1623-Pos Board B533
Stress Accumulation Originating from Mechanical Asymmetry Promotes Actin Filament Severing at Boundaries of Bare and Cofilin-Decorated Segments
Jérémy Roland, Cristian Suarez, Laurent Blanchoin, Jean-Louis Martiel, **Enrique M. De La Cruz**.
The regulatory protein, cofilin, severs actin filaments and increases the number of ends from which subunits add and dissociate. Structural and biochemical analyses demonstrate that cofilin binding alters the conformation and mechanics of actin filaments such that cofilin-decorated filaments are ~20-fold more compliant in bend and twist than native actin filaments. Equilibrium and kinetic binding models as well as direct visualization of cofilin binding to filaments favor a mechanism in which severing occurs at or near boundaries of bare and cofilin-decorated segments. It is hypothesized that shear stress associated with conformational fluctuations accumulates locally at boundaries of mechanical asymmetry, thereby leading to preferential severing at junctions of bare and cofilin-decorated segments. In this work, we evaluate if mechanical and conformational periodicity in filaments promotes stress accumulation at junctions of asymmetry (i.e. boundaries). We have derived mathematical expressions of the actin filament elastic free energy, accounting for contributions from bending, twisting and twist-bend coupling, and used a computational modeling approach to evaluate the distribution of energy and stress of model filaments strained by external mechanical (buckling or torque) loads applied to filament ends. Our results indicate that mechanical asymmetry introduced by cofilin binding promotes the accumulation of shear stress at boundaries between bare and cofilin-decorated segments that likely increases the probability of failure (i.e. severing) under active or passive, thermal deformation, analogous to the fracture of some non-protein materials. Elastic coupling between twisting and bending is critical for stress accumulation at boundaries.

1624-Pos Board B534
Fast Magic Angle Sample Spinning NMR Yields a View of the F-actin - Cofilin Complex with Atomic Resolution
Andrew H. Butterworth, Si Yan, Dmitri Kudryashov, Emil Reisler, Tatyana Polenova.
Proteins of the ADF/cofilin family are vital regulators of the actin cytoskeleton in eukaryotes. Binding of cofilin results in dramatic reorganization of the F-actin structure and potentiates filament severing, and its accelerated depolymerization from the pointed end. Although the X-ray structure of monomeric actin with cofilin-homology domain of twinfilin has been recently solved, F-actin evades crystallization and therefore the analysis of cofilin interaction with F-actin at the atomic level calls for alternative approaches. While considerable insight into the cofilin F-actin complex has been gained through chemical cross-linking and radiolytic footprinting, these approaches were unable to generate high resolution information on this interaction While considerable insight into the cofilin F-actin complex has been gained through chemical cross-linking and radiolytic footprinting, novel approaches are still desirable. Here we demonstrate that Fast-MAS Solid State NMR is a high sensitivity approach to studying this system. Isotopically labeled S. cerevisieae yeast cofilin, in complex with polymerized yeast actin, allows for an atomic resolution view of cofilin within the complex. Intramolecular conformational changes occurring in cofilin upon binding to actin can be deduced from dipolar and scalar coupling based spectra. Additional studies of the free cofilin have been performed in the solution and solid states for comprehensive comparisons. Therefore, our data demonstrates the general feasibility of the SS-NMR approach for studying the interaction between F-actin and actin binding proteins.

1625-Pos Board B535
Observations of Twist and Disorder in F-actin from Cofilin Binding
Diana Y. Wong, David Sept.
The regulation of actin polymerization is vital for cellular function. Cofilin is one important regulatory protein and has increasingly been credited as being a player in a cell's homeostasis. Cofilin binds and severs actin filaments, thereby leading to depolymerization and creation of new barbed ends for elongation. Mutagenesis experiments and cryo-EM work have provided critical information about the interaction of cofilin with the actin filament, however the molecular details of cofilin binding and the mechanism of twisting and severing have not been elucidated. We have performed a series of molecular docking and molecular dynamics studies using muscle actin and human cofilin I. After determining a model for bound cofilin, we performed both all-atom and coarse-grained molecular dynamics simulations on bare actin filaments, fully decorated filaments, and filaments with cofilin bound at isolated sites. We find that the binding of cofilin as domains or in isolated sites affects the average twist angles as well as the twist fluctuations. Decorated filaments not only have a greater average twist, in agreement with cryo-EM studies, but also a lower local fluctuation of twist angle. Additionally, we show how cofilin introduces local disorder in a filament. These results shine light on the cofilin's effects on F-actin twisting and bending and provide some clues about cooperative binding kinetics and filament severing.

1626-Pos Board B536
Actin Filaments Stabilize Locally at Random Sites
Thomas Niedermayer, Antoine Jégou, Guillaume Romet-Lemonne, Marie-France Carlier, Reinhard Lipowsky.
After the polymerization of actin monomers into filaments, the actin-bound ATP is hydrolyzed into ADP, a process that was believed to decrease the filament stability. Recent experiments suggest the opposite behavior, however, namely that actin filaments become increasingly stable with time. Several mechanisms for this stabilization have been proposed, ranging from structural transitions of the whole filament helix to pure artifacts arising, e.g., from the capping or surface attachment of the filament ends. We performed novel fluorescence microscopy experiments on single filaments to clarify this controversial issue. We find that filaments do indeed cease to depolymerize in an abrupt manner, and that this transition happens on relatively long time scales that exceed those of both ATP cleavage and phosphate release. We also developed a theory that allows us to distinguish the different possible stabilization mechanisms. A detailed comparison of theory and experiment implies that the sudden truncation of the shrinkage process does neither arise from artifacts nor from a collective transition of the whole filament. Instead, our results provide strong evidence for a local stabilization process occurring at random sites within the filament.

1627-Pos Board B537
Mechanism of Actin Nucleation by Arp2/3 Complex Visualized by Single Molecule Fluorescence
Benjamin A. Smith, Karen Daugherty-Clarke, Bruce Goode, **Jeff Gelles**.
Nucleation of actin filaments by the Arp2/3 complex is a critical process in cell motility and endocytosis. It is well established that activity of Arp2/3 complex is stimulated by interactions with the VCA domain of WASP family proteins and pre-existing (mother) filaments. However the kinetic mechanism by which these proteins, together with monomeric actin, cooperate to rapidly assemble a critical nucleus for new (daughter) filament growth is not fully understood. We used multi-wavelength single molecule fluorescence microscopy to directly visualize in vitro the dynamic interactions of individual fluorescently labeled S. cerevisiae Arp2/3 complexes with actin filaments and to follow the pathway of new filament nucleation. We observed that Arp2/3 complex associated with mother filament sides, not ends. The majority of Arp2/3 complexes rapidly dissociated from filament sides after a characteristic distribution of lifetimes, while the remaining sub-population nucleated daughter filaments. Pointed ends of daughter filaments remained stably associated with the mother filament for durations estimated to be orders of magnitude longer than the dissociation times of Arp2/3 complexes that did not generate daughter filaments. Arp2/3 complexes nucleated some daughter filaments even in the absence of VCA, consistent with a mechanism in which VCA shifts an equilibrium between nucleation-inactive and nucleation-primed states of filament-bound Arp2/3 complexes. Taken together, these novel measurements reveal key features of the kinetic mechanism of actin nucleation by Arp2/3 complex and will help to more clearly define the VCA-regulated steps in the pathway.

1628-Pos Board B538
Actin Polymerization Dynamics - Insights from *In vitro* TIRF Microscopy
Balakrishnan Kannan, Mårten Larsson, Wei Lin Lee, Maria Hernandez-Valladares, Robert C. Robinson.
Actin elongation is a bi-molecular reaction between monomeric actin (G-actin) and filamentous actin (F-actin), in the first approximation. It can be controlled by changing the ability of either G-actin or F-actin to participate in the reaction. Either of the two mechanisms alone is not sufficient to maintain a large pool of G-actin ready to polymerize in a signal-controlled fashion [1]. Mammalian cells have hundreds of actin-binding proteins (ABP) which bind either or both the forms of actin. Profilin sequesters G-actin and makes them predisposed towards F-actin barbed-end addition, cofilin severs F-actin and depolymerizes it into G-actin. On the other hand, capping protein (CP) caps the barbed-end and stops further elongation of F-actin [2]. Gelsolin-family of proteins [3] sever F-actin as well as cap filaments. *In vitro* TIRF microscopy [4] has been used to monitor real-time actin dynamics in the presence of ABPs [5], [6]. Representative results on some ABPs which alter actin assembly will be presented.
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1629-Pos Board B539

ATP Hydrolysis Energy Transfer in the Profilin-Mediated Actin Polymerization

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Profilin regulates actin polymerization in cells playing important role in cell motility and division. Actin polymerization involves ATP hydrolysis which occurs both in the absence and the presence of profilin. There is a hypothesis suggested in literature that profilin promotes actin polymerization through direct transfer of the energy of this spontaneous hydrolysis to polymerization utilizing tight coupling of the hydrolysis and corresponding polymerization events. Recently we suggested an alternative hypothesis based on an indirect energy transfer and pointed out that recent experimental and theoretical findings require re-evaluation of the direct transfer hypothesis.

Our thermodynamically rigorous model of actin steady state dynamics in the presence of profilin describes all events in terms of chemical reactions and allows both energy transfer mechanisms, each corresponding to certain ranges of the rate constants (parameters) for these reactions. In fact, the difference between the two mechanisms is defined by a single ratio r of the two rates: the rate of hydrolysis by the profilin-and-ATP-bound subunit at the filament end, and the rate of dissociation of this subunit (in complex with profilin) from the end. The direct transfer cannot exist unless $r>1$ while indirect transfer can occur at any r.

Our model predicts specific shapes for the dependence of actin critical concentration on profilin concentration depending on the ratio r. For values $r>1$, curves have peaks, which persist for wide ranges of other model parameters. Our experimental dependence (obtained with our new technique) shows no peak but a steady decline, contrary to the direct transfer hypothesis. This result provides strong support for our indirect transfer hypothesis.

Moreover, using our theory and routine experimental techniques we determined relative activities of the two specific molecular mechanisms of profilin action (both based on indirect transfer) predicted earlier with our model.

1630-Pos Board B540

The Effect of Toxofilin on the Structure of Monomeric Actin

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The actin cytoskeleton of eukaryotic cells plays a key role in many processes, including motility and cytokinesis. The structure and dynamics of the cytoskeleton are regulated by a large number of proteins that interact with monomeric and/or filamentous actins.

Toxoplasma gondii is an intracellular parasite, which can utilise the actin cytoskeleton of the host cells for their own purposes. One of the expressed proteins of *T. gondii* is the 27 kDa-sized toxofilin. The 245 amino acid long protein is a monomeric actin-binding protein involved in the host invasion. It can bind to actin monomers and to the ends of the actin filaments as well. The protein has three actin-binding sites which makes it capable to interact with an antiparallel actin dimer.

In our work we studied the effect of the actin-binding site of toxofilin$_{69\text{-}196}$ on the monomeric actin. We determined the affinity of toxofilin to the actin monomer with fluorescence anisotropy measurement (K_D = 1.3 μM). The flourescence of the actin bound ϵ-ATP was quenched with acrylamide in the presence or absence of toxofilin. In the presence of toxofilin the accessibility of the bound ϵ-ATP decreased, which indicates that the nucleotide binding cleft is shifted to a more closed conformational state.

The results of the completed experiments can help us to understand in more details what kind of cytoskeletal changes can be caused in the host cell during the invasion of the host cells by intracellular parasites.

1631-Pos Board B541

***Saccharomyces Cerevisiae* Glycolytic Enzymes are Stabilized by Association with Actin**

Daniela Araiza, Olivera Toro, Armando Zepeda Bastida,
Adela Mújica Miranda, Salvador Uribe Carvajal.

The cell contains constant concentrations of solutes and macromolecules except during stress, when compatible solutes accumulate in the cytosol. Molecular crowding in the cell results in protein association that allows the channeling of intermediates and thus increasing metabolic efficiency. Multienzymatic complexes (or metabolons) are anchored in a dynamic cytoskeleton. It is suggested that the efficiency of cellular metabolism depends on the enzymatic organization. In addition, metabolon probably protect enzymes in a metabolic pathway from the deleterious effects of stress. It was decided to examine whether glycolytic enzymes associate with actin and whether association confers higher stability to the different enzymes. Enzyme association was assessed by co-immunoprecipitation of actin with glycolytic enzymes in the presence or absence of compatible solutes. The whole fermentation pathway was also assayed in the presence of increasing compatible solutes. Actin stabilized the glycolytic pathway making a more efficient pathway even in the presence of a compatible solute. By contrast, depolymerization of actin did not affect fermentation.

1632-Pos Board B542

Acyl Chain Specificity of the Inhibition of Actin Polymerization by the Interaction of Lysophosphatidic Acid and Villin

Richard M. Epand, Seema Khurana, Raquel F. Epand.

Lysophosphatidic acid binds specifically to villin resulting in the inhibition of villin-induced polymerization of actin (Tomar; George; Mathew and Khurana (2009) J. Biol. Chem. 284, 35278). We have identified an amino acid pattern contributing to the arachidonoyl specificity of several proteins (Shulga, Topham, Epand (2010), submitted). The protein villin contains this pattern and was therefore studied for its specificity for arachidonoyl binding. The binding affinity of lysophosphatidic acid to villin was studied using quenching of the intrinsic Trp fluorescence of this protein by lysophosphatidic acid. We observed that 1-arachidonoyl-lysophosphatidic acid bound more tightly to villin than did 1-oleoyl-lysophosphatidic acid. The region of villin expected to confer arachidonoyl specificity comprises residues 4-18. A mutant of villin was constructed in which residues 4-14 were deleted. Using this mutant villin, there is very little difference in binding affinity between the two species of lysophosphatidic acid. This is a result of a loss of affinity for the arachidonoyl form in the mutant and little change in the binding of the oleoyl form. This is consistent with this domain conferring acyl chain specificity to the lipid interactions of villin. We also determined the effects on actin polymerization. Actin polymerization was promoted to similar extents by either the wild type villin or the deletion mutant. However, this is inhibited by lysophospatidic acid with the arachidonoyl form being more potent than the oleoyl and this inhibition is greater with the wild type protein than with the mutant. These results are consistent with the binding studies and demonstrate acyl chain specificity for this regulation of cytoskeletal rearrangements. The preference for the arachidonoyl form suggests a relationship of this mechanism of regulation of actin polymerization to the lipid intermediates of the PI-cycle.

1633-Pos Board B543

***O*-GlcNac Modification of Human Cardiac α-Actinin**

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Steven B. Marston.

We measured *O*-GlcNAcylation of myofibrillar proteins from human hearts by two assays: (1) Western blotting with an antibody (CTD110.6) specific to *O*-GlcNAc modified proteins and (2) specific enzymatic labelling of *O*-GlcNAc with *N*-acetylgalactosamine (UDP-GalNAz) by mutant enzyme Y289L beta-1,4-galactosyltransferase (Y289L GalT), which allows coupling of tetramethylrhodamine (TAMRA) fluorescent tag for direct imaging following SDS-PAGE. In every sample, the predominant modified protein was alpha-actinin, which made up $65 \pm 4.48\%$ of the enzymatically labelled myofibrillar proteins. We found that alpha-actinin exists as two bands on 12% SDS-PAGE. EA-53 antibody to alpha-actinin revealed that the faster migrating alpha-actinin band is on average $<1.4 \pm 0.31\%$ of the slower migrating band. Interestingly, CTD110.6 showed signals from both bands with similar intensities (mean ratio=1.02 ± 0.10), indicating that *O*-GlcNAc modification is more abundant in the minor band (>80x more concentrated). This was confirmed by the enzymatic labelling method; *O*-GlcNAc was often not detectable in the slower migrating band, with an average ratio of faster/slower migrating band of 2.45 ± 0.49. Further tests showed that the higher mobility band was not a degradation product of the lower mobility band. *O*-GlcNAc can be removed from the myofibril proteins by beta-N-acetylglucosaminidase. Using CTD110.6 detection, increasing enzyme concentration decreased the faster migrating band to near zero, but the slower migrating band did not show the same trend, suggesting that the antibody may cross react slightly with unmodified alpha-actinin. We conclude that *O*-GlcNAcylation is highly concentrated in the low abundance faster migrating species of alpha-actinin. The possibility that the two bands represent two isoforms of alpha-actinin differentially modified by the potentially regulatory *O*-GlcNAc modification is being investigated.