molecules per filament to determine the relationship between the number of heads and velocity. SMM:rod co-filaments (at 50:50, 25:75, and 15:25 ratios) were approximately the same length as normal SMM filaments (~0.6 μm), and the number of molecules incorporated was directly related to the ratio of SMM to rods. As the number of heads decreased, the maximal velocity at saturating ATP also decreased, and the K_{ATP} was lower for all of the co-filaments compared to the normal filaments. These findings provide evidence that the velocity of SMM filaments is influenced by attachment limited kinetics. The longer SMM filaments (average length of 2.3 ± 0.81 μm) will allow us to visualize the geometry of acto-myosin interactions during motion. Also, the relationship between velocity and filament length will be determined at various [ATP]. The long filaments also better demonstrate our previously described "parking" behavior where filaments move to the end of actin filaments but remain attached for long periods even at saturating [ATP] (Haldeman et al, JBC, 2014). Interestingly, most of the SMM filament hangs off the end of the actin suggesting the parking is mediated by a small fraction of the heads.

1485-Pos Board B436
The Structure of the Actin-Smooth Muscle MYOSIN II Complex in the Rigor State
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The actin-based motility of myosin utilizes catalysis of ATP to drive relative sliding of actin and myosin. Attachment to F-actin triggers conformational changes in the myosin head that accelerates release of catalytic products coupled to changes in actin affinity and motion of a structural domain called the lever arm. The earliest detailed model based on cryoEM and X-ray crystallography postulated that higher F-actin affinity and lever arm movement were coupled to closure of a feature of the myosin head dubbed the actin-binding cleft. Several studies since then using crystallography of myosin-V and cryoEM of myosin-I and -II have provided details of this model. Compared with non-muscle myosins, there are comparatively few catalytic intermediates available for myosin II. Details of the myosin II interaction with actin may differ from those for non-muscle myosins due in part to different lengths of important surface loops. Here we report on a 0.8 nm reconstruction of actin decorated with the smooth muscle myosin-II motor domain (MD) in the rigor state obtained by cryoEM and Iterative Helical Real Space Reconstruction. Quasiatomic models for both F-actin and the MD were obtained independently three times using molecular dynamics flexible fitting. SD3 and SD4 of F-actin are nearly identical while SD1 and SD2 show the most difference. The MD density is less well defined compared to the actin filament due to incomplete saturation. A significant density that could be assigned to loop1 is present. Loop2 and the myopathy loop show significant contacts with actin but these contacts appear variable between the three independent fittings. The comparison between nucleotide free acto-MD structure and the prepower-stroke crystal structure of the smooth muscle myosin MD suggests significant conformational changes occurred that may relate to the weak to strong transition.

Actin Structure, Dynamics, and Associated Proteins

1486-Pos Board B437
Role of H2 Calponin in Myoblast Differentiation, Fusion and Myogenesis
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Actin cytoskeleton plays a pivotal role in myoblast motility, adhesion, fusion and differentiation into myotubes. However, how the actin cytoskeleton is regulated during myogenesis is largely unknown. H2-calponin is a regulatory protein that stabilizes the actin cytoskeleton and regulates cell motility and adhesion. In the present study, we investigated the role of h2 calponin in the regulation of myoblast migration, differentiation and fusion. The results showed that h2-calponin is expressed at significant levels in undifferentiated mouse primary satellite cells and C2C12 myoblasts. In migrating satellite cells and C2C12 myoblasts, h2-calponin co-localizes with actin stress fibers at both the leading edge and the tail regions. In newly fused myotubes derived from primary satellite cells, h2-calponin is mainly present at the ends of the myotubes. The level of h2-calponin significantly decreases during satellite cell and myoblast differentiation and fusion, and becomes undetectable in mature myocytes. Primary satellite cells isolated from h2-calponin gene knockout mice had significantly decreased cell spreading area indicating reduced substrate adhesion and increased rate of cell proliferation as compared with wild type controls. Biochemical and histological studies revealed that skeletal muscles of h2-calponin knockout mice retain normal muscle weight and myofilament protein compositions. Myotubes derived from h2-calponin-null satellite cells showed apparently normal sarcomere striations and expression of myofilament proteins. Therefore, deletion of h2-calponin does not abolish the maximum capacity of myoblast function and myogenesis. However, in vitro differentiation studies revealed that h2-calponin-hull myoblasts had higher efficiency of fusion, as indicated by increased fusion index, than wild type controls. The data suggest that the function of h2-calponin provides a novel mechanism in regulating actin cytoskeleton activity during myoblast differentiation, fusion and myogenesis.

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Palladin Nucleates Actin Assembly and Regulates Cytoskeleton Architecture
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The actin scaffold protein palladin regulates both normal cell migration and invasive cell motility, processes that require the coordinated regulation of actin dynamics. Palladin localizes to actin-rich protrusions and has a well-documented effect on metastasis of invasive cancers. However, its potential effects on actin dynamics have remained elusive. Here, we show that the C-terminal immunoglobulin-like domain of palladin (Ig3) that is directly responsible for actin binding and bundling also potently nucleates the formation of actin filaments in vitro. Palladin eliminated the lag phase that is characteristic of the slow nucleation process of actin polymerization under both G- and F-actin buffer conditions. Furthermore palladin did not alter the critical concentration, and only had a modest effect on the rate of elongation of actin filaments. Therefore the increase in polymerization rate brought about by palladin can be attributed to a direct role for palladin in stabilizing the formation of actin nuclei. We also present evidence that nucleation is likely achieved by a mechanism involving actin-induced dimerization. In addition, we monitored actin polymerization in real-time using TIRF microscopy and found that palladin bundles the actin filaments while promoting polymerization. Finally, we examined whether the Ig3 domain of palladin is required for actin organization in a cellular context. In cells transfected with a full-length palladin construct containing either a deletion of the actin-binding domain or point mutations that disrupt actin-binding we observe dramatically altered cellular distributions of both palladin and actin, which suggests that this direct interaction with actin is critical for regulating cytoskeletal organization and dynamics. These observations define a new function for palladin and support an emerging view of actin-binding proteins that exhibit a dual cellular-nuclear localization and also participate in the regulation of the actin cytoskeleton architecture.

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Unraveling the Mystery of ATP Hydrolysis in Actin Filaments
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Numerous properties of actin filaments are modulated by the nucleotide state of the comprising actin monomers. This is evident in the typical life cycle of a bare actin filament (F-actin) during which ATP bound globular actin (G-actin) binds to the filament, ATP hydrolyzes in the filament and finally ADP bound G-actin dissociates from the filament. This is achieved by a 4.3×10^4 fold increase in the rate constant of ATP hydrolysis in F-actin as compared to G-actin. We investigate the cause of this dramatic rate increase using novel QM/MM simulations of ATP hydrolysis in both G-actin and F-actin forms. The F-actin system is modeled as a monomer with restraints on the coarse-grained variables to maintain its F-actin configuration. The free energy of ATP hydrolysis is computed along two reaction coordinates in each system with the aid of metadynamics. The simulations reveal a barrier height reduction for ATP hydrolysis in F-actin as compared to G-actin of 8 ± 1 kcal/mol, in good agreement with the experimentally measured barrier height reduction of 7 ± 1 kcal/mol. The barrier height reduction is influenced by an enhanced rotational diffusion of water in F-actin as compared to G-actin and shorter water wires between Asp154 and the nucleophilic water in F-actin, leading to more rapid proton transport.