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