on actin dynamics affect elastic properties of actin-mediated linkages with the sarcolemma. Future experiments using expressed fragments of dystrophin and utrophin and their functionally relevant mutants will determine which structural elements of these proteins are critical in determination of the flexibility of actin filaments and what level of actin flexibility is physiologically optimal.

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Myosin-induced Movement Of Tropomyosin Isoforms On Actin Filament

Joanna Moraczewska, Małgorzata Śliwińska, Magdalena Zukowska, Danuta Borys.
Kazimierz Wielki University of Bydgoszcz, Bydgoszcz, Poland.

Tropomyosins (TMs) are a family of proteins which regulate actin interactions with myosins and other actin-binding proteins. TMs flexibility is thought to underlie the mechanism of TM-dependent regulation. In this work involvement of C-terminal sequence of TM in myosin-induced shift of TM during filament activation was analyzed. Recombinant αTM variants with C-terminus encoded by exon 9a (TM1b9a) or 9d (TM5a) were used. Both isoforms belong to short class of TMs with N-terminus encoded by exon 1b. To assess the degree of TM flexibility we measured FRET between AEDANS, which was attached to each TM isoform either in Cys^{153} or in Cys^{28}, and DABMI bound to actin in Cys^{374}. TM's Cys^{153} is a native residue located in central region of TM, Cys^{28} was introduced in N-terminal region using recombinant DNA methods. When bound to actin alone both TM isoforms show moderate flexibility with TM-actin distances ranging between 38.5 and 44.3 Å. Myosin S1 induced >10 Å movement of N-terminal region of TM5a, whereas it's central region was moved by about 4 Å. More uniform movement of the central and N-terminal regions was observed for TM1b9a. We conclude that TM's C-terminal sequence determines flexibility of the molecule. Supported by The Wellcome Trust.

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Interactions of the Pleckstrin Homology Domains of M-RIP (p116Rip) with F-actin

Ana M. Garcia, **Terence C. Tao**.
Boston Biomedical Research Institute, Watertown, MA, USA.

M-RIP has been shown to interact with actin, myosin, RhoA and the targeting subunit of myosin phosphatase, and has been proposed to be a scaffolding protein that anchors RhoA and Rho kinase onto myosin phosphatase and the actomyosin cytoskeleton. The N-terminal portion of M-RIP has 2 pleckstrin homology domains at residues 44–152 and 387–484 (PH1 and PH2, respectively). Mulder *et al.* (*J. Biol. Chem.* **278**, 27216–23, 2003) showed that the actin-binding activity of M-RIP resides in its N-terminal portion, and characterized the interaction between PH1 and F-actin, but did not investigate the role of PH2 in M-RIP's actin binding activity. In this work we examined the contributions of PH1 and PH2 to the M-RIP-F-actin interaction by, first, constructing 3 deletion mutants of M-RIP: M-RIP(1-386) (PH1), M-RIP(146-492) (PH2) and M-RIP(1-492) (PH1 and PH2). Co-sedimentation experiments with F-actin were then carried out at low or high speeds to sediment M-RIP-bound to F-actin bundles or filaments, respectively. The following results were obtained: 1) The extent of bundle formation increases in the order M-RIP(1-386) < M-RIP(146-492) < M-RIP(1-492). 2) The extent of total actin binding increases in the order M-RIP(1-386) < M-RIP(146-492) < M-RIP(1-492). Sequence homology analysis revealed that certain basic residues known to be important for actin binding in other PH domain-containing proteins (Yao *et al. J. Biol. Chem.* **274**, 19752–61, 1999) are present in PH2. Mutagenesis of three such residues, Lys404, 405 and 396, into Ala eliminated the binding between M-RIP(1-492) and F-actin. Taken together our results show that the binding of M-RIP to F-actin is mediated primarily via its PH2 domain, in particular via ionic interactions between certain basic residues in PH2 and acidic residues in actin. (Supported by NIH AR41637 and AR49066).

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βCaMKII Regulates Actin Assembly and Structure

Hugo Sanabria, Kolodziej J. Steven, Matthew T. Swulius, Jun Liu, M. Neal Waxham.
The University of Texas Medical School at Houston, Houston, TX, USA.

Recently Ca^{2+}-Calmodulin-dependent protein kinase II (CaMKII), an abundant synaptic protein, was shown to be important in the organization of actin filaments at synapses. We report the binding properties and mechanistic role of βCaMKII as an actin binding protein *in vitro*. A particular case is the ability of βCaMKII to bundle actin filaments, which we corroborated by cryo-electron tomography. In addition, a pyrene-actin fluorescent polymerization assay was used to determine that the enzyme reduced the rate of polymerization by ~ 80%, suggesting that βCaMKII either serves as a capping protein or binds monomeric actin reducing the amount of freely available monomers to nucleate polymer assembly. Finally, by means of fluorescent cross correlation spectroscopy we determined that the β isoform of CaMKII does bind to monomeric actin, reaching saturation at a stoichiometry of 6:1 actin mononmers per βCaMKII holoenzyme with a binding affinity of ~2 μM. In conclusion, βCaMKII has a dual functional role; it can sequester monomeric actin to reduce actin polymerization and can also bundle actin filaments. Together, these effects would impact both the dynamics of assembly of actin filaments and enhance the structural rigidity of the filaments once formed, significantly impacting the structure of synapses.

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Coactosin, A Cofilin Like Protein, Does Not Change The Twist of F-actin

Albina Orlova[1], Vitold E. Galkin[1], Pekka Lappalainen[2], Edward H. Egelman[1].
[1]University of Virginia, Charlottesville, VA, USA, [2]Institute of Biotechnology, University of Helsinki, Helsinki, Finland.

Actin cytoskeletal rearrangements are orchestrated by a vast number of actin binding proteins. Coactosin is a 17kDa actin binding protein originally discovered in *Dictyostelium discoideum*. The protein has an ADF-homology domain (ADF-H-domain) also found in cofilin, twinfilin, and drebrin. It was shown that coactosin interferes with capping proteins, but in the absence of capping proteins it does not influence on actin polymerization. We used electron microscopy and the IHRSR method to reconstruct actin filaments stoichiometrically decorated with coactosin. In contrast toADF/cofilin, coactosin does not change the twist of F-actin. Nevertheless, coactosin utilizes a similar interface on F-actin to that used by ADF/cofilin. Our data are consistent with the solution structural studies on coactosin showing that the regions of ADF/cofilin involved in the interaction with F-actin are structurally conserved in coactosin.

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Conformational Changes Of Arp2/3 Complex During Activation

Paul Dalhaimer, Thomas D. Pollard.
Yale University, New Haven, CT, USA.

Kinetic and structural studies of Actin-related protein (Arp) 2/3 complex point to a transition from inactive to active conformations at an actin branch point. The inactive conformation is greatly favored for purified Arp2/3 complex. Crystal structures of inactive Arp2/3 complex show that Arp2 and Arp3 are separated by about 3 nm compared to their positions as the first two subunits of the daughter filament in low resolution models based in reconstructions from electron tomograms of actin filament branch junctions. Here, we use atomistic-scale molecular dynamics simulations to study activation of Arp2/3 complex. Starting in the ATP-bound inactive crystal structure, we apply forces to Arp2 and Arp3 so that the energy of the system is at a minimum when Arp2 and Arp3 are positioned like the first and second subunits of the daughter filament. Arp2 does not detach from the other subunits during activation as proposed from biochemical experiments. The long C-terminal α-helix of p34 rotates along with Arp2 and p40 through interactions with p20. Residues His^{30}-Leu^{35} of p20 stay in contact with the residues of the αG helix of Arp2 throughout the course of the simulation. Also, residues Arg^{106}-Lys^{107} of p20 interact with the region of Arp2 surrounding Leu^{235} between the αF and αG loops. The main impediment to a smooth transition is the αK helix of subdomain 3 of Arp3, which collides with the DNase binding loop of Arp2. This study provides the first insights into the conformational changes of Arp2/3 complex forms an actin filament branch.

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Acidic Calponin controls ERK1/2 translocation and l-Caldesmon phosphorylation in fibroblasts

Sarah Appel[1], J.-P. Jin[2], Kathleen G. Morgan[1].
[1]Boston University, Boston, MA, USA, [2]Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL, USA.

Calponin is an actin binding, troponin-like molecule, first isolated from chicken gizzard smooth muscle cells where it is involved in regulation of contractility. So far there are three known isoforms, basic, acidic and neutral Calponin, each encoded by a separate gene. Expression of the acidic Calponin isoform is, unlike basic Calponin, not restricted to smooth muscle cells but is expressed in a variety of tissues and cell types, especially in the brain. For basic Calponin it is known that the molecule affects smooth muscle cell contractility by regulation of the PKC/ERK signaling pathway. Here we could show that acidic Calponin co-precipitates together with ERK1/2 and PKCalpha in the cultured rat fibroblast cell line REF52.2. Moreover it colocalises with ERK1/2 and PKCalpha in podosome-like structures and at the cell cortex after Phorbol-12,13-Dibutyrate (PDBu) treatment. Knockdown of acidic Calponin expression in these cells results in inhibition of PDBu-mediated ERK1/2 translocation to these structures, whereas PKCalpha targeting is not affected. Both endogenous ERK1/2 activity and phosphorylation of the ERK1/2 substrate l-Caldesmon are blocked by acidic Calponin knockdown, indicating an important role for the acidic Calponin isoform in regulation of ERK1/2 function in REF52.2 cells. Abstract sponsored by John Gergely, Boston Biomedical Research Institute, Watertown 02472, MA, USA
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