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788-Pos Board B574

'Catch Mechanism' found in Molluscan Animals other than Bivalves

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Some muscles of bivalve molluscs (phylum Mollusca, class Bivalvia) such as the smooth part of the adductor and the byssus retractor are known as 'catch muscles' that can maintain high passive tension for long periods with little energy expenditure after their active contraction. The state of this high passive tension is called 'catch' and it has been hypothesized that some special 'catch mechanism' exists for the maintenance of the tension. We have developed an *in vitro* catch assay in which the catch state is reconstituted by using proteins purified from bivalve catch muscles. With this assay, we have revealed that the catch tension is due to binding of actin filaments to myosin filaments which can be directly visualized under a light microscope. We have also found that twitchin, a giant protein associated with myosin filaments structurally related to connectin/titin, and to less extent, vertebrate cardiac myosin binding protein C, is an essential key component for the catch mechanism. Twitchin was originally found in a nematode *Caenorhabditis elegans*, and was later found widely in many invertebrates. This fact raises a question whether animals having twitchin other than bivalves also have the catch mechanism. To answer this, we prepared synthetic thick filaments containing myosin and twitchin from organs of molluscan animal species other than bivalves. We used foot of a spiral shellfish *Monodonta labio* (class Gastropoda) and a chiton *Liolophura japonica* (class Polyplacophora), and inner wall of suckers of an octopus *Octopus vulgaris* (class Cephalopoda). We performed the *in vitro* catch assay and found that the catch mechanism, at least at the molecular level, exists also in these molluscan animals. The results suggest that the catch mechanism is distributed more widely in Animal Kingdom than we know.

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Phosphorylated Smooth Muscle Heavy Meromyosin Shows an Open Conformation: Implications for the Structure of Myosin with One Head Phosphorylated

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Smooth muscle myosin (smM) and heavy meromyosin (smHMM) are activated by regulatory light chain (RLC) phosphorylation but the mechanism remains unclear. Dephosphorylated, inactive smHMM assumes a closed conformation with asymmetric intramolecular head-head interactions involving motor domains and the essential light chain (ELC) [Wendt et al., PNAS 98: 4361 (2001)]. The ''free head'' can bind to actin, but the actin-binding interface of the ''blocked head'' is involved in interactions with the free head. We report here a 3-D structure for phosphorylated, active smHMM obtained using electron crystallography of 2-D arrays, and an atomic model obtained by fitting using normal mode flexible fitting. Head-head interactions of phosphorylated smHMM resemble those found in the dephosphorylated state, but occur between separate molecules. The interface between heads of phosphorylated smHMM is less extensive and somewhat altered in orientation compared with that of dephosphorylated smHMM. The light chain binding domain of phosphorylated and several dephosphorylated myosin structures show systematic differences. However, the major difference appears to be the relationship between the motor domain and the ELC in a phosphorylated head compared to that of the ''blocked head'' of dephosphorylated smHMM. We hypothesize that RLC phosphorylation disrupts the inhibited conformation primarily by its effect on the ''blocked head'' rather than the ''free head''. Singly phosphorylated smHMM is not compatible with the closed conformation if the ''blocked head'' is phosphorylated. The implications of this observation for myosin activation at low levels of phosphorylation in smooth muscle will be discussed. Supported by grants from the NIAMS, NHLBI and NSF-MCB.

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Synthesis and Functional Characterization of Azido-Blebbistatin, a Photoreactive Myosin Inhibitor

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Photoreactive compounds are important tools in life sciences that allow precisely timed covalent crosslinking of ligands and targets. Using a novel large-scale technique we have synthesized azidoblebbistatin, which is a new derivative of blebbistatin, the most widely used myosin inhibitor. Without UV irradiation azidoblebbistatin exhibits identical inhibitory properties to those of blebbistatin. Using UV irradiation azidoblebbistatin can be covalently crosslinked to myosin, which greatly enhances its in vitro and in vivo effectiveness. Photocrosslinking also eliminates limitations associated with the relatively low myosin affinity and water solubility of blebbistatin. Irradiating light used for photocrosslinking is not toxic for cells and tissues, which confers a great advantage in in vivo tests. As the crosslink results in an irreversible association of the inhibitor to myosin and the irradiation eliminates the residual activity of unbound inhibitor molecules, azidoblebbistatin has a great potential to become a highly effective tool in both structural studies of actomyosin contractility and the investigation of cellular and physiological functions of myosin II.

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In Vivo Investigation of Calpain Activity by Lifetime Imaging of Genetically Encoded FRET Sensors

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Calpains belong to a family of calcium-dependant cysteine proteases that are ubiquitously expressed in mammals and have roles in numerous physiological processes. Inappropriate calpain activation, caused by a loss of calcium homeostasis, has been implicated in various disease states. To date, *in vivo* studies looking at the activity of calpains have relied on invasive procedures and the use of intensity-based measurements. We created a calpain-sensitive FRET biosensor (CSFB) and applied time-lapse fluorescence lifetime imaging (FLIM) to measure calpain activation in skeletal muscle *in vivo*. CSFB consists of the red fluorophores TagRFP-T and mPlum, linked by a calpain-cleavable peptide. In the uncleaved state CSFB allows FRET. Upon calpain activation CSFB is cleaved and FRET ceases. FLIM was used to measure the proportion of cleaved versus uncleaved CSFB, as the fluorescence lifetime of TagRFP-T can be discriminated in the two states.

Plasmid DNA was electroporated into the tibialis anterior (TA) hind-leg muscle of mice. At the peak of protein expression mice were positioned on an imaging platform and the TA imaged in reflection geometry. Calpains were activated by electrical stimulation of the leg muscle. A series of wide-field time-gated images were acquired allowing fluorescence decay profiles to be fitted and fluorescence lifetimes calculated over the course of calpain activation. A calcium FRET sensor, TN-L15, was also used to monitor the calcium transient elicited upon muscle stimulation.By multiplexing TN-L15 with CSFB we have been able to record raised intracellular calcium and calpain activation simultaneously in live mice. This non-invasive methodology allows temporal assessment of calpain activation *in vivo*, enabling evaluation of its role in translational studies such as in the mouse model for Duchenne muscular dystrophy.

Bacteria & Motile Cells: Signal Transduction

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Second-Chance Signal Transduction is a Model for Bacterial Flagellar Switching and Tropomyosin-Based Motility

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The reversal of flagellar motion (switching) results from the interaction between a switch complex of the flagellar rotor and a torque-generating unit (motor unit) of the stator. To explain the steeply cooperative switching response to ligand, present models propose allosteric interaction between subunits of the rotor but have yet to address the reaction that stimulates a motor unit to reverse directions. An individual motor unit could exist in ground and excited states corresponding to counterclockwise and clockwise rotation, respectively. After a passing ligand-bound switch complex excites a motor unit, the independent decay rate of the excited state determines the probability that a fresh switch complex will reach the dwell site owing to the steady-state rotation of the rotor before the motor unit returns to ground state. Here, we derive an analytical expression, based on our muscle model, for the energy coupling between a switch complex and a motor unit in the stator complex of a flagellum, and demonstrate that it accounts for the cooperative switching response without the need for allostery. This analytic function becomes the Hill equation as a special case. We found that the analytical result can be reproduced by simulation if the motion of the rotor provides a motor unit with a second chance to remain excited and the outputs from multiple independent motor units are constrained to a single all-or-none event. A motor unit and switch complex represent switch and reader