Actin-binding Proteins in Physiology & Disease

648-Pos Board B527
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, L165V 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 dynamical regulation of rapid polymerization and depolymerization of actin. As part of its defense mechanism, certain species of fungi and marine sponges produce cyclic peptide compounds like Jasplakinolide and Phalloidin that interfere with the actin polymerization in foreign species. In this work we use computational methods like molecular dynamics, docking and QM/MM with computational methods like molecular dynamics, docking and QM/MM 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 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 Ca2+ -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 Ca2+ concentration; and ii) the unloaded shortening velocity and the power output at different loads at saturating Ca2+ (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 Ca2+ sensitivity of the contractile system. Stiffness measurements accompanied by analysis of the crosshead speed 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.