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 laevis (class Gastropoda) and a chiton Liolepadora 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 knew.

Phosphorylated Smooth Muscle Heavy Meromyosin Shows an Open Conformation: Implications for the Structure of Myosin with One Head Phosphorylated Kenneth A. Taylor1, Bruce A.J. Baumann1, Dianne W. Taylor1, Zhong Huang1, Florence Tama2, Patricia M. Fagnant2, Kathleen M. Trybus2, 1Florida State University, Tallahassee, FL, USA, 2University of Arizona, Tucson, AZ, USA, 2University of Vermont College of Medicine, Burlington, VT, USA.

Smooth muscle myosin (sM) and heavy meromyosin (sHMM) are activated by regulatory light chain (RLC) phosphorylation but the mechanism remains unclear. Dephosphorylated, inactive sHMM 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 sHMM 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 sHMM resemble those found in the dephosphorylated state, but occur between different molecules. The interface between heads of phosphorylated sHMM is less extensive and somewhat altered in orientation compared with that of dephosphorylated sHMM. 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 sHMM. We hypothesize that RLC phosphorylation disrupts the inhibited conformation primarily by its effect on the “blocked head” rather than the “free head.” Singly phosphorylated sHMM 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 NIAAMS, NHLBI and NSF-MCB.

Bacteria & Motile Cells: Signal Transduction

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 counts 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