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A Single Actin Filament Works as a Mechanosensor

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Force plays a variety of roles in cell biology, including cell signal pathways for cell survival, growth, development, and migration. Cells may use molecular mechanisms other than mechanosensitive ion channels such as cytoskeletons to sense force, since intracellular and extracellular mechanical environments greatly affect the disassembly of stress fibers, suggesting that the tension is sensed by unknown, intracellular mechanisms and the stress fibers are disassembled. Here, we report that loss of tension in stress fibers induced disassembly of stress fibers in permeabilized semi-intact cells, which was dependent on mouse brain derived cytosolic factor(s) in the bath solution, and purified Cofilin/ADF, a major actin modulating protein ubiquitously distributed in eukaryotes, was sufficient for this stress fiber disassembly. We hypothesize that the tension decline is sensed by the actin filament, which allows cofilin to sever the filament. To test this, we have prepared a single actin filament tensed with optical tweezers and examined the tension dependent severing of the filament by cofilin. The time to sever the filament by cofilin was prolonged about 2 times in the presence of mechanical tension in the filament compared with that of a filament that was not tensed. These results indicate that tension prevents binding of cofilin to actin filaments, or prevents severing of actin filaments by cofilin. We directly observed binding of Alexa488-labeled-cofilin to actin filaments tethered on the surface of cover slip via NEM-myosin by TIRF microscopy. Scratching the ''motion restricted'' tethered actin filaments by a fine tip of glass capillaries created freely moving filament ends. The rate of binding of cofilin to the actin filaments near (<2 μm) the scratching was significantly increased. These results strongly support the hypothesis that the actin filament acts as a ''tension-sensor'' and changes susceptibility of cofilin to the filament.

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The Kinetics of Cooperative Cofilin Binding to Actin Filaments

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The interaction of cofilin with actin filaments displays positive cooperativity. The equilibrium binding and associated thermodynamic parameters of this interaction are well described by a simplified, one-dimensional Ising model with nearest neighbor interactions [De La Cruz (2005) *J. Mol. Biol. 346*, 557-564]. Here we evaluate the ability of the model to account for cooperative association kinetics and to determine the kinetic contributions to cooperative binding. A Monte Carlo based simulation protocol that allows for nearest-neighbor interactions between adjacent binding sites was employed to globally fit time courses of cofilin binding. A consistent set of binding parameters in good agreement with the equilibrium thermodynamic parameters describes well the experimental data across a wide range of cofilin concentrations. We conclude that despite its simplicity, the one-dimensional Ising model with nearest neighbor cooperative interactions, is a reliable model for analyzing and interpreting the thermodynamics and kinetics of cooperative cofilin-actin filament interactions. The methods developed for this system will be applicable to the kinetics analysis of cooperative ligand binding to linear biological polymers.

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Molecular Interaction of Cofilin with F-Actin and Implications for Filament Severing

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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 protein-protein docking and molecular dynamics simulations. 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. Based on our binding models, we have performed simulations of sparsely and fully decorated filaments in order to gain insight into the mechanism of F-actin twisting and severing. The implications of this binding model in the function and severing action of cofilin are discussed.

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Single-Molecule Study of Actin Filament Severing by Gelsolin using Total Internal Reflection Fluorescence Microscopy

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Gelsolin-family proteins are regulators of actin polymerization dynamics.[1] To understand the mechanism of actin filament (F-actin) severing by gelsolin at the single-molecule level an *in vitro* total internal reflection fluorescence (TIRF) microscopy[2] assay has been developed. Preliminary TIRF data confirm that gelsolin sever F-actin.[3] These real-time microscopic observations are in concert with the structural and biochemical studies that have established activation of gelsolin by calcium ions. [3],[4],[5] To further ascertain a) if gelsolin preferentially binds to F-actin rather than sequestering actin monomers (G-actin), b) if it preferentially binds and severs ADP-F-actin or ATP-F-actin, and c) if gelsolin or cofilin, a member of actin depolymerizing factor (ADF), sever gelsolin-capped F-actin better than native filaments, dual color TIRFM assays are undertaken. Results will be discussed in the context of structural and biochemical data on gelsolin-family of proteins.

[1] P. Silacci et al, Cell Mol Life Sci 2004, 61: 2614-2623
[2] J.R. Kuhn, T. D. Pollard, Biophys. J 2005. 88:1387-1402
[3] S. Nag et al, Proc. Natl. Acad. Sci. 2009, 106 :1371313718
[4] R.C. Robinson et al, Science 1999. 286: 1939-1942
[5] K. Narayan et al, Febs Lett. 2003. 552:82-85

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Measurement of Filamin a Torsion In-Singulo

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Filamin A (FLNa) is one of the most ubiquitous actin binding proteins that cross-link actin filaments. The protein is a v-shaped homodimer. Each monomer consists of twenty-four β-sheet ''rod-like'' domains which separate the actin binding domain at the N-terminus from the C-terminus at the dimerization site. The rod-like domains are divided by two unstructured ''hinge'' sequences between repeats 15 and 16 and repeats 23 and 24. While it has been reported that these hinge sequences play a key role in determining the nonlinear elastic response of stressed actin networks, how the hinge segment elicits such changes in network elasticity remains unknown. To elucidate this mechanism, we measure the torsional dynamics of single FLNa molecules in real-time by using high-resolution, fluorescence microscopy. Using a native-like single-molecule assay consisting of the complex formed by a surface-bound actin filament cross-linked to a freely-rotating actin filament by FLNa we are able to track the instantaneous angle between the cross-linked filaments as the cross-linked filament undergoes a thermally-driven rotational motion. By estimating the torsional stiffness of both wildtype and hingeless FLNa isoforms, the role of the hinge sequence in determining the torsional rigidity of FLNa is inferred. Both hinged and hingeless FLNa form cross-links with comparable near-acute angles. Moreover, the torsional dynamics of each cross-link can be distinctly categorized by using this strategy to image the molecular interactions present in each cross-link. This work may illuminate the molecular origins of the nonlinear elasticity observed in FLNa-cross-linked F-actin networks. Support from the Singapore-MIT Alliance for Research and Technology and the NSF Career Award (0643745) are gratefully acknowledged.

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Actin Crosslinking Proteins Recognize Distinct Arrangements of Actin Filaments

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Self-assembly of complex structures is commonplace in biology but often poorly understood. In the case of the actin cytoskeleton, a great deal is known about the components that comprise higher order structures, such as lamellar meshes, filopodial bundles and stress fibers. Each of these cytoskeletal structures contains actin filaments and crosslinking proteins, but the role of crosslinking proteins in the initial steps of structure formation has not been clearly elucidated. We employ a novel optical trapping assay to investigate the behaviors of fascin and alpha-actinin during the first steps of structure assembly. Here we show that these proteins have distinct binding characteristics that cause them to recognize and crosslink filaments that are arranged with specific geometries. Alpha-actinin is a promiscuous crosslinker, linking filaments over a range of angles. These angles include parallel (0^0), anti-parallel (180^0), and series of intermediate crossing angles ($15\text{-}165^0$). It is also an extremely flexible crosslinker, maintaining connection even when the link is rotated. Conversely, fascin is extremely selective, only crosslinking filaments in a parallel

orientation. This means that for fascin bundles to form the actin filaments must occupy a parallel orientation before the structure can be stabilized. In the case of alpha-actinin structures, crosslinks could form at any point during the process of structure assembly. We have shown that crosslinking proteins recognize specific orientations of actin filaments, which places constraints on how cytoskeletal structures assemble and organize.

803-Pos

A Structural and Biochemical Study of the Interaction Between Actin and the Mammalian Formin FRL2

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Formins are a class of proteins that influence the rate of actin filament nucleation and elongation. Mammals possess 15 formin isoforms, providing a myriad of possibilities for regulating actin-based structures in cells. The dimeric formin homology 2 (FH2) domain is capable of accelerating the nucleation rate of new actin filaments and subsequently influences filament elongation via direct interaction with the barbed end of an actin filament. The FH2 domain moves processively with the barbed end as the filament elongates. A subset of formins, including FRL2, can also bundle filaments. Our goal in this study is to examine FRL2's interaction with actin in detail. A portion of FRL2 containing the FH2 domain forms a stable interaction with tetramethylrhodamine-malamide labeled actin (TMR-actin) or Latrunculin B-bound actin, two forms of actin that are unable to polymerize. The actin/FRL2 complex is mono-disperse, as judged by analytical ultracentrifugation and gel filtration. FH2 domain-containing constructs from three other mammalian formins (mDia1, mDia2, and INF2) do not show an equivalent interaction. A mutation in the FH2 domain of FRL2 that prevents barbed-end binding also prevents interaction with TMR-actin, suggesting that the interaction interface is similar to that of FRL2 with the filament barbed end. These properties make the actin/FRL2 complex an ideal system for a structural study of actin/formin interactions. Both biochemical and structural experiments are being carried out with FRL2 constructs and both LatB treated and TMR labeled actin in order to characterize the details of their binding interactions.

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The Effect of Heavy Meromyosin on the Flexibility of Formin-Bound Actin Filaments

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Formins are conservative proteins with important roles in the regulation of the microfilamental system in eukaryotic cells. They have several domains including FH1, FH2, GPB and DAD domains. In the interaction between actin and formin the FH2 domain plays a key role. This domain builds antiparallel dimers with the help of the 'linker region' between FH1 and FH2 domains. The 'mammalian Diaphanous-related 1′ constitutes one of the subfamilies of the formins. It was shown that formins could make the actin filaments more flexible (Bugyi et al. 2006), and another actin binding protein, tropomyosin reduces this effect (Ujfalusi et al. 2008). In our work we investigated whether the flexibility of the filaments could be restored by the binding of the common binding partner of actin, myosin. Skeletal muscle and non-muscle 2B isoforms of HMM were used. Temperature dependent Förster-type resonance energy transfer (FRET) and fluorescence anisotropy decay experiments showed that the formin (mDia1-FH2) induced an increase in the flexibility of actin filaments, which was reversed by the binding of heavy meromyosin HMM. Our previous measurements showed that tropomyosin had a similar stabilizing effect on the formin-bound actin filaments. These observations together indicated that actin-binding proteins played a central role in the molecular mechanisms that regulate the dynamic properties of the actin filaments. This mechanism provides the opportunity for the formin-induced actin filaments to become similar to those that are polymerized in the absence of formins.

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The Calponin Regulatory Region is Intrinsically Unstructured: Novel Insight into Actin-Calponin and Calmodulin-Calponin Interfaces using Nmr Spectroscopy

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h1-calponin is an actin and calmodulin binding protein. Previous studies established that the recombinant calponin fragment 131-228 binding to actin involves two distinct sites. The first actin binding site was attributed to amino acids Ala145-Ile163 while amino acids Lys172-His187 formed the second actin binding site. Here we have used nuclear magnetic resonance spectroscopy to investigate the structure of this functionally important region of calponin and map its interaction with actin and calmodulin at amino acid resolution. Our data indicates that the free calponin peptide is largely unstructured in solution, although 4 short amino acid stretches corresponding to residues 140-146, 159-165, 189-195 and 199-205 display the propensity to form α-helices. Our data identified the amino acids involved in actin binding within the first actin binding site and demonstrated for the first time that the N-terminal flanking region of Lys137-Tyr144 is an integral part of this actin binding site. We have further delineated the second actin binding site to amino acids Thr180-Asp190. Binding to calmodulin extends beyond the previously identified minimal sequence of 153-163 and includes most amino acids within the stretch 143-165. In addition we found that calmodulin induces chemical shift perturbations of amino acids 188-190 demonstrating for the first time an effect of Ca^{2+}-calmodulin on this region. The spatial relationship of the actin and calmodulin contacts within the regulatory region of calponin provides a structural framework for understanding the Ca^{2+} dependent regulation of the actin-calponin interaction by calmodulin.

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Evidence from the Laser Trap for Two Closed States of Tropomyosin

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The position of tropomyosin (Tm) on the thin filament is often described by a three state model: 1) blocked, Ca^{2+} is absent and steric hindrance by Tm blocks myosin from binding to actin, 2) closed, Ca^{2+} binds to troponin C which unlocks Tm and partially unblocks myosin binding, and 3) open, initial myosin binding shifts Tm further, exposing downstream myosin binding sites and cooperatively activating the thin filament (McKillop and Geeves, 1993). We previously showed that Tm phosphorylation enhances force production by myosin - an effect independent of steric hindrance and thus not predicted by the current three-state model. We therefore tested the hypothesis that Tm phosphorylation affects the on-rate of single actin-myosin bonds. Heavy meromyosin (HMM) was adsorbed to immobilized, nitrocellulose-coated pedestals, and biotinylated actin filaments with natively phosphorylated or dephosphorylated Tm were coupled to streptavidin-coated beads. Using a laser trap, we measured the time necessary for the first bond to form (1/on-rate) between actin and rigor HMM. Measurements were repeated in the presence 10-20 nM N-ethylmaleimide modified myosin-S1 to force Tm from the closed to the open state. Actin-myosin on-rates were increased by Tm, but only in the closed state. Phosphorylation of Tm enhanced this effect. However, the frequency of actin-HMM bond formation was reduced in the closed state in the presence of Tm. Together, these data suggest that there may be at least two closed states of Tm in equilibrium with one another. In the first, Tm hinders myosin binding resulting in a relatively low on-rate, while in the second Tm becomes a ''guide'' to myosin binding and accelerates the on-rate.

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Precise Modulation of Tropomyosin Polymer Length is Crucial for its Association with Actin and Ability to Regulate Myosin Function

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Tropomyosin (Tm) is an evolutionarily conserved dimeric α-helical coiled-coil protein, which interacts end to end to form polymers capable of associating with and stabilising actin-filaments and thereby regulate myosin function. The fission yeast Schizosaccharomyces pombe possesses a single Tm, known as Cdc8. Cdc8 is an essential protein, which can be acetylated on its amino terminal methionine in vivo. This acetylation increases the affinity of Cdc8 for actin, and also enhances its ability to regulate myosin function (J. Cell Sci. 120: 1635-1645). We have recently undertaken an extensive analysis on the physical properties of acetylated and unacetylated Cdc8 together with a series of novel amino terminal Cdc8 mutants, in an attempt to explore the effect acetylation has upon the regulatory function of the Cdc8 protein. By correlating the stability of each protein and its propensity to form polymers with its ability to associate with actin and regulate myosin, it has been possible to establish that precise modulation of Tm-polymer length is crucial for its function. Cdc8 mutants capable of forming Tm-polymers significantly longer than the wild-type protein had a reduced affinity for actin, and in contrast to both wild type forms of the protein were unable to regulate myosin. The longer Tm-polymers are unable to efficiently coil around the already formed actin filament, and together our data are consistent with a mechanism by which acetylation regulates the formation of short Tm-polymers (normally up of ~2 Cdc8 dimers) which associate with actin-filaments and are subsequently stabilised by electrostatic interactions with actin.