

STRUCTURAL MODELS FOR THE REGULATORY SWITCH OF MYOSIN

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Regulation of muscle contraction depends on calcium-sensitive protein switches that control the force-producing interactions of myosin and actin. All muscle myosins contain light chains, which in many cases function as regulatory subunits (Szent-Györgyi et al., 1973). In the absence of calcium, they inhibit myosin's enzymatic activity and restrict myosin-actin interactions. The "off" states of various regulated myosins are probably similar, and depend on cooperative interactions between myosin heads (Stafford et al., 1979; Persechini and Hartshorne, 1981); that is, single heads (S1) cannot be turned off. The mechanisms that trigger the "on" state vary, however: molluscan myosin is switched on when Ca^{2+} binds to a specific site on the head of the molecule; but in some invertebrate muscles, as well as in vertebrate smooth muscle and many nonmuscle cells, Ca^{2+} activates myosin indirectly by inducing light-chain phosphorylation. Scallop myosin has proved suitable for study of light-chain function because of the simplicity of its regulatory switch, and because the "regulatory" light chains (R-LC) can be reversibly dissociated from the myosin molecule (see review by Szent-Györgyi and Chantler, 1985).

We have used the property of light-chain dissociation, in combination with electron microscopy and three-dimensional image analysis, to show that the elongated regulatory light chain spans a region ~ 100 Å long in the neck of the scallop myosin molecule (Craig et al., 1980; Vibert and Craig, 1982; Flicker et al., 1983). The R-LC and the other essential light chain (SH-LC), together with a segment of the myosin heavy chain, appear to constitute a distinct regulatory domain of each myosin head (Bennett et al., 1984; Szentkiralyi, 1984; Winkelmann et al., 1984). The extended structure of this domain depends on the presence of the R-LC; moreover, cross-linking experiments reveal movement of the SH-LC relative to the R-LC between the "on" and "off" states of the regulatory switch (Hardwicke et al., 1983). The light chain motions occur at one end of the regulatory domain, near the head-rod junction of myosin; in this region, the NH_2 -terminal regions of the R-LCs on the two heads of a single myosin molecule are also close enough to be cross-linked (Hardwicke and Szent-Györgyi, 1985; Vibert et al., 1985). Interactions at the head-rod junction are critical for light chain regulation, as shown by the cooperative aspects of both inhibition (Stafford et al., 1979; Chantler and Szent-Györgyi, 1980) and activation (Chantler et al., 1981).

Regulation by light chains probably affects more than

one step in the cycle of myosin-actin interactions. Ca^{2+} -binding induces light-chain motions within the regulatory domain and elevates the ATPase activity of scallop HMM in the absence of interaction with actin (Wells and Bagshaw, 1985). The nature of the myosin-actin interaction is also altered by the presence of Ca^{2+} , and the ATPase is then fully activated through acceleration of a kinetic step in the cross-bridge cycle (Chalovich et al., 1983; Wells and Bagshaw, 1985). Thus light chains may control conformational changes in the myosin head associated both with the cycle of ATP hydrolysis and with the specific linkages to actin.

Structural studies on various myosins indicate that the enzymatic and actin-binding sites are located near the distal end of the head, 150 Å or more from the head-rod junction (Vibert and Craig, 1982; Winkelmann et al., 1983). The light chains themselves are elongated (Stafford and Szent-Györgyi, 1978), but probably do not extend into the actin- or ATP-binding sites. Proteolytic cleavage indicates that within the head the myosin heavy chain may fold into at least two major domains. One of these, with which the light chains interact (the COOH-terminal 24,000 d peptide of scallop S1 [Szentkiralyi, 1982, 1984], or the corresponding $20\text{--}26 \times 10^3$ d peptide of vertebrate skeletal and smooth muscle S1 [Sellers and Harvey, 1984]), is probably highly extended (Winkelmann et al., 1984), and may reach from the head-rod junction to the actin-binding site. Structural studies by x-ray diffraction and spectroscopy indicate that interdomain motions may be critical in force generation by all myosin (cf. Huxley and Kress, 1985). Moreover, recent electron microscopy of myosin heads cross-linked to actin suggests that head shape, as well as apparent angle of attachment to actin, may change significantly during active ATP hydrolysis (Craig et al., 1985). Thus a key aspect of light-chain regulation could involve control of internal motions of the myosin head.

Earlier models for light-chain regulation envisaged either a "steric blocking" role for the R-LC at the actin-binding site, or that R-LC altered head-head interactions by controlling the conformation of the head-rod junction (Kentrick-Jones and Jakes, 1977; Bagshaw, 1980; Cohen et al., 1980; Chantler, 1983). Recent results on the domain structure of the myosin head, together with our evidence on light chain location, structure, interactions, and motions, suggest another class of models in which light chains prevent internal head motions.

In this picture of myosin head topography, at least two

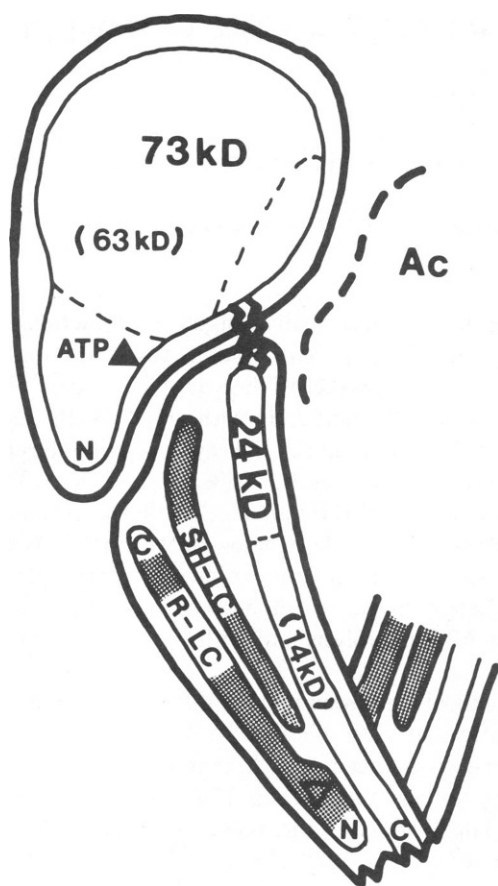


FIGURE 1 Topography of the scallop myosin head. Two major structural domains are shown, connected by a flexible hinge at the proteolytically sensitive junction of the 24,000 d and 73,000 d heavy chain peptides (Szentkiralyi, 1982, 1984) (the 73,000 d peptide may, by analogy with vertebrate myosin, comprise two domains of mass ~50,000 d and 23,000 d, the latter containing the nucleotide site and the NH₂-terminus of the heavy chain). The 24,000 d peptide is assumed to be highly extended (Winkelman et al., 1984); its COOH-terminus is at the S1/S2 junction, and its NH₂-terminal region is probably close to the actin-binding site. (It is not yet known whether this peptide binds specifically to actin, as the homologous 20,000 d peptide of vertebrate myosin is reported to do [Sutoh, 1983]; but cleavage at the 73/24 junction is inhibited by actin binding.) Other heavy chain cleavage sites (and possible hinges) occur ~10,000 d distant on either side of the 73/24 × 10³ d junction, producing a 14,000 d COOH-terminal peptide (which carries light chain binding sites) and a 63,000 d NH₂-terminal peptide (Szentkiralyi, 1982, 1984). The two light chains (R-LC and SH-LC) are shown as extended rods that overlap for most of their length; the R-LC, however, has a compact Mg²⁺-binding domain near its NH₂-terminus.

major structural domains within the head are envisaged (Fig. 1). A globular distal region would contain the ATP-binding site and correspond to the 73,000 d NH₂-terminal peptide of the scallop heavy chain. (By analogy with vertebrate myosin, this might comprise 23,000 d and 50,000 d peptides, possibly as separate structural domains [Mornet et al., 1981; Sutoh, 1983].) A second extended regulatory domain would contain the two light chains and the 24,000 d peptide. The actin-binding site(s) would be close to the junction of the two domains. Both force

production during myosin-actin interaction, and elevated myosin ATPase in regulated myosins would involve substantial relative motions of the domains. The "off" state of regulated myosins would depend on immobilization of the domains, thus inhibiting both the ATPase cycle and adoption of the head geometry required for tight actin binding. Light chains could function either by locking an interdomain hinge, or by fixing the domains through additional interdomain contacts (Fig. 1). Interactions between regulatory light chains near the head-rod junction would provide one pathway for the cooperation between myosin heads that is essential in regulation; the light chains would also promote the intermolecular contacts responsible for the stable orderly array of myosin heads on assembled filaments in the "off" state (Vibert and Craig, 1983, 1985). On this view, switching myosin "on" by local light chain rearrangements would permit motions both within and between heads to trigger cooperative activation of the entire myosin filament.

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HIGH-RESOLUTION EQUATORIAL X-RAY DIFFRACTION FROM SINGLE SKINNED RABBIT PSOAS FIBERS

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Recently it was shown by mechanical measurements (Brenner et al., 1982) and low-angle equatorial x-ray diffraction (Brenner et al., 1984) that in a relaxed skeletal muscle fiber, a substantial fraction of myosin heads (cross-bridges) is attached to the thin filaments at low temperature (5°C) and low ionic strength ($\mu = 20$ mM), and that the fraction of attached cross-bridges decreases with increasing μ . These findings raised the question whether cross-bridges attached in the relaxed state assume the same structure as those found in other physiological states, such as in rigor. Results from the equatorial x-ray diffraction work (Brenner et al., 1984) and two-dimensional x-ray diffraction study by Matsuda and Podolsky (1984) suggested that the respective configurations of the attached cross-bridges in the relaxed and rigor states are probably different. To obtain additional evidence, in the present study we obtained improved electron density maps in axial projection of muscle fibers under relaxed and rigor conditions.

The unit cells of skeletal muscle consist of hexagonal arrays of interdigitating thick and thin filaments. Changes

in the intensities of the two strongest x-ray equatorial reflections *10* and *11* have been interpreted to reflect mass movement associated with cross-bridge formation. However, with two reflections, the spatial resolution is limited to ~22 nm. The electron density maps of the projected thick and thin filaments are shown to be featureless cylinders (Yu et al., 1985). To improve the spatial resolution, higher orders beyond *10* and *11* are necessary.

In the past, most of the x-ray diffraction work was performed on intact whole muscle or bundles of skinned fibers. The present series of studies uses single, chemically skinned fibers where the sarcolemma is made permeable to large particles, whereby the medium surrounding the contractile proteins is directly controlled. Furthermore, with a single fiber preparation, the myofilament lattice is apparently better ordered for both relaxed and rigor fibers. This enables us to record several equatorial reflections not reported previously.

In the present study, fiber preparation and procedures of x-ray diffraction experiments mostly follow those reported previously (Brenner et al., 1984). Solutions were also