ring structure and molecule itself in wild and mutant types. We studied the dynamics of FtsZ-ring width during different stages of cell cycle by using photoactivated localization microscopy (PALM) technique. In addition, tracking individual FtsZ molecules in live E. coli cell expressing with or without their interacting molecules we measured the difference in physical parameters such as diffusion coefficient and local compartment size. Preliminary data shows the width of Z-ring increases during Z-ring development, The diffusion coefficient of filamentous FtsZ molecule is $0.017 \pm 0.0012 \mu m^{2}/s$ (n = 332) and the diffusion coefficient of globular FtsZ is $0.75 \pm 0.04 \mu m^{2}/s$ (n = 30).

2428-Pos Board B414

Single Molecule Dynamic Studies of UgtP, an Inhibitor of FtsZ

Assembly of the highly conserved cytoskeletal protein FtsZ into a ring structure at the nascent division site is the first step in bacterial cell division. In the soil bacterium Bacillus subtilis, the UDP-glucose binding protein, UgtP, plays a central role in cell size homeostasis, by inhibiting FtsZ and increasing cell size in during growth in carbon rich medium. Previous work indicates that UgtP activity is controlled via UDP-glucose dependent changes in localization. Under carbon rich/high UDP-glucose conditions, UgtP is distributed throughout the cell and concentrates at the FtsZ ring where it delays division until cells reach the appropriate size. Conversely, under carbon poor/low UDP glucose conditions, UgtP is sequestered away from FtsZ in small, punctate foci [1]. Using novel single-molecule fluorescence image analysis methods, SIMA [2] and IPMC [3], we study the spatial distribution, kinetics, and dynamics of UgtP in live B. Subtilis cells. We observed randomly distributed specific binding sites where the UgtP aggregate, and nonspecific membrane binding events where the mean UgtP association time is seconds. Our images indicated that approximately half of the proteins diffuse in the cytoplasm, while the other half was bound to the membrane. A helical diffusion pathway in the membrane has also been observed, suggesting possible connections with previous reports of a helical diffusion pathway for FtsZ.


2429-Pos Board B415

Cryo-Electron Microscopy, Tomography, and Single-Particle Analysis of MSP Filaments Derived from the Ameobic Spord of Amano Suum
Andrew J. Maloney, Murray Stewart, Thomas M. Roberts.

Locomotion of nematode sperm is remarkably similar to that of most other crawling cells but is powered by a system of filaments composed of major sperm protein (MSP) instead of the actin-myosin machinery typically associated with amoeboid motility. The MSP motility apparatus has been reconstructed in vitro, and individual MSP filaments can be generated by the addition of ATP to detergent-treated *Amano* sperm cytoplasm. Filaments formed in this way have been successfully vitrified and examined using cryo-electron microscopy, and this has allowed for the structural analysis of filaments formed in the presence of MSP accessory proteins. Single particle reconstruction techniques have been applied to individual filaments sampled along their lengths, and whole filament meshworks have been reconstructed using electron cryotomography. These resulting physiological models were compared to earlier models derived using purified MSP, including a helical reconstruction of filaments polymerized in ethanol (King et al. 1992. JCS 101:847) and an x-ray crystal model of MSP subfilaments (Bullock et al. 1998. NSB 5:184). Comparisons suggest important differences between filaments formed under physiological and nonphysiological conditions. There are currently six *Amano* sperm proteins known to modulate MSP filament dynamics in sperm; these same proteins are absent from filaments prepared using purified MSP. Comparing MSP filament models with and without these accessory proteins allows us to map the binding sites of these accessory proteins and provides a greater understanding of how they effect MSP filament dynamics and influence motility. Supported by NIH Grant R37 GM29994 and by the American Heart Assoc.

2430-Pos Board B416

Structure and Mechanics of Desmin Protophilibrils Explored with Scanning Force Microscopy
Balazs Kiss, Miklos S.Z. Kellermayer.

Desmin filaments form the intermediate filament system of muscle cells where they play important role in maintaining mechanical integrity and elasticity. Although the importance of desmin elasticity and assembly/disassembly dynamics in cellular mechanics is being increasingly recognized, the molecular basis of neither desmin’s elasticity, nor its disassembly pathway are well understood. In the present work we explored the topographical structure of purified and reconstructed desmin filaments by using scanning force microscopy. Upon the addition of divalent cation chelators EGTA or EDTA the filaments disassembled on a time scale of hours to days into stable, thin fibrillar components with variable (up to micrometer) length, smooth surface and uniform thickness. Based on topological considerations the filamentous structures were identified as protophilibrils. Desmin protophilibrils appear as elastic structures with a persistence length of 51.5 nm. The Young’s modulus of the protophilibrils (10.6 MPa) far exceeds that of the mature filament (3.7 MPa). Thus, protophilibril bundling within the desmin filament results in high longitudinal tensile strength at a large bending flexibility. The stability of protophilibrils suggests that desmin may disassemble along a pathway quite distinct from that of the assembly.

2431-Pos Board B417

Modeling the Self-Organization Property of Keratin Intermediate Filaments

Keratins, a group of eukaryotic intermediate filaments, have an important function toward structural support in epithelial cells and tissues. Formation of crosslinked networks, e.g., bundles, is necessary to achieve the mechanical properties necessary to sustain this vital role. In progenitor basal keratinocytes of the epidermis, intermediate filaments made of keratin 5 (K5) and keratin 14 (K14) exhibit a bundled organization. Recent findings from our laboratory showed that bundle formation can be self-mediated through interactions involving K14’s non-helical tail domain and two distinct regions within the central rod domain of K5. Here, we exploit theoretical principles and computational modeling to investigate how these biochemical interactions best promote filament bundling. We develop a simple model where keratin filaments are treated as rigid rods to apply Brownian dynamics simulation. Our result shows that there should be long-range filament interactions, along with tail domain-mediated stabilization of filament-filament interactions, for stable bundle formation. Our model can also explain the difference of the mechanical properties of keratin networks between wild type and disease-causing mutant keratin proteins. The possible source of long-range filament interactions is discussed.