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Measuring motions in muscles

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If one could attach a fluorescent tag to a macromolecule and then measure its orientation in a cellular environment, then one would have a powerful tool by which to study conformational changes under physiological conditions. Apply this approach to the study of myosin and it should be possible to measure the movement that is thought to drive muscle contraction. This method is not new but until now it has yielded rather ambiguous results because the fluorophores themselves could move independently of the protein to which they were attached. By dint of much hard chemistry, Corrie *et al.* (*Nature* **430**, 425–430, 1999) have managed to fix both ends of the bifunctional fluorophore rhodamine to the myosin regulatory light chain (RLC), thereby uniquely defining its orientation. They have then used the rhodamine-labeled RLC, in suitable muscle preparations that allow exchange of endogenous RLCs, to measure changes in the orientation of the myosin lever arm during contraction.

Conformational changes in the myosin head regions (catalytic domain in red, heavy chain in blue, regulatory light chain in magenta and essential light chain in yellow) that bridge myosin and actin filaments (brown, gray, green) are thought to drive force generation and the relative sliding between these two filaments. Atomic resolution structural data, together with biochemical and physiological studies of the interaction between myosin, actin and ATP have led to the lever arm model for the force-generating conformational change in the myosin head. According to this model, the light chain binding domain changes orientation relative to the actin-bound catalytic domain during the ATPase cycle. Corrie *et al.* fixed rhodamine through iodoacetate groups to different locations on the regulatory light chain so as to measure changes in orientation of the lever arm during a contraction. In each case, the rhodamine has been fastened to pairs of cysteine groups engineered into the regulatory light chain at an appropriate distance apart, based on the known crystal structure, to provide a fixed attachment. The average orientpation of the fluorescence dipole is expected to be parallel to a line connecting the two cysteines. The three dimensional orientation of the RLC can be deduced from orientations of the bifunctional rhodamine dipoles, knowing the crystal structure coordinates of the residues where each rhodamine probe was attached.

Shortening of a contracting muscle fiber results in a decrease in force, followed by a very rapid recovery that is thought to be due to the movement of the lever arms. Corrie and coworkers measured changes in orientation of the RLC region upon a muscle shortening of 3.6 nm per crossbridge with a 50 µs time resolution. Measurement of the tilt and twist angle of the RLC with respect to the actin filament showed a rotation corresponding to a 3.6 m average movement of the lever arm. Thus, the rotation of the lever arm was closely linked to the tension recovery. This result strongly favors the swinging lever arm model for muscle contraction. However, there are two caveats. The magnitude of the force recovery after the muscle shortening was modest, although this could be readily explained by the fact that the muscle fibers had been through a number of manipulations. Furthermore, the number of myosin crossbridges taking part in the contraction was small. A number of other experiments suggest that the number of attached crossbridges is small even in normal contracting muscle, but this issue is still rather contentious. Nevertheless, this recent study demonstrates a highly sensitive method for measuring the most basic movement in muscle in a physiological environment. This method should find wider applicability in many different areas of biology, where it will complement high-resolution structures.

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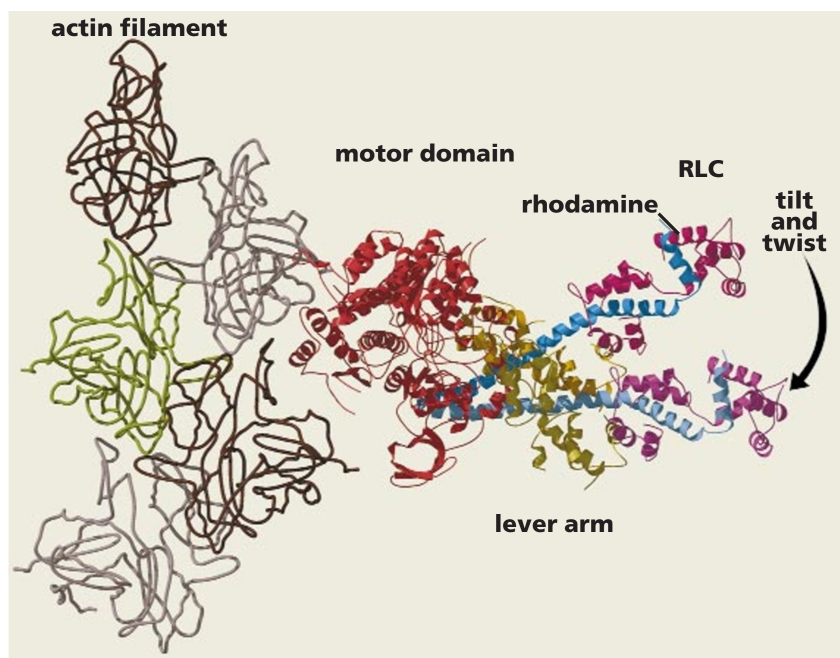

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