the repeating motif demonstrates a molecular mechanism for gain of function mutations.

3322-Pos Board B477

Compliant Or Stiff: Two Differing Mechanisms of Actin Network Stabilization by Calponin and Tropomyosin

Mikkel H. Jensen[1], Eliza J. Morris[2], Cynthia Gallant[1], Philip Graceffa[3], Paul Leavis[3], Kathleen G. Morgan[1], David A. Weitz[2], Jeffrey R. Moore[1].
[1]Boston University, Boston, MA, USA, [2]Harvard University, Cambridge, MA, USA, [3]Boston Biomedical Research Institute, Boston, MA, USA.

The cellular actin cytoskeleton plays a central role in the ability of cells to properly sense, propagate, and respond to external stresses and other mechanical stimuli. Calponin, an actin-binding protein found both in muscle and non-muscle cells, has been implicated in actin cytoskeletal organization and regulation. While currently thought to stabilize actin in the cell, the mechanisms of this stabilization are poorly understood due to the complicated nature of the living cell. Here we use a simplified in vitro model system to dissect the specific roles of individual actin binding proteins within a complex network environment.

We studied the mechanical properties of actin networks in the presence of basic calponin and smooth muscle aortic tropomyosin. These two actin-binding proteins have distinct effects on single actin filaments; while calponin binds over subdomain 2 of actin and makes actin more flexible, tropomyosin lies along the long-pitch helix of actin and buttresses the filament. We constructed in vitro crosslinked actin networks and studied their strain behavior both macro- and microscopically using bulk rheology and active microrheology. Actin networks decorated with either calponin or tropomyosin exhibited increased tensile strength despite the very different effects these proteins have on individual actin filaments. While calponin-decorated actin networks exhibited delayed strain stiffening, tropomyosin-decorated actin networks underwent stress relaxation through local network failure. When the two actin-binding proteins were present together, each of these effects were apparent, and the network tensile strength were further increased.

This work demonstrates two very different mechanisms by which calponin and tropomyosin increase the tensile strength of actin networks through purely mechanical interactions.

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3323-Pos Board B478

Modeling of Multi-Domain Assemblies in α-Actinin-4 Reveals How External Cues Regulate its Binding to Actin

Timothy Travers, Hanshuang Shao, Alan Wells, Carlos J. Camacho.
University of Pittsburgh, Pittsburgh, PA, USA.

α-Actinin-4 (ACTN4) is an actin-crosslinking protein that is essential to a number of processes in non-muscle cells, including motility and maintenance of cell shape. The binding of ACTN4 to actin filaments is dynamically regulated by a variety of external cues such as tyrosine phosphorylation, binding of calcium ions, binding of phosphoinositides, and calpain proteolysis, but the underlying molecular mechanisms are poorly understood. To address this, we developed an atomic model for the full ACTN4 homodimer, whose end regions contain a novel ternary complex between the C-terminal calmodulin-like domain of one monomer, and the N-terminal actin-binding domain (ABD) and an adjacent helical ''neck'' region of the opposite antiparallel monomer. This assembly is predicted to reduce actin binding by blocking the binding site on the CH1 subdomain of the ABD, which is validated by mutagenesis experiments designed specifically to disrupt the complex. using this atomic model, we are able to rationalize changes in actin binding due to external effectors as changes in the assembly of the ternary complex. Integrating these structural insights into a network model has allowed us to make detailed predictions that are consistent with a broad set of semi-quantitative experimental results. The similarity between ACTN4 and other actin-binding proteins suggests that many of our findings will apply for this whole class of proteins.

3324-Pos Board B479

R1 Motif is the Major Actin Binding Domain of TRIOBP-4

Jianjun Bao[1], Elizabeth Bielski[1], Ankita Bachhawat[1], Kavitha Thirumurugan[2], Shin-ichiro Kitajiri[3], Takeshi Sakamoto[1].
[1]Wayne State University, Detroit, MI, USA, [2]School of Biosciences & Technology, VIT University, Vellore, India, [3]Kyoto University Graduate School of Medicine, Kyoto, Japan.

The objective of this study is to identify the actin-binding domains of TRIOBP, an actin-bundling protein associated with human deafness DFNB28. Previous study shows that *in vitro*, TRIOBP isoform 4 (TRIOBP 4) forms dense F-actin bundles resembling the inner ear hair cell rootlet structure. Deletion of TRIOBP isoforms 4 and 5 leads to hearing loss in mice due to their inability to form rootlets. Despite the importance of TRIOBP in hearing, the mechanism of actin bundle formation by TRIOBP is not fully understood. The amino acid sequences of TRIOBP isoforms 4 and 5 contain two repeated motifs, referred to as R1 and R2, respectively. To examine the potential role of R1 and R2 motifs in F-actin binding, we generated TRIOBP-4 mutant proteins deleted with R2, and/or R1, and assessed their F-actin binding activity and bundle formation *in vitro* by actin co-sedimentation assay, fluorescence and electron microscopy. Cellular distributions of the TRIOBP-4 mutants were examined by confocal microscopy. We showed that deletion of both R1 and R2 motifs completely disrupted actin binding activity of TRIOBP-4 and impaired its localization to cellular actin cytoskeleton structure including filopodia. By contrast, TRIOBP-4 lacking only R2 motif retained F-actin binding and bundling ability and localized to actin filaments in cells, similar to full length TRIOBP-4. Moreover, the R1 motif-deleted TRIOBP-4 mutant which mainly contains R2 motif, formed thin F-actin bundle *in vitro*, but failed to co-localize to actin filaments in cells. These results indicate that R1 motif is the major actin-binding domain of TRIOBP-4 and R2 motif may make secondary contacts with actin filaments within a bundle.

3325-Pos Board B480

Relative Affinity of VCA Domain of Wave to Actin, Arp3, and Arp2 using Molecular Dynamics Simulations

Amruta C. Mahadik[1,2], Brian W. Beck[1,2].
[1]Texas Womans University, Denton, TX, USA, [2]Texas Woman's University, Denton, TX, USA.

Initiation of branching in actin cytoskeletal filaments requires an Arp2/3 complex bind to the side of an existing actin filament. Arp2 and Arp3, the major subunits of the Arp2/3 complex, nucleate actin branches by forming the first short pitch dimer of the daughter actin filament. Nucleation promoting factors like Wiskott-Aldrich (WA) family proteins are thought to activate the Arp2/3 complex and promote the elongation process by orienting and enhancing actin monomer binding to the daughter actin filament. Previous kinetics and cross-linking studies have established the importance of binding the VCA/WH2 domains of WA proteins in Arp2/3 complex activation, but the quaternary interactions are not well understood. We hypothesize that as in actin binding, the VCA domain of the WA protein WAVE also binds the hydrophobic cleft present between subdomain 1 and subdomain 3 of either Arp3 or Arp2, displacing the relative C-terminal extension of each present in the cleft. Binding and displacement allows conformational changes in the Arps to occur that result in the formation of a short-pitch Arp3:Arp2 dimer. We suggest that the binding of a VCA domain and ATP thus facilitates the activation of the Arp2/3 complex for branching. Here, we use molecular dynamics simulations to study complexes of V and C fragments of VCA domains individually with actin and both Arps in different nucleotide bound states in order to assess the relative energetics and conformational variation of different binding orientations.

3326-Pos Board B481

Model of Capping Protein and Arp2/3 Complex Turnover in the Lamellipodium based on Single Molecule Statistics

Laura M. McMillen, Matthew B. Smith, Dimitrios Vavylonis.
Lehigh University, Bethlehem, PA, USA.

Capping protein (CP) and Arp2/3 protein complex regulate actin polymerization near the leading edge of motile cells. They assemble near the edge of the lamellipodium, undergo retrograde flow, and dissociate into the cytoplasm as single subunits or as part of actin oligomers. To better understand this cycle, we modeled the kinetics of CP and Arp2/3 complex in the lamellipodium using data from prior single molecule microscopy experiments [Miyoshi et al. JCB, 2006, 175:948]. In these experiments speckle appearance and disappearance events corresponded to assembly and dissociation from the F-actin network. We used the measured dissociation rates of Arp2/3 complex and CP (0.048 s^{-1} and 0.58 s^{-1}, respectively) in a Monte Carlo simulation that includes particles in association with F-actin and diffuse in the cytoplasm. We explored the effect of slowly diffusing cytoplasmic pool to account for a big fraction of CP with diffusion coefficients as slow as 0.5 $\mu m^2/s$ measured by single molecule tracking [Smith et al. Biophys. J., 2011,101:1799]. These slowly diffusing species could represent severed actin filament fragments. We show that such slow diffusion coefficients are consistent with prior FRAP experiments by Kapustina et al. [Cytoskeleton, 2010, 67:525] who fitted their data using larger diffusion coefficients. We also show that the single molecule data are consistent with FRAP experiments by Lai et al. [EMBO J., 2008, 28:986] who found that the Arp2/3 complex recovers more quickly at the front of the lamellipodium as compared to the back. We discuss the implication of disassembly with actin oligomers and suggest experiments to distinguish among mechanisms that influence long range transport.