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. Besides, 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.

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 sulphhydryl-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 BRM in response to ADP release. Similar experiments aimed at visualizing conformational changes in kinesin motors are in progress.

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 diffraction 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 Rfree=23.6% 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-actin forms 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.