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1634-Pos Board B544
Mapping the Actin-Binding Region in the Tarp Protein from Chlamydia
James L. Tolchard, Lawrence A. Eaglen, Leah Morris, Ted Hackstadt, **Tharin M.A. Blumenschein**.
Chlamydia trachomatis is a gram negative obligate intracellular parasite responsible for the most common bacterial sexually transmitted disease in developed countries, and the leading cause of preventable blindness worldwide. Chlamydiae have a unique life cycle, with a metabolically inert, yet infectious, spore-like phase and a morphologically distinct metabolic phase inside the infected cell. During infection and multiplication, Chlamydiae affect the cellular processes in the host cell through a number of type-III secreted effectors. Early in the infection process, the 1005 residue protein Tarp (translocated actin recruiting protein) is injected into the host cell, where it causes remodelling of the host cell's actin cytoskeleton, forming an actin pedestal, which aids in the internalisation of the parasite. Tarp is predicted to be largely intrinsically disordered, and is capable of interacting directly with actin monomer to nucleate the formation of acting filaments. The minimal actin-binding region has been mapped to a 100-residue fragment, $Tarp_{726\text{-}825}$ (Jewett *et al* (2006) *PNAS*, **103**:15599-15604). We show that a shorter region of Tarp, $Tarp_{726\text{-}808}$, is capable of binding actin and displays the characteristic NMR spectrum of a disordered protein. Using a combination of 2D and 3D nuclear magnetic resonance (NMR) experiments, the backbone resonances of this fragment were assigned. Comparison between $\{^1H, ^{15}N\}$-HSQC 2D NMR spectra in the presence and absence of actin reveals the amino acid residues directly affected by the binding.

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Molecular Moedling of Actin-Vinculin Interactions
Shayna M. Atkins.
Cytoskeletal rearrangement is a critical event during cell motility. This complex event is orchestrated by actin and tubulin in coordination with numerous other cytoskeletal protein components. Vinculin is one of the structural proteins that directly interacts with actin via its tail domain and is involved in bundling actin filaments. However, the structural organization of vinculin tail in association with actin remains elusive. Recent efforts by our collaborators have yielded low-resolution information about the overall organization of vinculin tail and actin monomers. Here, we propose a structural model for association of vinculin tail monomers with the actin filament, by fitting the crystal structures of actin and vinculin tail monomers into the low-resolution cryo-EM density map of an actin filament decorated by the vinculin tail. We further attempt to validate the structural accuracy of the model by rationally designing mutations using Medusa - a comprehensive protein design toolkit developed in-house. We believe that the generated models provide tremendous insight into the structural basis for actin-vinculin association and further our understanding of the role of vinculin in cell motility.

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Structural Dynamics of the Actin-Binding Domains in Dystrophin and Utrophin
Ava Y. Lin, Ewa Prochniewicz, Zach James, Davin Henderson, James Ervasti, David D. Thomas.
We are using time-resolved phosphorescence anisotropy (TPA) and dipolar electron-electron-resonance (DEER) to determine and compare the structural dynamics of the actin-binding domains in dystrophin and utrophin. Dystrophin and utrophin bind actin *in vitro* with similar affinities, but with different molecular contacts (Rybakova et al, 2006, *J. Biol. Chem*). We hypothesize that these differences alter the elasticity of dystrophin-actin and utrophin-actin linkages to the sarcolemma, affecting the cell's response to muscle stretches, with important implications for muscular dystrophy and its therapy. Our previous TPA studies, detecting the microsecond dynamics of phosphorescent-labeled actin, showed that both proteins have novel effects on actin flexibility, with utrophin more effective than dystrophin (Prochniewicz et al., 2009, *PNAS*). We have now compared the effects of the isolated actin-binding domains of dystrophin, ABD1 and ABD2. TPA shows that the enhanced rate of actin rotational dynamics is induced primarily by ABD1, while both ABD1 and ABD2 contribute to the restriction in rotational amplitude. Disease-causing point mutations in ABD1 decrease the effects on actin's rotational rate. We propose that this in turn causes the dystrophin-actin complex to be less resilient and thus less able to prevent damage to the muscle cytoskeleton during contraction. Finally, we have attached probes directly to ABD1 in dystrophin and utrophin, to detect changes in structure upon actin binding. High-resolution distance measurements, provided by DEER, show that the two lobes (calponin-homology domains) within ABD1 undergo a dramatic opening upon actin binding, helping to resolve a previous controversy. Analogous studies with dystrophin are in progress.

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Monitoring the Real-Time Binding of Tropomyosin to Actin using Total Internal Reflection Fluorescence Microscopy
William M. Schmidt, Paul Leavis, William Lehman, Jeffrey Moore.
Tropomyosin, an elongated actin binding protein, regulates the access of numerous other proteins onto the thin filament in virtually all cell types. This association, in turn, regulates various functions. Tropomyosin monomers assemble end-to-end to form a continuous polymeric chain that lies along the length of the actin filament. A number of different tropomyosin isoforms are present in all three muscle types: *viz.* smooth, cardiac, and skeletal. In cardiac and skeletal muscle, tropomyosin and troponin are the primary regulators of contraction. A plethora of tropomyosin isoforms are also present in non-muscle cells where they associate with cytoskeletal actin and play a role in numerous cellular processes including lamellipodia formation and actin-based cell motility. Moreover, various mutations in tropomyosin are associated with diseases such as congenital fiber type distortion (CFTD), ulcerative colitis, nemaline myopathy, and various cardiomyopathies.
Tropomyosin is a modular protein, and individually charged residues within its repeating domains are thought to be critical for actin binding. However, the mechanism of tropomyosin binding to actin is still uncertain. In the present study, we have acquired real-time videos of skeletal and smooth tropomyosin isoforms binding to actin filaments using total internal reflection fluorescence microscopy (TIRF). The videos suggest that weak monomer binding facilitates the gradual formation of small "nuclei" of tropomyosin monomers and/or oligomers. Once the nucleus is formed, the affinity of tropomyosin for actin increases exponentially and additional monomers are able to rapidly add to either end of the growing tropomyosin chain lying on F-actin.

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Tropomyosin Isoforms Exert Different Effects on Polymerizing Actin
Renjian Huang, **Chih-Lueh Albert Wang**.
Tropomyosin (Tm) isoforms are known to target different subpopulations of actin filaments in cells. For example, Tm2 is typically co-localized with stress fibers, whereas Tm5a/b are localized to cell peripheries where the actin cytoskeleton is more dynamically remodeled. We have reported previously that actin undergoes an obligatory conformational transition during polymerization, called maturation. Both Tm5a and caldesmon (CaD) interfere with this process and maintain actin filaments in the pre-transition (or "young") state if present at early stages of polymerization, but stabilize the matured actin filaments when binding occurs after this transition. When Tm5a and CaD are present together, they synergistically modulate actin maturation. The commonality between Tm5a and CaD apparently is not shared by all actin-binding proteins. Here we found that Tm2, when included in the F-buffer used to initiate actin polymerization, the pyrene-actin fluorescence enhancement was only slightly suppressed. When Tm2 and CaD were added together at the beginning of actin polymerization, the pyene-actin emission enhancement was greatly suppressed, although not as much as that caused by CaD alone. When calmodulin was added to dissociate CaD from actin in the presence of Tm2, the suppressed pyrene-actin fluorescence recovered quickly, with the final emission intensity approaching the level of Tm2•actin, which is about the same as that of actin alone. Upon addition of EGTA, on the other hand, the pyrene fluorescence only changed slightly to the level of CaD-bound mature F-actin. Clearly, actin had already passed the critical transition even Tm2 was still present. Our observations thus indicated that the maturation process can be blocked by Tm5a, but not by Tm2. Whether such a difference is related to their subcellular localizations and whether this finding holds true for other Tm isoforms await future investigations.

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The Interplay of Nonlinearity and Architecture in Cytoskeletal Mechanics
Shenshen Wang.
The interplay between cytoskeletal architecture and the nonlinearity of the interactions due to bucklable filaments plays a key role in modulating the cell's mechanical stability and affecting its structural rearrangements. We study a model of cytoskeletal structure treating it as an amorphous network of hard centers rigidly cross-linked by nonlinear elastic strings. Using simulations along with a self-consistent phonon method, we show that this minimal model exhibits diverse thermodynamically stable mechanical phases that depend on excluded volume, crosslink concentration, filament length and stiffness. Within the framework set by the free energy functional formulation and making use of the random first order transition theory of structural glasses, we further estimate the characteristic densities for a kinetic glass transition to occur in this model system. Network connectivity strongly