cofilin and cofilin bound to the filament in our model is expressed as a function of the torsion angle on the cofilin-bound filament based on the Gibbs-Duhem relation. According to our analyses, binding and dissociation of cofilin from the filament are equivalent at a critical torsion angle. This critical angle is about -164[deg/2.75 nm] and is almost constant along the length of the filament, except in case of very short actin filaments. The chemical potential difference is negative above and positive below the critical angle. Therefore, untwisting the double helix of the cofilin-bound filament causes cofilin to dissociate from the filament. Furthermore, the double helical nature of the filament likely induces stretch-twist coupling. Thus, tensile forces induce changes in the torsion angle of the filament, causing a positive chemical potential difference leading to the dissociation of cofilin from actin.

632-Pos Board B511
Slow skeletal muscle actin
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It had been thought that vertebrates synthesize the same isoform of sarcomeric actin in all skeletal muscles. The present work demonstrates the existence of a unique variant which accounts for all of the sarcomeric actin in the slow skeletal trunk muscle of Atlantic herring. Residues 48–375 of the primary structure have been inferred from nucleotide sequencing (Acc# EF495203) and residues 48–61 confirmed by Edman based sequencing of a fragment generated by subtilisin cleavage. EF495203 differs from the same segment of slow skeletal muscle actin from salmonids (Mudalige et. al. FEBS J. (2007) 274, 3452-3461) by a single residue (# 353), but there are eleven and ten substitutions, respectively, between EF495203 and salmon fast skeletal actin and rabbit skeletal actin. At least half of these substitutions are of a non-conservative nature. Actins isolated from different skeletal muscles from herring and salmon, but not rabbit, chicken and frog, can be differentiated by electrophoretic mobility at alkaline pH in the presence of 8M urea; digestion with various proteases, including thrombin, subtilisin and V8, and resistance to induced-denaturation. The melting temperatures of various G-actins (Ca.ATP) are: ~45 (salmon slow skeletal muscle); ~50 (herring slow skeletal muscle) and ~55 degrees C (salmon, herring and rabbit fast skeletal muscle). Possible sources of the enhanced chain flexibility will be discussed. The demonstration of slow skeletal muscle actin in two unrelated teleosts indicates that it is not a lone occurrence.

633-Pos Board B512
Binding Studies Between Cofilin And Actin Using Fluorescence Resonance Energy Transfer And Molecular Modeling
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The actin cytoskeleton is a 3-dimensional network within all eukaryotic cells. The assembly of actin monomers (G-actin) to form filaments (F-actin) is regulated by a number of actin binding proteins (ABPs). Cofilin is a principle regulator of actin dynamics and is essential for cell division and changes in cell shape. A full understanding of the functional effects of cofilin on actin is not possible due to the lack of an atomic-resolution model of the actin-cofilin complex. To date, several predicted models have been proposed based on the putative cofilin-actin interface. The actin-binding surface of cofilin was identified from structural homology with other ABPs, mutagenesis and NMR. Recently a new model was proposed (Kamal JKA et al, 2007, Proc Nat Acad Sci USA 104:7910) which identified the cofilin-binding interface on actin using radiolytic footprinting. Additionally, we have generated 16 possible cofilin-actin complexes *ab initio* using RosettaDock software (http://www.rosettacommons.org) by inputting the atomic models of actin and cofilin.
In vitro mutagenesis within cofilin has been undertaken to provide sites suitable for modification with extrinsic fluorescent probes, while preserving the capacity of cofilin to bind to G-actin. Selective mutation of several amino acids in cofilin has allowed us to identify mutants that remain functional. To test the proposed actin-cofilin models, intermolecular fluorescence resonance energy transfer (FRET) spectroscopy has been performed to calculate distances between several sites on cofilin and actin. These FRET distances will be used as constraints to generate a molecular model of the cofilin-actin complex.

634-Pos Board B513
Starting Actin Filaments Anew - Adenomatous Polyposis Coli Is an Actin Nucleator
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Adenomatous polyposis coli (APC) protein has emerged as a complex, multifunctional regulator in the Wnt-signaling pathway and in controlling the actin and microtubule cytoskeleton during basic cellular events such as cell polarization, migration, adhesion, chromosome segregation, and apoptosis. Here we demonstrate that APC directly binds F-actin and also affects actin dynamics by acting as a potent actin nucleator regulated by the microtubule (+)-end tracking protein, EB1. In addition, we show APC mediates the formation of cytoskeletal networks by actin bundling and regulated crosslinking of actin filaments with microtubules (MTs). These newly-identified functions of APC suggest that APC may function at the intersection of microtubule (+) - ends and actin rich zones to direct cell polarity and motility, and that these cytoskeletal APC-based functions may be important for the initiation and progression of cancerous tumors.

635-Pos Board B514
Measuring Molecular Interaction between Actin Filament and Actin Binding Protein Governing Mechanical Properties of Cross-Linked F-Actin Network
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Actin binding proteins (ABPs) regulate the assembly of actin filaments (F-actin) into various structures that provide physical support for the cell and play important roles in numerous cellular processes. Although the mechanical properties of F-actin networks have been extensively studied, a full understanding of how molecular interactions between ABPs and actin filaments influence the network mechanical properties of F-actin network is lacking. Here, we study the molecular interaction between ABPs and actin filaments and the mechanical rupture of a cross-linked F-actin network. A single molecule assay was used to measure the rupture force of a complex formed by an ABP filamin linking two actin filaments by pulling one of the filaments with the optical tweezers. The rupture forces for filamin/F-actin complex were 88 ± 34 pN for loading rates of 1209 ± 119 pN/s. About 20% of the force-extension traces exhibited a sawtooth pattern with intervals ~30 nm comparable to length changes due to unfolding of individual immunoglobulin domains of filamin. We also prepared the F-actin gels and monitored their mechanical responses while an embedded bead was displaced at constant speed. Compared to similar experiments with an entangled F-actin solution, the force-extension curves for the filamin/F-actin network exhibited multiple transitions in the force of 37 ± 17 pN, suggestive of bond rupture. In addition, using the optical tweezers, active microrheology was used to measure the strain-dependent mechanical properties of the filamin/F-actin network. Results demonstrate the network to be highly nonlinear and irreversible. Support from the NIGMS (GM-076689) is gratefully acknowledged.

636-Pos Board B515
Interaction of CapZ with Actin: Molecular Mechanism and Regulation
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The heterodimeric actin-capping protein (CP) is a major capper of barbed ends of actin filaments in eukaryotes, which prevents the incorporation or loss of actin subunits. CP regulates actin-dependent events in cells, including controlling cell shape and movement. CP is regulated by CARMIL, which inhibits CP in vitro and proposed to be able to physically remove CP from actin filaments. Here, we have identified the residues on the surface of CP that are important for binding to actin and to CARMIL. Previous cryo EM studies and computational docking studies predicted the residues involved in the interaction of CP and actin filaments, and functional assays with site-directed mutants of CP confirmed the predictions. Using TIRF (total internal reflection fluorescence) microscopy, we observed that adding CARMIL rapidly changed capped actin filaments to grow, consistent with uncapping. Together, these results extend our understanding of how CP binds to the barbed end of the actin filament.

637-Pos Board B516
Structural Binding Model of Cofilin and F-actin
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The regulation of actin polymerization within the cell is critical for many cell functions. Cofilin plays an important part in this process since it binds and severs actin filaments leading to depolymerization as well as the creation of new barbed ends. Although the details of cofilin's interaction with G-actin have been elucidated through a range of experimental studies, the specific interactions with F-actin have remained more elusive. Here we present the results of a detailed computational study involving a combination of molecular dynamics simulations and protein-protein docking. The resulting structural model for the cofilin/F-actin complex matches very well with existing cryoEM and mutagenesis data. Further insight is gained from a sequence and secondary

structure alignment of cofilin analogs that strongly supports our binding model. The implications of this binding model in the function and severing action of cofilin are discussed.

638-Pos Board B517

Energy Coupling In Profilin-Dependent Actin Polymerization

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Profilin regulates actin polymerization in cells and is required for normal cell proliferation and differentiation. The molecular mechanism of profilin has been extensively studied and debated in the last 25 years, however no satisfactory explanation consistent with the laws of thermodynamics has yet been provided. In our recent article (Biophys. J. BioFAST: doi:10.1529/biophysj.108.134569) we demonstrate that the mechanism of profilin action could be based a general principle of indirect energy coupling which, as emerges from recent theoretical and experimental research, underlies many biologic processes.

In presence of profilin, there are two possible pathways for actin filament elongation. First pathway (g) is direct elongation through binding of G-actin subunits to the barbed end to obtain a filament one subunit longer. The second pathway (pg) is elongation through binding of profilin to G-actin, formation of profilin-actin complex, then binding of the profilin-actin complex to the barbed end with subsequent dissociation of profilin. In the absence of profilin, there is only one pathway g_0, which is the pathway g in the absence of profilin.

Our analysis shows that the pathway g for filament elongation in presence of profilin is not energetically equivalent to the pathway g_0 for the filament elongation in the absence of profilin due to the dependence of standard free energy change for both pathways g and pg on profilin concentration. Conventional calculations of energy imbalance have neglected the difference between the pathways g and g_0. We found that profilin can lower actin critical concentration even when the pathways g and pg are energetically equivalent. In this case the existence of the pathway pg can drive the filament nucleotide profile toward ATP-bound F-actin, making both pathways g and pg more energetically favorable than the single pathway g_0 available in the absence of profilin.

639-Pos Board B518

Computational Study Of Viscoelasticity Of Crosslinked Actin-like Networks

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Mechanical force is very significantplays many important roles in eukaryotic cells in whichwhere the cross-linked actin cytoskeleton consisting largely of actin and actin binding proteins is one of the major structural components. Thus, the investigationit is critically crucial to study the of rheological properties of actin networks is indispensable in order to elucidate cell mechanics and various related cellular processes. Using Brownian dynamics, we develop a computational model equipped withthat incorporates virtues features such as including repulsive forces between actin filaments, realistic network morphology, and the consideration oftakes into account the stiffness, binding, and unbinding of actin cross-linking protein (ACP). Via bulk rheology and the analysis of thermal fluctuations of actin filaments, we elucidate investigate the viscoelastic properties of actin networks in under diverse aspectsconditions. We first validate ourThe model is first validated model by comparison with an experiments performed under similar conditions. Then, we study the influences of prestrain and ACP concentration on viscoelastic moduli, G' and G'', are examined. The storage modulus, G', tends is found to increase and becomes almost nearly independent of frequency at high ACP concentration or at large prestrain. We also find that the behavior of networks underAlso, under conditions of high prestrain, network rheology is governed by only a small portion of filaments that are highly stretched. Inclusion of ACP unbinding events under high prestrain results in stress relaxation and also leads to a power law behavior in G' as observed in many cells, and causes the loss modulus, G'', to increase at low frequency. We observe Nnonlinear stress-strain behaviors of actin networks are observed that are dependent on shear strain rates and the concentration and rupture of ACP. [Supported by GM076689]

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Weak to Strong Transition at the Actin-Myosin Interface Detected by Sensitized Emission Luminescence Resonance Energy Transfer (SELRET)

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Force is generated upon the transition of the actin-myosin complex from weak- to strong-binding states. To detect changes at the actin-myosin interface during this transition, we have detected sensitized emission luminescence resonance energy transfer (SELRET) from a luminescent Tb-chelate donor (attached to C374 of actin) to two kinds of acceptor-labeled myosin heads: (1) skeletal muscle myosin S1 labeled with tetramethylrhodamine iodoacetamide (TMRI) at C707 or (2) *Dictyostelium* myosin II motor domain (S1dC) labeled with iodoacetamidofluorescein (IAF) at a site engineered near the proposed binding interface (S619C). The emission of the Tb-donor, excited by a microsecond pulse, has an excited-state lifetime of 2 ms and is negligible at wavelengths where emission of acceptors is detected. Since the acceptors have ns excited-state lifetimes, the observed ms emission kinetics of acceptors is exclusively due to SELRET. Experiments on muscle S1 (labeled at C707) showed that the strong-to-weak transition results in a change of the distance from 5.5 nm (distribution width FWHM = 1.7 nm) to 6.0 nm (width 2.3 nm). The same transition with S1dC (labeled at C619) results a change of the distance from 4.0 nm (width 0.9 nm) to 4.2 nm (width 3.2 nm). Thus for both systems, the weak-to-strong transition results in a small change in the mean distance and a large decrease in the width of the distance distribution. These results add to the evidence that the weak-to-strong transition is fundamentally a disorder-to-order transition, whether it is measured within actin, within myosin, or at the interface between the two proteins.

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Crystal Structures of Monomeric Actin Bound to Cytochalasin D

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The fungal toxin cytochalasin D (CD) interferes with normal actin cytoskeletal dynamics by binding to the barbed end of actin filaments. Despite being extensively used as a tool for studying actin-mediated processes, the exact location and nature of its binding to actin are unknown. We have determined two crystal structures of a cytoplasmic actin, engineered to remain monomeric, with CD. One was obtained by soaking actin crystals with CD, and the other by co-crystallization. The CD-binding site, in the hydrophobic cleft between actin sub-domains 1 and 3, is identical in the two structures. Polar and hydrophobic contacts play equally important roles in CD binding, with six hydrogen bonds stabilizing the actin-CD complex. While many actin-binding proteins and marine toxins target this cleft, they primarily target the front half of this cleft (viewing actin with sub-domain 2 on the upper right). CD differs in that it targets the back half of this cleft. Our analysis suggests that contacts with this region of the cleft would ensure filament capping without severing. Importantly, the actin molecule in the co-crystallized actin-CD structure shows novel conformational changes in response to ligand binding. These include an ~6° inter-domain rotation, causing small changes in crystal packing that enables the ordering of the D-loop (DNase I-binding loop), which is disordered in most structures of actin. The D-loop adopts an extended, non-periodic conformation and is stabilized by contacts with neighboring actin monomers. Based on the shift in position of a putative nucleophilic water, we postulate a mechanism for CD-induced enhancement of actin-catalyzed ATP hydrolysis. We speculate that these changes represent a potential conformation that the actin monomer can adopt on the pathway to polymerization or in a filament.

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Structure of an Actin Trimer Stabilized by a Tandem W Domain Hybrid Construct

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The study of the actin filament is one of the major problems of structural biology. Uncontrollable polymerization has interfered with our ability to obtain crystals of the filament. Proteins that initiate actin polymerization in cells have the natural ability to stabilize multiple actin subunits into a filament-like conformation, allowing them to overcome the rate-limiting step in polymerization, i.e. the formation of actin dimers and trimers. With the exception of formins, known filament nucleators use the Wiskott-Aldrich syndrome protein (WASP) homology 2 (WH2 or W) domain for interaction with actin. The W domain is a short (17–27 aa) actin-binding motif. A common architecture, found in filament nucleators such as Spire, Cobl, VopL, and VopF, consists of tandem W domains that tie together three to four actin monomers to form a polymerization nucleus. We have engineered a stable actin trimer stabilized by a tandem W domain hybrid construct that also includes filament barbed and pointed end capping elements. The structure of the actin trimer was first studied in solution using x-ray scattering. Different crystal forms of the trimer have been obtained. We will present these studies. In particular, we will show how tandem W domains stabilize a polymerization nucleus by organizing actin subunits into a filament-like conformation.
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