

MS04.13.02 WHAT CAN THE REGULATORY DOMAIN OF MYOSIN TELL US ABOUT REGULATION? A. Houdusse, C. Cohen, Rosenstiel Basic Med. Sci. Res. Ctr., Brandeis University, Waltham, MA

The activity of a large class of myosin molecules is controlled by a regulatory mechanism built into the molecule itself. As in all muscle myosins, the globular head portion (S1) of the molecule consists of both a motor domain that binds actin and has ATPase activity and an elongated regulatory domain (RD) where two different light chains (LCs) are bound. In molluscan muscles, direct binding of Ca^{2+} ions triggers activity; in vertebrate smooth muscle myosin and some non-muscle myosins, a Ca^{2+} -dependent phosphorylation of the molecule acts as the trigger. The unusual state in the regulated myosins is the "off" state of enzymatic activity which appears to require interactions of both heads together with a stable portion of the coiled-coil rod region. In this state, the loss of Ca^{2+} (or dephosphorylation) produces conformational changes in the RD of each head which are transmitted to the motor domains some 100 Å distant.

Here we report the structure of the Ca^{2+} -bound form of the RD of scallop myosin refined to 2.0 Å resolution. This proteolytic fragment consists of two calmodulin (CaM)-like LCs that stabilize a long helical heavy chain (HC) fragment. The quality of the electron density is improved sufficiently over that in the previous 2.8 Å structure to establish the precise coordination of the Mg^{2+} ion in the open lobe of the regulatory light chain (RLC) allowing us to account for the preferential binding of Mg^{2+} rather than Ca^{2+} in this site. The unusual coordination of the triggering Ca^{2+} site in the closed lobe of the essential light chain (ELC) is also accounted for by distinctive structural features of this lobe. Because of the network of critical linkages between the Ca^{2+} -binding site of the ELC, and both the RLC and HC, the structure implies that in the Ca^{2+} -bound "on" state, this domain is likely to be a rigid structure. Correspondingly, we describe how the loss of the Ca^{2+} ion might induce flexibility in the structure. Comparison of the structures of Ca^{2+} -bound scallop RD with that of the same region of chicken S1 also shows two places where the HC is bent differently. We describe how these differences might affect the relative positioning of the two motor domains in the molecule. The structural results suggest a model for myosin regulation in which the RD acts as a switch so that in the Ca^{2+} -free state, the transient flexibility of the RD permits specific intra-molecular linkages to be made in the myosin molecule that were sterically inaccessible in the Ca^{2+} -bound structure. In this "off" state, motions within the motor domain required for activity are effectively switched off. According to this view, regulated myosins belong to a class of structures that require dimerization for the full expression of function.

MS04.13.03 STRUCTURE OF MOLECULAR TRACKS AND MOTORS. R.A. Milligan, Department of Cell Biology, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, CA 92037 U.S.A

Molecular motors belonging to the myosin and kinesin superfamilies utilize ATP to move along their respective F-actin and microtubule tracks. The track-motor complexes have not been amenable to crystallization so x-ray crystallographic investigations have focused on structure determinations of the individual proteins. Although providing detailed descriptions of the structure of each protein, this approach cannot reveal the geometry of interaction of the proteins or the conformational changes which occur during the mechanochemical cycle. To obtain this information, we use electron microscopy and image analysis to calculate three dimensional maps of the track-motor complexes at moderate resolution (1.5-3.0nm). Recently, we have been investigating conformational changes in the motors in the response to nucleotide binding. In smooth muscle myosin (a collaboration with H.L. Sweeney, U. Penn.) and brush border myosin I, there are large changes in the orientation of the light-chain-containing tail of the motor when MgADP binds to the actin-myosin complex. The light-chain region of the molecule seems to act as a rigid lever arm, pivoting about a point located near the

sulphydryl-containing region of the myosin motor domain. The reorientation of the light-chain regions that we have observed could account for a step of 3.5nm in smooth muscle myosin and <7nm in BBMD in response to ADP release. Similar experiments aimed at visualizing conformational changes in kinesin motors are in progress.

MS04.13.04 AN OPEN-STATE OF β -ACTIN AND THE SOLID-STATE TRANSFORMATIONS OF PROFILIN: β -ACTIN CRYSTALS John K. Chik (NIH), Uno Lindberg, Stockholm U. and C.E. Schutt, Princeton U.

The 2.65Å structure of an "open-state" of bovine β -actin reveals many differences to our previously solved "tight-state" structure (C.E. Schutt et al., *Nature*, **365**:810-816 (1993)) such as solvent accessible ATP phosphates. It was also possible to estimate the energy difference between these two states using osmotic pressure. The conformation of β -actin complexed with profilin is sensitive to the environment surrounding the crystal. Profilin: β -actin crystals normally grown in potassium phosphate were sensitive enough to solution condition so as to make them unsuitable for isomorphous heavy-atom methods (Schutt et al., *J. Mol. Biol.*, **209**:735-746 (1989)). Transferring the 1.8M potassium phosphate grown crystals into 3.2M ammonium sulphate yielded better diffracting crystals that were also amenable to heavy-atom methods. This transition from potassium phosphate, open-state, to ammonium sulphate, tight-state, buffer caused the c-dimension of the unit-cell to shrink from 185.7Å to 171.9Å without deleterious damage to the crystal. These tight-state crystals lead to the 2.55Å structure of the complex (C.E. Schutt et al., *Nature*, **365**:810-816 (1993)). The structure of the open-state complex was solved using molecular replacement ($R=20.1\%$ and $R_{\text{free}}=32.8\%$ from 8.00-2.65Å). The open- to tight-state transition buries the solvent exposed ATP phosphates, closes the cleft between the domains and rotates sub-domain 2 by 14.7°. Also, as seen in tight-state crystals, open-state β -actins form extensive contacts to symmetry related actins along the 2₁ axis parallel to the b-direction. This "ribbon" feature is maintained in the open- to tight-state transition. Using osmotic pressure, it was possible change the unit-cell dimensions to a similar degree as seen in the open- to tight-state transition and thus, by inference, provides an experimental estimate of the energy difference between the states. The estimated difference is only a fraction of thermal energy and is consistent with the observed sensitivity of the crystals.

MS04.13.05 FROM STRUCTURE TO FUNCTION OF PLASMA GELSOLIN. L.D. Burtnick*, E.K. Koepf*, J.M. Grimes+, E.Y. Jones+, D.I.H. Stuart+, P. McLaughlin# and R.C. Robinson+, *Chemistry Dept. UBC, Vancouver, Canada, V6T 1Z1, #Edinburgh University, Edinburgh, U.K., EHS 9XD and +LMB, Oxford University, Oxford, U.K., OX1 3QU.

The structure of horse plasma gelsolin to 2.5 Å resolution can be coupled with biochemical and biophysical results to explain the calcium-dependent severing of actin filaments by gelsolin. Gelsolin contains six similarly folded domains organized into two nearly independent halves. Connections between the halves are formed firstly by a long polypeptide loop that covalently links the terminus of S3 to the start of S4, and then by the C-terminal helical tail of the protein reaching back to lie parallel to a long helix in S2. We suggest that the binding of calcium to the second half of gelsolin releases the C-terminal tail from its interactions with S2. The two halves of the gelsolin molecule then can act relatively independently, restrained only by the 50 residue linker, to bind to actin units on opposite sides of an actin filament. We suggest that S2 binds first and positions S1 near to its binding site. The binding of S1 introduces sufficient steric conflict between S1/S3 and the next actin protomer in the filament to induce severing. These actions would be mirrored at an actin unit across the filament due to the binding of S4-S6, completing the severing and capping activity. Slack in the lengthy chains that link various of the domains of gelsolin would enable the required relative motions amongst the segments, without requiring significant changes in the secondary or tertiary structure of any individual domain.