including the kidney filter protein nephrin. In addition to discrete binding sites for actin and nephrin, CD2AP possesses three SH3 domains and a proline-rich region containing, in turn, binding sites for SH3 domains. CD2AP is implicated in dynamic actin remodeling and membrane trafficking that occurs during receptor endocytosis and cytokinesis. We have initiated structural studies of recombinant CD2AP protein using electron microscopy and single particle image analysis. Negative stain electron microscopy of revealed uniform particles with a size and morphology suggesting a tetrameric organization, subsequently verified with chemical crosslinking. Single particle image analysis was used to generate a three-dimensional map of the CD2AP tetramer at 21 Å resolution. The electron density map reveals an extended structure allowing the identification of specific subdomains. The tetramer is organized around a central core, including density assigned to the C-terminal coiled-coil domain, surrounded by four loosely attached arms radiating out from the center, which we have assigned to the N-terminal SH3 domains. We have further identified CD2AP as a substrate for cytoplasmic cathepsin L, a protease that is induced in early podocyte damage. Cleavage of CD2AP with cathepsin L results in a C-terminal core domain that is structurally competent but releases the CD2AP binding partner dendrin resulting in translocation of dendrin to the nucleus where it promotes apoptosis. Based on our analysis of the cathepsin L cleavage sites within CD2AP we conclude that cytosolic cathepsin L releases the N-terminal arms producing a structurally competent C-terminal core domain.

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Prestress-dependent Rheology of Semiflexible Polymers of the Cytoskeleton

Arnab Majumdar[1], Noah Rosenblatt[2], Adriano M. Alencar[3], Bela Suki[1], **Dimitrije Stamenovic[1]**.
[1]Boston University, Boston, MA, USA, [2]University of Illinois at Chicago, Chicago, IL, USA, [3]University of Sao Paulo, Sao Paulo, Brazil.

Rheological properties of living cells are essential for their physiological functions. Microrheological measurements have shown that cytoskeletal contractile stress (or prestress) and weak power-law viscoelasticity are governing principles of cell rheology, and that these two properties are closely associated in living cells for reasons that are largely unknown. In this study, we develop a stochastic model of a semiflexible polymer of the cytoskeleton that links the power-law rheology to the prestress. We describe a semiflexible polymer chain as a three-dimensional elastically-jointed chain composed of nonlinearly elastic bonds jointed by linearly elastic torsional springs. Assuming that the chain dynamics is thermally driven, we use a Monte-Carlo-based algorithm to obtain numerical simulations of the chain's creep behavior during uniaxial stretching. We obtain that the creep curves follow a power-law and that this behavior changes with prestress in a manner that is consistent with previously reported data from living cells and reconstituted crosslinked actin gels. We show that the power-law creep results from a finite-speed propagation of free energy from the chain's end points towards the center of the chain in response to externally applied stretching force. We also show that the power-law dependence on the prestress results from the chain's nonlinear, stiffening behavior that originates from both entropic and enthalpic contributions. Based on qualitative similarities between model simulations and experimental data from living cells and actin gels, it is conceivable that the mechanisms embodied in our model may also be key determinants of the overall viscoelastic properties of living cells and actin gels.

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Straining the Laws of Attraction: Mechanotransduction Studied Through Changes in Intracellular Binding Energy

Nur Aida Abdul Rahim[1,2], Mohammad R.K. Mofrad[3], Peter T.C. So[1], Roger D. Kamm[1].
[1]MIT, Cambridge, MA, USA, [2]National University of Singapore (NUS), Singapore, Singapore, [3]University of California, Berkeley, Berkeley, CA, USA.

Mechanical force modulates myriad cellular functions including migration, alignment, proliferation, and gene transcription. Mechanotransduction, the transmission of mechanical forces and its translation into biochemical signals, may be mediated by force-induced protein conformation changes, and subsequently result in the modulation of protein signaling cascades. For the paxillin and focal adhesion kinase interaction, we demonstrate that mechano-induced changes in protein complex conformation, dissociation constant, and Gibbs free energy of binding can be quantified by lifetime-resolved fluorescence energy transfer microscopy and fluorescence correlation spectroscopy. A comparison with in vitro data shows that this interaction is allosteric in vivo, and spatially resolved imaging indicates that this binding constant is equal in both the cytosol and focal adhesion complexes. Further, inhibitor assays show that the mechano-sensitivity of this interaction must be mediated by soluble factors not based on protein tyrosine phosphorylation.

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Salt Dependence of Neurofilament Gel Phase Behavior - A Synchrotron X-ray Scattering Study

Joanna Deek, Jayna Jones, Roy Beck, Cyrus R. Safinya.
University of California, SB, Santa Barbara, CA, USA.

Neurofilaments (NFs) are cytoskeletal proteins expressed in neuronal cells, and are believed to play a role in the determination and maintenance of the neuronal cell shape and mechanical integrity. NFs self-assemble as flexible cylinders from 3 protein subunits: NF-Low (NF-L), NF-Medium (NF-M), and NF-High (NF-H). The three subunits are structurally conserved with the exception of their ''tail'' domains, composed of amino acid strands of increasing length and charge respectively. Screening of tail charges is achieved by varying the salinity of the in vitro buffer. At high concentrations, the filaments interact amongst themselves through their unstructured tails that branch out from the filament core and form a viscous gel. Polarized microscopy was used to map out phase diagrams of the resultant neurofilament hydrogels, and thus characterize salt dependent phase behavior. Reassembled separate networks of NF-L + NF-M and NF-L + NF-H show phase transitions from isotropic to nematic gel phases at distinct salt concentrations, thus reflecting the differences in the governing sidearm interactions [1].
We will describe synchrotron x-ray scattering experiments that have allowed us to quantitatively study the microscopic structure of the NF gels: shifts in average interfilament spacing demonstrate the phase boundary between the isotropic and anisotropic NF-liquid crystal gel phases and how they are shifted as a result of varying the in vitro buffer salt concentrations. Funding provided by DOE DE-FG-02-06ER46314, NIH GM-59288, NSF DMR-0503347.
[1] J.B. Jones, C.R. Safinya, Biophys. J. 95, 823 (2008).

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Multi-Parameter Analysis of Spindle and Cell Cycle Dynamics in Asymmetric Cell Division

Kemp W. Plumb, Vincent Pelletier, Susi Kaitna, Jackie Vogel, Maria L. Kilfoil.
McGill University, Montreal, QC, Canada.

Budding yeast is a unicellular organism which undergoes an asymmetric cell division. Successful completion of this process requires the assembly and translocation of the mitotic spindle from the mother cavity interior to the plane of cell division prior to segregation of the chromosomes. We use confocal fluorescence microscopy and automated image analysis algorithms to quantify the movement of the mitotic spindle, throughout the cell life cycle, at high spatial and temporal resolution. By selecting fluorescent proteins which specifically label the spindle poles and cell periphery, the spindle dynamics can be characterised within a coordinate system relevant to the cell division. Spindle length and orientation as well as the mother and bud cavity volumes have been extensively quantified in wild type cells. Our observations reveal fluctuations in spindle length and angle in the short pre-anaphase spindles that are distinct from longer anaphase spindles. Furthermore they indicate an apparent correlation between cell morphology and spindle dynamics. To elucidate the contributions of individual intercellular force generators to the fluctuations observed during spindle assembly and positioning, we apply the above methods to cells bearing deletions of the kinesins cin8, kip1 and kip3. It is expected that this work will ultimately enable a connection between observable single cell dynamics and biochemical signal pathways measurable by bulk assays.

Microtubular Motors I

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Synthesis Of Novel Fluorescent Atp Analogue And Interaction With Nucleotide Dependent Motor Proteins

Taro Kimura, Masafumi Yamada, Masato Ito, Shinsaku Maruta.
Soka University, Hachiohji, Japan.

Previously, several kind of fluorescent ATP analogues have been synthesized for the application to the kinetic study of ATPase. However, some of the ATP analogues exist as mixture of isomers and showed small fluorescence changes during ATP hydrolysis.
For instance, 2′(3′)-O-NBD-ATP and 2′(3′)-O-Mant-ATP have isomer of 2′ and 3′ in their ribose moiety and each isomer performs differently as substrate for the ATPases. In the present study, we have tried to synthesize new fluorescence ATP analogues that have no isomer and show significant fluorescence change during ATP hydrolysis. The fluorescent ATP analogue 6-(N- (7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino) ethyl triphosphate (NBDTP) and N-methylanthraniloyl amino ethyl triphosphate (MANTTP) have been designed and synthesized, which are similar to non-nucleotide ATP analogue 2-[(4-azido-2-nitrophenyl) amino] ethyl triphosphate (NANTP). It is known that NANTP