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LRR Domain of Tropomodulin is Responsible for Targeting it to the Pointed End of the Actin Filament

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Tropomodulin, a tropomyosin-dependent actin filament capping protein, consists of two structurally and functionally different domains. Tropomodulin lacking its C-terminal domain can cap actin filaments with an affinity close to the full-length protein in vitro. To investigate the functional properties of the C-terminal domain in live cells, truncated GFP-tagged tropomodulin was expressed in rat cardiac myocytes. Three fragments were analyzed: Tmod1(1-159) that lacks the entire C-terminal domain, Tmod1(1-320) and Tmod1(1-349). GFP-Tmod1(1-159) did not assemble well at the pointed ends of the filaments (~80% of the cells demonstrated a diffuse distribution, while ~20% showed faint, inconsistent assembly). Together, the in vitro and live cell studies indicate that the C-terminal domain is not required for capping actin filaments but is important for specifically targeting tropomodulin to the pointed ends of the actin filaments in sarcomeres. In the cells where GFP-Tmod1(1-320) was expressed most (~70%) of the assembly was faint and inconsistent. Tmod1(1-320) lacks the 39 C-terminal residues that include both the C-terminal helix that is not a part of LRR fold, and the tropomyosin independent actin filament capping site. The precise location of the tropomyosin independent actin filament capping site is not known, although removal of 15 residues from the C-terminus destroys the actin filament capping ability of Tmod1 in the absence of tropomyosin. GFP-Tmod1(1-349) is missing the ten C-terminal residues which discriminates Tmod1 from other tropomodulin isoforms; this fragment consistently assembled at the thin filament pointed ends, comparable to the cells expressing wild type GFP-Tmod1. Based on these data we suggest that both the LRR fold (residues 160-320) and residues 321-349 are important for regulating tropomodulin's pointed end capping activity though the specific role each of these regions play in this phenomenon may be different.

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Organization of F-Actin by Avian Smooth Muscle Synaptopodin 2 (Fesselin)

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Fesselin is a member of the synaptopodin family of actin binding proteins and it is rich in smooth muscle tissue. Fesselin accelerates actin polymerization in a calcium-calmodulin dependent fashion and forms insoluble aggregates with actin. The polymerization of actin occurs even in the absence of salts usually required to initiate polymerization. The question is whether the properties of fesselin are indicative of a function in forming structures rich in fesselin such as dense bodies and Z-lines. Electron microscopy of actin filaments shows that fesselin initially produces long actin filaments that become organized into thick bundles. The structure of the bundles depends on the ratio of fesselin to actin. Most frequently there are dense parallel ordered bundles. In some cases, the bundles are frayed and thus display single actin filaments. The filaments within a bundle are in-register and have either common initiation or termination points. Decoration with S1 showed that the filaments of a bundle had the same orientation. Actin filaments were occasionally seen to be radiating from densely stained complexes. Solution studies support the idea that fesselin stimulates pointed end growth of actin filaments. That is, fesselin (a) increased the critical concentration for polymerization and (b) induced actin growth even in the presence of barbed end blockers. When added to actin in the absence of other factors, fesselin can nucleate networks of linear actin filaments in which growth occurs primarily at the pointed ends of actin filaments.

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Generation of a De Novo Actin Pointed-End Binding Protein

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In order to create disease specific drugs, molecules that are designed to bind to any given target with high affinity and high specificity have been the motivation of the biotechnology field for some time. The natural immune system can produce antibodies that essentially recognize any antigenic target with high affinity and specificity. Therefore, employing this specificity through antibody related technologies should yield bioengineered proteins that are very useful to biomedical research. We performed a high-throughput phage display screen, which used restricted amino acid diversity phage display libraries to generate antibody fragments to F-actin. We generated synthetic actin-binding antibody fragments (sABs) that were further selected to bind to specific actin conformations. These sABs resemble the Fab fragment of an antibody, with a 55kDa heterodimer of heavy and light chain variable fragments, forming an antigen-binding site that was engineered to bind to a specific actin conformation by specific library sorting and subsequent phage ELISA characterization. A series of these generated sABs were characterized in vitro and in vivo. We identified three interesting candidates for study. The first construct severs F-actin in vitro and in vivo. The second sAB bundles F-actin and inhibits G-actin from polymerizing. And the most interesting sAB is a pointed-end actin binding molecule. This construct shows clear affinity for the pointed-end of actin by in vitro TIRF analysis. Furthermore, this construct also inhibits in vitro polymerization of actin over time. The application of this technology to generate molecules to specific conformations of proteins for analysis will be pivotal in the bioengineering field.

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Binding of N-Terminus Fragments of Cardiac Myosin-Binding C-protein to Actin

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Myosin-binding protein C (C-protein) is a sarcomeric protein regularly associated with thick filaments of vertebrate skeletal and heart muscle and is involved in the regulating of muscle contraction. C-protein belongs to the immunoglobulin- fibronectin superfamily of proteins and it consists of 11 tandemly arranged immunoglobulin-like domains. Four Ig-domains at the N-terminus of the cardiac isoform (C0-C1-m-C2 or C0C2) influence the actin -myosin S1 interaction whereas the C-terminal domains (C7-C10) play a structural role in the sarcomere, binding to myosin thick filaments. It has been shown that C0C2 fragment stabilizes F-actin, decorating the filaments in a highly regular arrangement.
We used electron microscopy and single particle image analysis to reconstruct complexes of F-actin with cardiac C-protein fragments containing 4 Ig-domains (C0C2) and 2 Ig-domains (C0C1). Both fragments regularly decorated actin filaments and image analysis has revealed substantial rod-shaped density connecting adjacent monomers along the actin filament. The fragments induced dendritic growth of actin filaments in the first several minutes of co-polymerization followed by formation of regular sheets of filaments and ordered bundles. The results will be discussed in the terms of suggested involvement of C-protein in the modulation of contractile cycles of heart muscles.

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Mechanisms Of Filament Twirling In Gliding Assays

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Helical motion of filaments in a gliding assay is a ubiquitous phenomenon that has been observed in non-processive myosins [1,2], processive myosins [2], non-processive axonemal dyneins [3] and in some cases in processive kinesins. Here we present a theoretical study about the mechanism of such filament rotation. We show that this mechanism is different in all 4 combinations of processive/non-processive motors moving actin/microtubules.
In the case of processive motors the pitch of the filament motion is simply determined by the step-size of the motor (myosins) or its stepping pattern, as well as protofilament helicity (microtubule-based motors). The interpretation is more complex in the case of non-processive motors. For non-processive myosins we show that rotation results from the fact that a myosin head preferentially binds to to the actin filament within ''target zones'' in which the subunits have the right orientation [4]. This leads to a left-handed rotation whose pitch depends non-monotonously on the ATP concentration. Combined with an off-axis component of the power-stroke, the model also allows for a cross-over between left- and right-handed rotation, providing a possible explanation for the difference between Refs. [2] and [1]. Finally, in non-processive microtubule motors (e.g., single headed dyneins or kinesins) the filament rotation is determined by the properties of the motor, rather than the filament geometry. It can either result from an asymmetry in the attachment rate, or from at least one off-axis power stroke within the duty cycle.

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