Actin-binding Proteins in Physiology & Disease

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Structure Function Analysis of Disease-Causing Missense Mutations in Dystrophin

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Duchenne muscular dystrophy (DMD) affects 1 of every 3500 males and results in death during the mid to late twenties. Mutations in the dystrophin gene leading to DMD commonly result in loss of protein expression or expression of a truncated protein lacking essential ligand binding domains. In some cases, point mutations leading to a single amino acid change in the dystrophin protein cause DMD, Becker muscular dystrophy or X-linked cardiomyopathy. Of the known disease causing mutations, 9 are located in the N-terminal actin-binding domain of dystrophin. Examining the effects of these mutations on actin binding activity will lead to a better understanding of key residues for dystrophin function in vivo. With this in mind, we engineered all 9 N-terminal disease-causing mutations into the full-length dystrophin cDNA and have begun to characterize the biochemical properties of each mutant protein expressed in the baculovirus system. We have found that R82P and A172P mutants did not express well enough to enable further biochemical characterization. The 7 remaining mutants were consistently less soluble and more aggregated than WT dystrophin. We have analyzed four mutants K18N, L54R, D165V and L172H for their ability to bind F-actin and found that K18N and L54R decreased the affinity of dystrophin for F-actin by 3–4 fold. The L172H mutation affected solubility but not actin binding properties of the full-length dystrophin protein. These data suggest that disease phenotypes associated with missense dystrophin mutations are caused by either loss of solubility or a combination of insolubility and decreased F-actin affinity. We also found that mutations that cause a more severe disease phenotype (K18N and L54R) bound actin with a lower affinity and were less soluble than WT dystrophin.

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Computational modeling of the binding interaction of Jasplakinolide and Phalloidin with mammalian and parasite F-actin

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Actin,a component of the cytoskeletal system, is a polymer that is critical for maintaining the shape and motility of a cell. This is achieved by a complex dynamic regulation of rapid polymerization and depolymerization of actin. As part of its defense mechanism, certain species of fungi and marine sponges produces cyclic peptide compounds like Jasplakinolide and Phalloidin that interferes with the actin depolymerization in foreign species. In this work we use computational methods like molecular dynamics, docking and QM/MM with to elucidate the molecular details of the interaction of these compounds with mammalian and parasite actin filament. Our analysis, in addition with experimental observations from our collaborators, also helps us to propose possible mechanisms for the polymer stabilizing property of these compounds.

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Vinculin Expression Regulates Tumor Cell Invasion In 3-D Matrices

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The process of tumor metastasis formation involves cell invasion into 3-D extracellular matrices and mechanical properties of the matrices as well as focal adhesion protein complex formation are believed to regulate cell migration. We analyzed high vinculin expressing breast carcinoma cells and wildtype and vinculin-deficient mouse embryonic fibroblasts to test their ability to invade into 3-D collagen type I fiber matrices. High vinculin expressing breast carcinoma cells invaded further into collagen matrices at 2.4 mg/ml collagen concentration compared to low vinculin expressing cells, whilst at 1.2 mg/ml collagen concentration, low vinculin expressing cells invaded deeper into the collagen matrices than high vinculin expressing cells. To determine the influence of vinculin, we used wildtype and vinculin-deficient mouse embryonic fibroblasts in 3-D matrix invasion assays at these collagen concentrations. We found that the invasion depth of fibroblasts expressing vinculin was greater at high compared to low collagen concentrations, whilst vinculin-deficient fibroblasts invaded deeper into collagen gels at low compared to high collagen concentrations.
These results indicate that breast carcinoma cells and fibroblasts use at least two invasion modes which depend on collagen concentration, i.e. mesenchymal and amoeboid. In conclusion, we assume that high vinculin expressing cells follow an invasion mode at high collagen concentration through contractile force generation (mesenchymal invasion), whereas at low collagen concentration, low vinculin expressing cells follow an invasion mode at negligible contractile force generation (amoeboid invasion).

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Role of Nebulin on Actomyosin Interaction Studied *in situ* in Demembranated Skeletal Muscle Fibers from Newborn Mice

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The effects of absence of nebulin, an actin filament associated protein, on mechanical and kinetic properties of Ca^{2+}-activated, chemically skinned, psoas fibers were investigated comparing mechanical performance of fibers from 1-day-old wildtype (*wt*) mice and 1-day-old nebulin deficient ($nebulin^{-/-}$) mice. With fast mechanics (Linari et al. *Biophys J* 92:2476, 2007) on fiber bundles (sarcomere length 2.5μm, temperature 13 °C) we determined *i*) the relation between isometric force, stiffness and Ca^{2+} concentration; and *ii*) the unloaded shortening velocity and the power output at different loads at saturating Ca^{2+} (pCa, 4.50). Actin filament length in psoas fibers is not affected by the absence of nebulin, as proven by immunofluorescence imaging. Our results show a reduction in isometric force in the absence of nebulin without changes in the Ca^{2+} sensitivity of the contractile system. Stiffness measurements accompanied by analysis of the compliance of the half-sarcomere indicate that the reduction in isometric force is due to a proportional reduction in the number of myosin motors attached to actin without change in the average force of the motor. In addition, the absence of nebulin increases the unloaded shortening velocity by 63%, while decreases the maximum power output by 80%. These results indicate that the absence of nebulin induces a decrease of the rate of attachment of the myosin motors to actin and an increase of the rate of detachment of negatively strained motors under zero load, revealing a direct role for nebulin in stabilizing the actomyosin interaction. Supported by NIH and MiUR.

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Tropomyosin Specifically Regulates Type II Myosin In Yeast

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Tropomyosin (Tm) is an evolutionarily conserved α-helical coiled-coil protein that forms polymers which coil around actin filaments to regulate their integrity and function within cells. In yeast, tropomyosin stabilised actin filaments are used by molecular motors to transport cargoes or generate motile forces. Acetylation of the amino terminal methionine of mammalian Tms is required for these proteins to associate with actin and is also crucial in regulating the formation of the actomyosin complex, however it is unclear whether this post-translational modification affects myosin regulation.
The fission yeast, *Schizosaccharomyces pombe*, contains a single Tm isoform, Cdc8, which localises to the cytokinetic actomyosin ring and is absolutely required for its formation and function during cell division. Our previous work has revealed that both acetylated and unacetylated forms of Cdc8 are present within fission yeast cells, and whilst we have shown that acetylated Cdc8 is capable of regulating myosin function *in vitro,* the role of the unacetylated form remains unresolved.
S. pombe possesses five myosin's representing 3 individual myosin classes, Class I (Myo1), II (Myo2 & Myp2) and V (Myo51 and Myo52). We have examined the role that acetylated and non-acetylated Cdc8 play in regulating each class of myosin within the cell. Myo2 is found localised exclusively to the cytokinetic actomyosin ring and its function is to facilitate cell division, whilst Myo1, and Myo52 are associated with actin polymers throughout the cell cycle. In this study we show that acetylated Cdc8 specifically affects Myo2 function, but does not affect the motor activity of Myo1 or Myo52. This is not only consistent with the fact that acetylated Cdc8 is found associated predominantly with actin filaments within the cytokinetic ring, but also with the cytokinetic defects observed in cells lacking acetylated Cdc8.

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Neurospora Crassa possesses a novel ultra short tropomyosin

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Tropomyosins are alpha-helical coiled coil proteins. They interact with actin filaments; binding along the major grooves, forming a continuous filament along the actin strand. In higher eukaryotes their most well understood role is in the regulation of muscle contraction, where they regulate the myosin II - actin interaction that generates force. Their non-muscular functions are not well characterised, and in lower eukaryotes regulatory function is less clear. However, it has been clearly shown they are fundamental to maintaining the actin cyoskeleton in yeast. We have identified that *Neurospora crassa* possess two tropomyosins: a 161 residue, 4 actin spanning protein, and a 123 residue, 3