Modulation of striated muscle contraction by binding of myosin binding protein C to actin

Pradeep K. Luther  
*Imperial College London*

Roger W. Craig  
*University of Massachusetts Medical School, Roger.Craig@umassmed.edu*

Follow this and additional works at: [http://escholarship.umassmed.edu/craig](http://escholarship.umassmed.edu/craig)  
Part of the [Cell Biology Commons](http://escholarship.umassmed.edu/cellbiology)

Repository Citation  
[http://escholarship.umassmed.edu/craig/19](http://escholarship.umassmed.edu/craig/19)

This material is brought to you by eScholarship@UMMS. It has been accepted for inclusion in Craig Lab by an authorized administrator of eScholarship@UMMS. For more information, please contact Lisa.Palmer@umassmed.edu.
Modulation of striated muscle contraction by binding of myosin binding protein C to actin

Pradeep K. Luther 1,* and Roger Craig 2
1 Molecular Medicine Section; National Heart and Lung Institute; Faculty of Medicine; Imperial College London; London, UK; 2 Department of Cell Biology; University of Massachusetts Medical School; Worcester, MA USA

Keywords: C-protein, sarcomere structure, thick filament structure, cardiac muscle regulation, electron tomography

Submitted: 12/20/11
Revised: 01/10/12
Accepted: 01/11/12
http://dx.doi.org/10.4161/bioa.1.6.19341
*Correspondence to: Pradeep K. Luther; Email: p.luther@imperial.ac.uk

Myosin binding protein C (MyBP-C or C-protein) is a protein of the thick (myosin-containing) filaments of striated muscle thought to be involved in the modulation of cardiac contraction in response to β-adrenergic stimulation. The mechanism of this modulation is unknown, but one possibility is through transient binding of the N-terminal end of MyBP-C to the thin (actin-containing) filaments. While such binding has been demonstrated in vitro, it was not known until recently whether such a link between thick and thin filaments also occurred in vivo. Here we review a recent paper in which electron microscopy (EM) is used to directly demonstrate MyBP-C links between myosin and actin filaments in the intact sarcomere, suggesting a possible physical mechanism for modulating filament sliding. Molecular details of MyBP-C binding to actin have recently been elucidated by EM of isolated filaments: the results suggest that MyBP-C might contribute to the modulation of contraction in part by competing with tropomyosin for binding sites on actin. New results on the structure and dynamics of the MyBP-C molecule provide additional insights into the function of this enigmatic molecule.

Mutations in the slow skeletal isoform also lead to skeletal muscle myopathy: distal arthrogryposis type 1, a disease of the distal limbs, is thought to result from restricted movement of the fetus in the uterus. MyBP-C has a bead-on-a-string structure consisting mainly of domains of the immunoglobulin (Ig) and fibronectin type III (Fn3) families (Fig. 1). The C-terminal domains anchor MyBP-C to the myosin tails and titin in the thick filament backbone, while the N-terminal region has been shown to interact with both the initial part of the myosin tail (subfragment 2, S2), with the myosin regulatory light chain, and also with actin. Immuno-EM studies have shown that MyBP-C is located on 7–9 stripes, 43 nm apart, in each half of the A-band, strips that can also be seen directly in well-preserved unlabelled muscle (Fig. 2).

Although MyBP-C was discovered nearly 40 y ago, its function is not yet fully understood. In the heart it appears to be involved in the modulation of contraction in response to β-adrenergic stimulation, in skeletal muscle its role is unclear. One way in which MyBP-C might in principle modulate cardiac contraction is through connection to the thin filaments. Interaction with both F-actin and Ca2+-regulated thin filaments (containing tropomyosin and troponin) has been shown in vitro to slow F-actin motility and to modulate the state of activity of thin filaments. Whether such interactions occur in situ, or are merely an in vitro artifact, has recently been clarified by electron tomography of sectioned skeletal muscle. Here we
review these findings—the first to directly demonstrate MyBP-C links between the two types of contractile filament. We relate these results to observations of N-terminal and of the flexible and dynamic structure of the MyBP-C molecule itself.29,30

Three-Dimensional Organization of MyBP-C in the Sarcomere

While the periodic distribution of MyBP-C within the two central regions (C-zones) of each half thick filament has long been known, its organization in three dimensions has remained a mystery. The narrowness of the stripes suggests that the elongated MyBP-C molecule (Fig. 1) is oriented perpendicular to the filament axis, rather than longitudinally; but whether it wraps around the backbone, or possibly extends out toward the thin filaments is not apparent from direct inspection of the electron micrographs. Fig. 2. Knowing the answer to this question could provide key insights into MyBP-C function, in particular whether it interacts with thin filaments in situ.

Deciphering the organization of this narrow, elongated, and labile protein in intact muscle has recently been achieved by 3D electron microscopy of exceptionally well preserved specimens.36 Tissue structure is conventionally preserved for EM by chemical fixation using glutaraldehyde, a bi-functional protein cross-linker. Major cellular components and organelles are well preserved in this way, but fine molecular details are usually lost. It has been found experimentally that the best preservation is achieved by cryo-fixation, which can instantaneously capture 3D cell and molecular architecture by the physical process of freezing. Freezing is achieved by “slamming” tissue against a polished copper block cooled with liquid helium, which vitrifies specimens (freezing them without ice crystal damage) within a millisecond to depths of up to 20 μm. Following vitrification, specimens can be sectioned at low temperature (cryo-sectioned) then imaged in the frozen state by cryo-EM.31 While this procedure preserves fine structure better than any other, it is complex and technically challenging. An alternative method that yields excellent results is freeze-substitution: instead of cryo-sectioning, the vitrified tissue is chemically fixed at low temperature (e.g., with osmium tetroxide in acetone) as the ice gradually dissolves. This is followed by embedding in epoxy resin and conventional thin sectioning (e.g., Padron et al.32). This is the procedure we used to preserve thick filament 3D molecular structure in sections of frog skeletal (sartorius) muscle.33 Comparison of the averaged Fourier transforms of the A-bands from several electron micrographs showed excellent agreement with X-ray diffraction patterns of living muscle, demonstrating that we had indeed achieved high quality filament preservation (Fig. 2).34,35

Visualization of fine structure in normal EM sections is hampered by superposition of components at different levels in the section. This problem can be overcome by collecting images at different tilt angles in the microscope and recombingining them computationally by the procedure of back projection (electron tomography).36 Tilt series were collected from the sections of
rapidly frozen, freeze-substituted sartorius muscle. The high density of the MyBP-C stripes in unifilar sections (Fig. 2) suggests that a large part of the protein is confined to a thin disc at the stripe location. However, direct inspection of the tomograms at this location does not reveal MyBP-C structure due to noise in the tomograms. To overcome this problem, we computationally extracted thick filaments from the 3D volume of three tomograms (207 filaments in all) and performed multiple rounds of alignment and averaging to obtain a final thick filament average.

Inspection of the averaged tomogram in 3D (using UCSF Chimera) showed clear “crowns” of myosin heads with a 14.3 nm spacing (Fig. 3), long known to be present based on X-ray diffraction patterns of muscle, but never before directly visualized in muscle sections. A radially extensive envelope of density (MyBP-C) was seen at every third level of heads in the C-zone, corresponding to the location of the dense stripes in the sections (Fig. 2). While MyBP-C appears prominent when visualized by this surface rendering (Fig. 3A), depiction as a density map reveals that it is in fact weak and diffusely localized. This becomes especially evident in viewing the reconstruction from different angles (see Movie S3 in Luther et al.), which shows that the intense stripes appear only when viewed exactly edge-on. Analysis of the tomogram shows that the bulk of MyBP-C density is at high radius, further from the filament backbone than the myosin heads. The density emanates at a fixed point on the filament circumference, has an initial radial component and then veers to a more circumferential direction, apparently making contact with the actin filaments (white in Figure 3B and C).

These observations demonstrate directly that MyBP-C can indeed interact with thin filaments in the intact sarcomere. We conclude that previous in vitro studies of MyBP-C-thin filament interaction, and the functional conclusions derived from them, may be relevant in vivo. While the binding of MyBP-C to actin in vivo must be weak, in order to allow for filament sliding during contraction, it could have profound physiological consequences, e.g., by sensing and modulating filament sliding. Weakening of actin binding by phosphorylation of the M-domain could contribute to the enhancement of cardiac contraction that occurs in response to β-adrenergic stimulation. Binding to actin could help to account for a long-standing puzzle—how MyBP-C, with its restricted location in the thick filament, can affect thick-thin filament sliding as a whole. This would not appear to occur via interaction with the small number of myosin molecules that are in contact with MyBP-C; direct connection to actin could explain effects at the whole filament level straightforwardly.

While the tomogram clearly reveals the extension of MyBP-C between thick and thin filaments, it does not provide molecular detail on the binding to the surface of either. Suggestive information on the binding of the C-terminal end to the thick filament has come from a 3D reconstruction of isolated filaments, which shows three 4-nm globular domains (coincident with the MyBP-C stripes) running longitudinally along the filament in contact with titin.

These may represent domains C8-C10, known to bind to the thick filament backbone; the rest of the molecule is not visualized, presumably due...
to its disordering in isolated filaments. Fitting of our tomogram to the isolated filament reconstruction suggests a simple model for MyBP-C organization, in which the C-terminal three or four domains bind along the thick filament while the N-terminal half extends out and binds to the thin filaments. This arrangement argues against the organization of MyBP-C molecules into a collar around the thick filament backbone, proposed on the basis of interactions observed between C5 and C8 and between C7 and C10 in yeast 2 hybrid screens38; indeed there is no visible evidence in the thick filament reconstruction for circumferentially arranged 4-nm domains, even though the resolution is clearly good enough to see such detail.39

Mode of Interaction of MyBP-C with Thin Filaments

Structural information on the binding of MyBP-C to thin filaments has recently come from observations of actin filaments decorated with expressed N-terminal fragments. Based on neutron scattering data from filaments decorated with C0C1C2 or C0C2 fractions, it has been suggested that binding occurs by interaction of the C0 and C1 domains with subdomain 1 and the DNase I binding loop of the C0 and C1 domains with subdomain 2. While the C2 and C3 domains appear to lie above the actin surface (Fig. 4A), the presence of the C0C2 or C1C2 fragment under high Ca2+ conditions, whereas at low Ca2+ their velocity is increased.40 The reconstruction offers a possible explanation. The absence of steric clash at high Ca2+ could allow uninhibited binding of cMyBP-C to actin, exerting a significant drag on thin filament sliding (consistent with a similar inhibition of the sliding of F-actin alone) and from reconstructions of F-actin decorated with C0 and C1,40 in vitro motility assays show that thin filaments are slowed by the presence of the C0C2 or C1C2 fragment under high Ca2+ conditions, whereas at low Ca2+ their velocity is increased.40 The reconstruction offers a possible explanation. The absence of steric clash at high Ca2+ could allow uninhibited binding of cMyBP-C to actin, exerting a significant drag on thin filament sliding (consistent with a similar inhibition of the sliding of F-actin alone). In contrast, the competition of cMyBP-C and tropomyosin for part of the same region of actin at low Ca2+ could destabilize the blocking position of tropomyosin, thus tending to

Figure 4. Binding of the N-terminal fragment C0C3 of cMyBP-C to actin. (A) F-actin reconstruction (gray) fitted with F-actin atomic model39 (monomers colored white, blue and cyan) and showing best-fit position of two C0C3 fragments based on C0C3-decorated reconstruction. The approximate fitting of an Ig domain atomic structure suggests that C0 and C1 bind to subdomain 1, while the M-domain may bridge over subdomain 2 and possibly contact subdomain 1 of the adjacent actin, while the C2 and C3 domains appear to lie above the actin surface (Fig. 4A). While this appearance agrees broadly with the neutron scattering model, the M-domain in the reconstruction appears to be attached to the actin filament, in agreement with solution assays showing that binding to actin occurs primarily through the C1 and M-domains.39 It is possible that the higher pH of the neutron scattering experiments caused the M-domain to dissociate. New in vitro motility and optical trap experiments localize the M-domain binding site to a cluster of highly conserved arginines within the first 17 amino acids of the domain.40 These appear to be involved in stereospecific binding of cMyBP-C to actin, and model the position of actin’s negatively charged N-terminus.

The most interesting finding to emerge from the reconstruction is the apparent steric clash between the C0 and C1 domains and tropomyosin. This appears to occur when tropomyosin is in the low Ca2+ (Fig. 4C) but not the high Ca2+ (Fig. 4B) position, consistent with the conclusions from neutron scattering and from reconstructions of F-actin decorated with C0 and C1.40 In contrast, the competition of cMyBP-C and tropomyosin for part of the same region of actin at low Ca2+ could destabilize the blocking position of tropomyosin, thus tending to
activate the thin filament, enhancing its motility. If comparable effects occur in vivo, cMyBP-C might thus contribute (together with troponin) to the balance between the low and high Ca\textsuperscript{2+} positions of troponomyosin.\textsuperscript{29} It will be of interest to test this model by directly determining the effect of MyBP-C binding on troponomyosin position in regulated thin filaments.

The reconstruction also shows a major clash between myosin head and MyBP-C binding (Fig. 4D). Because of the low stictioncoy of MyBP-C in muscle this would be unlikely to have a major physiological effect. Phosphorylation of cMyBP-C has been shown to weaken its binding to actin, which may reduce any effect of MyBP-C on thin filament activity, and allow an increased rate of filament sliding in muscle. This could be one means by which adrenergic stimulation of the heart (which leads to phosphorylation of cMyBP-C) increases cardiac contractility.

Overall, these recent and earlier data suggest a model in which cMyBP-C's ability to bind reversibly to actin provides an internal load to myosin power generation in muscle, a load that can be modulated by phosphorylation of the M-domain. cMyBP-C's potential interference with troponomyosin position on actin suggests additional means of regulating contraction.

**Binding of S2 to N-Terminal Domains of MyBP-C**

In addition to its actin binding capability, the N-terminal region of cMyBP-C has also been shown to interact with myosin subfragment 2 (the initial part of the myosin tail, emerging from the junction of the heads, which is thought to lie relatively loosely associated with the thick filament backbone) primarily through the M-domain.\textsuperscript{9,10} This interaction may also play a critical role in actin-myosin interaction in intact muscle, regulated by phosphorylation of the M-domain, although it would appear that this effect may be qualitatively different from that involving actin binding. The positioning of MyBP-C at only every third level of myosin heads in the middle third of each half thick filament (the C-zone, Fig. 2) means that only about 20% of all heads in the filament are likely to be directly affected by MyBP-C interaction. In contrast, binding of MyBP-C to actin, even in a limited region, could have a global impact, affecting sliding of the entire filament. In addition, if MyBP-C affects tropomyosin position on actin, this could be transmitted cooperatively along a substantial length of the filament, due to the high stiffness of the tropomyosin polymer.\textsuperscript{5,12}

While the work discussed above implicated the M-domain in binding to both actin and S2, a recent NMR study suggests that sites on the C1 domain might also serve such a dual purpose.\textsuperscript{12} Apparent overlap between putative N-terminal actin and S2 binding sites suggests the possibility that MyBP-C might switch between binding partners to a defined way, possibly controlled by phosphorylation of the M-domain.\textsuperscript{30,40}

**Flexibility and Disorder in MyBP-C**

Fascinating new insights into the structure of the isolated MyBP-C molecule, which may help to explain some of its enigmatic biological studies. Atomic force microscopy experiments, in which the mechanical properties of individual MyBP-C domains can be measured, show that the Ig and Fn3 domains that comprise most of the molecule are stably folded, but capable of unfolding under imposed stress, with different domains having different unfolding thresholds.\textsuperscript{30} The M-domain stands out in being much weaker than the Ig and Fn3 domains. It appears to behave like a highly extensible spring, a property that probably relates to its predicted intrinsic disorder.\textsuperscript{24} What function does this plasticity of MyBP-C serve? One speculation is that mechanical load on MyBP-C may affect its binding to or activation of signaling molecules. Similarly, binding sites for actin or S2 within the extensible M-domain may be modulated by stretch, allowing even the small C0C1 segment to serve such a dual purpose.43 Apparent overlap between putative N-terminal actin and S2 binding sites suggests the possibility that MyBP-C might switch between binding partners to a defined way, possibly controlled by phosphorylation of the M-domain.\textsuperscript{30,40}

**Comparison of proline/alanine content**

In the Pro/Ala-rich region of cMyBP-C from different species shows a direct correlation with mammalian body size and inverse variation with heart rate.\textsuperscript{44} These differences may be important in matching contractile speed to cardiac function in different species by differentially affecting crossbridge kinetics.\textsuperscript{45} Alternatively, differences in the extensibility of the Pro/Ala-rich region may have evolved to generate different distributions of extended and compact states, depending on the mechanical requirements of different hearts, possibly affecting the rate of the putative switching of cMyBP-C's N-terminus between binding sites on myosin\textsuperscript{11} and actin.\textsuperscript{46}

**Acknowledgments**

This work was supported by project grants from the British Heart Foundation (PG/06/010), EC Grant Agreement 241577 (BSG-HearT) FP7 Seventh Framework Programme Health, and NIH grants AR034711 and HL093408.
