

Cardiac Myosin Binding Protein C

Saul Winegrad

Abstract—Myosin binding protein C (MyBP-C) is one of a group of myosin binding proteins that are present in the myofibrils of all striated muscle. The protein is found at 43-nm repeats along 7 to 9 transverse lines in a portion of the A band where crossbridges are found (C zone). MyBP-C contains myosin and titin binding sites at the C terminus of the molecule in all 3 of the isoforms (slow skeletal, fast skeletal, and cardiac). The cardiac isoform also includes a series of residues that contain 3 phosphorylatable sites and an additional immunoglobulin module at the N terminus that are not present in skeletal isoforms. The following 2 major functions of MyBP-C have been suggested: (1) a role in the formation of the sarcomeric myofibril as a result of binding to myosin and titin and (2) in the case of the cardiac isoform, regulation of contraction through phosphorylation. The first is supported by the demonstrated effect of MyBP-C on the packing of myosin in the thick filament, the coincidence of appearance of sarcomeres and MyBP-C during myofibrillogenesis, and the defective formation of sarcomeres when the titin and/or myosin binding sites of MyBP-C are missing. The second is supported by the specific phosphorylation sites in cardiac MyBP-C, the presence in the thick filament of an enzyme specific for MyBP-C phosphorylation, the alteration of thick filament structure by MyBP-C phosphorylation, and the accompaniment of MyBP-C phosphorylation with all major physiological mechanisms of modulation of inotropy in the heart. (*Circ Res.* 1999;84:1117-1126.)

Key Words: myosin binding protein C ■ heart ■ filament structure ■ force generation ■ regulation of contraction

One of the basic mechanisms for movement in eukaryotic cells is the interaction of a molecular motor with a polymer. The type of movement and the particular function served by the movement vary considerably among cells, from generation of force or pressure by striated muscle to intracellular transport of organelles and vesicles. The properties of different molecular motors and polymers have evolved to allow this diversity. The breadth of function is also achieved by the presence of additional proteins that are crucial for the 3-dimensional organization of the contractile proteins and the modulation of their interaction. In striated muscle, force is generated by an interaction between the molecular motor myosin and filamentous actin, the major proteins of, respectively, thick and thin filaments. Although they generate force, actin and myosin alone cannot reproduce all of the fundamental properties of the contractile system of striated muscle. A pure actin-myosin system lacks the calcium-dependent switch that controls the transition between the resting and force-generating states. Regulatory proteins in the thin filaments, tropomyosin and the 3 subunits of troponin (TNI), provide this control by blocking the access of myosin to its binding site on actin in the absence of calcium.

In addition to its major component, myosin, the thick filament contains other proteins that have been identified as myosin binding protein-C, -H, and -X (MyBP-C, -H, and -X).¹ They constitute 1% to 2% of the myofibrillar mass. The extra proteins in the thick filament are located along 9 stripes

in the region of the A band containing crossbridges (C zone), with each stripe separated by 43 nm from the adjacent ones^{2,3} (Figure 1). With these additional proteins present, it is possible to synthesize thick filaments with central bare zones, tapered ends, and periodically distributed crossbridges arranged helically around the circumference of the filaments.⁴ Thick filaments that closely resemble natural thick filaments can be formed from myosin extracts of myofibrils.⁵ Apparently, proteins associated with myosin are required for the formation of the filaments. Without these proteins, the filaments are thicker, their lengths and thicknesses are very heterogeneous, clear central bare zones are not present, and myosin crossbridges are not clearly discernible.⁶⁻⁸

Structure of MyBP-C

Several different isoforms of MyBP-C from human, chicken, rabbit, and mouse have been cloned and sequenced, allowing an in-depth comparison of the structures.⁹⁻¹⁶ There are 3 isoforms of the protein: fast skeletal, slow skeletal, and cardiac. MyBP-C appears to exist only in striated muscle as probes for fast and slow skeletal MyBP-C do not hybridize with human pancreas, kidney, liver, lung, placenta, brain, or heart.⁹ The cardiac isoform is present only in the heart.⁹ In humans the 3 isoforms map to different chromosomes: slow skeletal to chromosome 12, fast skeletal to chromosome 19, and cardiac to chromosome 11, showing that the isoforms are not products of alternative splicing. In humans the fast type

Received November 2, 1998; accepted March 23, 1999.

From the Department of Physiology, School of Medicine, University of Pennsylvania, Philadelphia, Pa.

Correspondence to Saul Winegrad, Department of Physiology, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104-6085. E-mail bsg@mail.med.upenn.edu

© 1999 American Heart Association, Inc.

Circulation Research is available at <http://www.circresaha.org>

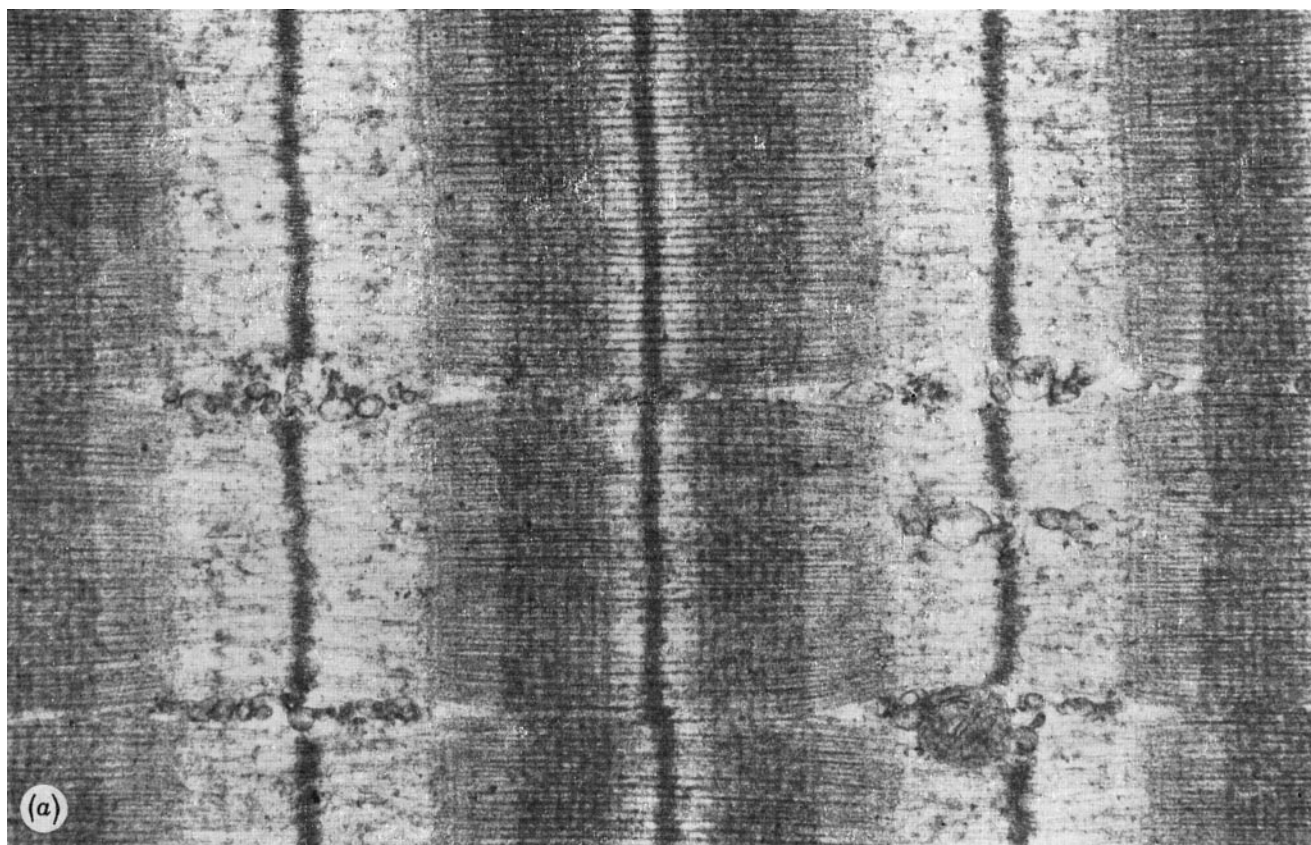


Figure 1. Electron micrograph of cardiac muscle stained with antibody to MyBP-C. Note the 7 lines in the A band. Reproduced with permission from The Royal Society (Craig R, Offer G. The location of C-protein in rabbit skeletal muscle. *Proc R Soc Lond B Biol Sci.* 1976;192:451–461 [this is part of Figure 2 in original source]).

consists of 1142 amino acids (based on the cDNA sequence) with a predicted molecular mass of 128 kDa, which is less than the value inferred from gel electrophoresis.⁹ Each skeletal MyBP-C contains 10 domains, consisting of 7 immunoglobulin (Ig) C2 motifs and 3 fibronectin (Fn) type III motifs arranged in the same order (Ig-Ig-Ig-Ig-Ig-Fn-Fn-Ig-Fn-Ig) (identified as modules I to X)¹⁷ (Figure 2). The electron microscopic image of MyBP-C is a mixture of rod shape, V shape, and U shape. The skeletal isoforms are ≈ 3 nm in diameter and 32 nm long,^{18,19} whereas the cardiac isoform is longer, ≈ 40 to 44 nm, and also shows the same shapes. There are indications that the molecules may form dimers.^{18,19} The cardiac isoform differs from the 2 skeletal isoforms in 3 important ways (see below).

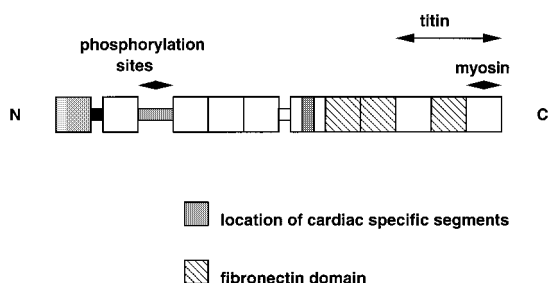


Figure 2. Diagram of the cDNA encoding cardiac MyBP-C with the binding sites for myosin and titin indicated. Also shown are the portions of the cDNA in cardiac muscle that are different from skeletal muscle.

The C-terminal module (module X, also known as CX) contains the binding site for myosin,^{11,17,20} but, although this module is necessary, it is not sufficient for maximal binding to myosin. The affinity of MyBP-C for myosin increases progressively as the 3 adjacent modules, VII through IX, are added at the C terminus.²⁰ The importance of these additional modules in the binding to myosin is also demonstrated by the inability of a peptide encoding module X to displace MyBP-C from myosin.¹⁷

The overall homology of amino acids deduced from the various cDNAs of MyBP-C is high.⁹ Fast human and fast chicken have 71% identity and 10% conservative substitutions. Human fast and slow isoforms have 50% sequence identity and 16% conservative substitutions. The most conserved immunoglobulin repeats are II and X. The longest region of identity among the chicken and human isoforms, 18 residues, is present in the spacer between modules I and II, and overall this region has $\approx 80\%$ identity. The phosphorylation sites that exist on the cardiac but not the skeletal isoforms are located in this region (see below).

In addition to the myosin binding site on MyBP-C, there are sites in the 3 C-terminal domains (VIII through X) that bind to a specific subset of immunoglobulin and fibronectin domains in titin.^{11,12,21,22} MyBP-C can also bind to actin in both regulated and unregulated filaments.^{23,24} In regulated filaments, calcium increases the amount of MyBP-C bound, producing a side-by-side association of the filament that does

not occur in the absence of calcium. In unregulated actin, the stoichiometry is ≈ 1 MyBP-C/3 to 5 actins, and calcium does not affect the binding. The dissociation constant is in the micromolar range, making a specific physiological role for the binding unlikely.

Two additional myosin binding proteins, MyBP-X and MyBP-H, have been identified.^{2,25,26} Both of these bind to myosin at 43-nm repeats in the C zone of the thick filament, although not necessarily in the same pattern. MyBP-X, found in red and mixed muscles, has subsequently been shown to be the slow skeletal isoform of MyBP-C based on amino acid composition, elution pattern from hydroxyapatite, and sedimentation coefficient.^{9,27} The second protein, MyBP-H, is much smaller than MyBP-C, with a molecular mass of 52 kDa based on amino acid sequence, and it is encoded in a separate gene from MyBP-C.^{13,14,28–30} A substantial amount of MyBP-H is present in the Purkinje cell, a cardiac cell specialized for impulse conduction. It is the only cell type containing striated myofibrils that does not contain MyBP-C.²⁶ It appears to be absent or in very low concentration in cardiac muscle¹⁴ and slow skeletal muscles. MyBP-H consists of 2 immunoglobulin and 2 fibronectin modules with a high level of conservation compared with MyBP-C and a myosin binding site that closely resembles that in MyBP-C. However, MyBP-C can still bind to myosin even if the myosin is saturated by MyBP-H.³⁰

The cardiac isoform of MyBP-C is encoded in a different gene on a different chromosome from the 2 skeletal isoforms (in human, chromosome 11). Although the 3 isoforms share a similar structure consisting of several immunoglobulin and fibronectin modules and a myosin binding site in its C-terminal immunoglobulin module, the cardiac isoform has 3 important differences in structure from the skeletal isoforms, as follows: (1) cardiac MyBP-C (cMyBP-C) contains an additional immunoglobulin module with 101 residues at the N terminus (module 0 or C0); (2) in the 105-residue linker between the C1 and C2 immunoglobulin domains (MyBP-C motif), it contains 9 additional residues and 3 phosphorylation sites that are unique to the cardiac isoform¹⁵; and (3) a 28-residue loop is added to the C5 immunoglobulin domain.

The importance of cMyBP-C in the structure and function of cardiac muscle has attracted greater attention since it was demonstrated that mutations in the gene can produce familial hypertrophic cardiomyopathy (FHC).^{16,31–36} The majority of mutations are splice donor, splice acceptor, deletion, or insertion mutations that produce at least 1 abnormal transcript and lead to functional proteins lacking the myosin binding site, in some cases the titin binding site, and even the phosphorylation sites.

In developing skeletal muscle cells, the 2 skeletal isoforms can be coexpressed while the cardiac isoform of MyBP-C is specifically expressed in human and mouse developing cardiac but not developing skeletal cells.^{36–38} Because the 2 skeletal isoforms can be coexpressed in skeletal muscle, but only the cardiac isoform is expressed in cardiac muscle, cardiac muscle should be more vulnerable to gene mutation in MyBP-C than skeletal muscle. No skeletal myopathy associated with cardiomyopathy due to mutations of MyBP-C has been described.

FHC associated with truncations in MyBP-C is more benign than those resulting from mutations in genes encoding myosin heavy chain (MHC) or tropomyosin. The clinical picture favors haploinsufficiency rather than a poison polypeptide as the cause of the pathology of the heart. Yang et al³⁹ have been successful in producing transgenic lines of mice in which there is overexpression of transcripts of the wild type of MyBP-C or a truncated form lacking the specific titin and myosin binding sites. Even in the presence of overexpression of transcripts of the wild type of MyBP-C, there is the normal stoichiometry of MyBP-C to myosin, indicating the presence of some mechanism for controlling the overall stoichiometry of MyBP-C with respect to other contractile proteins. The truncated form of MyBP-C is found in foci and has a diffuse rather than normal sarcomeric distribution. Some sarcomeres are in disarray or are abnormally formed. The extent of the pathology is related to the dose of truncated protein. Although there is no difference in contraction, relaxation, or the response to increased workloads in the isolated intact heart, skinned bundles from papillary muscles show a decrease in maximum power, a decrease in maximum force, and an increase in calcium sensitivity without any changes in maximum velocity. Extraction of a major portion of MyBP-C from either skeletal or cardiac muscle increases calcium sensitivity in both skeletal and cardiac muscle, but the effect is more pronounced in cardiac muscle.^{40,41} There is also a reduction in shortening velocity in skeletal muscle with low level of activation.

MyBP-C Binding to Myosin

Of the 9 stripes in the C zone of the A band, 7 are due to MyBP-C and the other 2 to other proteins, which may also be present in the 7 stripes that contain MyBP-C. The periodicity of MyBP-C corresponds very closely to that of the helically arranged crossbridges in a 3-stranded array of myosin molecules in the thick filament.⁴² Fast and slow skeletal isoforms may be present within a single sarcomere of skeletal muscle.^{43,44} Because MyBP-C is 32 to 44 nm long but the available protein antigenic sites have an axial extent of <10 nm, probably ≈ 7 nm, in the thick filament, it is likely that the molecule is oriented perpendicularly to the long axis of the filament. Its accessibility in the thick filament to antibodies suggests that at least part of MyBP-C lies on the surface of the thick filament.⁶ MyBP-C binding is restricted to the C zone, probably because its binding is sensitive to the packing of myosin in the thick filament, and this packing is different near the bare zone and the tapered ends of the filament.⁶ The 43-rather than 14-nm repeat (every 3 rather than every 1 crossbridge) suggests that all myosin molecules within the filament are not equivalent. Based on the stoichiometry of MyBP-C to myosin, each of the 7 stripes contains between 2 and 4, most likely 3, molecules of MyBP-C per thick filament.⁶

There is a myosin binding domain in the C-terminal module of MyBP-C²⁰ and 2 MyBP-C binding domains in myosin, one near the junction of the S2 and light meromyosin (LMM) fragments in the hinge region of the myosin molecule and the other in the LMM or rod portion of the molecule.^{45,46} The specific binding residues have not yet been identified.

MyBP-C does not bind to subfragment 1 (the head of the myosin or the crossbridge region). From the nature of the binding, one might predict that MyBP-C could have a significant effect on crossbridge movement and myosin packing. In myosin filaments formed at pH 8.0, which have diameters similar to those of native filaments, the presence of MyBP-C in physiological stoichiometry (0.1 to 0.3 molar ratio) increases the filament length, with MyBP-C restricted to the C zone.⁸ At higher molar ratios, MyBP-C appears to bind in the region between the bare zone and the C zone. From its effect on filament turbidity, Davis⁸ has proposed that there are 3 sites of MyBP-C binding to myosin, as follows: a high-affinity site that changes the structure of the synthetic filaments (0.1 to 0.3 molar ratio), a second site that is occupied at higher molar ratios (0.3 to 1.0) and more likely to be the one on subfragment 2, and a third site at much higher molecular ratios (1 to 2 to 1) that is more nonspecific and due to the adhesive nature of the immunoglobulin and fibronectin modules in MyBP-C.

MyBP-C contributes to the 43-nm reflections in the x-ray diffraction patterns in striated muscle and may also contribute to the “forbidden reflections” also found in resting striated muscle.^{42,47} The forbidden reflections are presumed to be due to imperfections in the order of protein arrangements in the thick filament. During contraction of some striated muscle, reflections associated with mass at the locations of MyBP-C and the forbidden reflections markedly diminish or disappear.^{40,45} There is also a rearrangement within the backbone of the thick filament that is associated with an increase of 1.5% in the periodicity of the 14.3-nm reflection.⁴⁷ These changes in the diffraction pattern suggest that a change in MyBP-C structure and the packing of the myosin in the backbone of the thick filament may occur during the contraction.

Filament Formation

The dimensions of synthetic myosin filaments are modified by the presence of MyBP-C, resulting in an increase in length, a decrease in the diameter, and increased uniformity of the diameters of the filaments apparently from greater compactness of the filament.⁷ The effect of MyBP-C on the structure of the myosin filaments is very sensitive to the conditions under which it is added to the myosin. Of particular importance are the molar ratio of MyBP-C to myosin and the timing of the addition of MyBP-C with respect to the formation of the myosin filaments. The individual myosin filaments formed in the absence of MyBP-C by decreasing the ionic strength of a myosin solution have uniform diameters along the filament, but the range of diameters is wide. There are 14-nm subunit and 43-nm axial periodicities as in the normal filament *in vivo* due to the uniform spacing and the helical arrangement of the myosin molecules, respectively. About 25% of the filaments are frayed, apparently from weak lateral myosin-myosin interactions. If the myosin filaments are produced in the presence of MyBP-C in the physiological molar ratio (1 MyBP-C:3 to 4 myosins), the range of diameters is much narrower.⁷ There is little fraying, suggesting that the lateral myosin-myosin interactions are strengthened. The periodicities of myosin and

LMM paracrystals with and without MyBP-C indicate that MyBP-C is probably present in native thick filaments with periodicity identical to that of myosin.^{8,46}

Myosin II (the form on skeletal muscle) expressed in transfected COS cells forms filaments that are much thicker than those in skeletal muscle.⁴⁸ When myosin and MyBP-C are coexpressed, the length of the myosin filaments is increased and the diameter is decreased.

Several conclusions about the effect of MyBP-C on the structure of the thick filament can be tentatively drawn. (1) Although MyBP-C is not necessary for the formation of myosin filaments, it is probably necessary for the formation of normal thick filaments. (2) MyBP-C is not necessary for the subunit repeat of 14 nm in a myosin filament. (3) MyBP-C has a major influence on the structure of the myosin filament, probably through an effect on the lateral myosin-myosin interaction. (4) MyBP-C binds to myosin at 2 different sites, 1 in the rod portion and the second in the subfragment 2. The affinity of the latter site may be lower than that of the former. (5) Binding of MyBP-C at 43-nm rather than 14-nm repeats and only within a portion of the thick filament suggests that the packing of myosin molecules is more complex and heterogeneous along the thick filament; all myosin molecules are not equivalent.^{6,46}

Sarcomere Formation

Three major types of evidence suggest that MyBP-C plays an important role in the formation of myofibrils in striated skeletal and cardiac muscle. First, the formation of thick filaments is altered by the presence of MyBP-C, and only in the presence of the normal content of MyBP-C do synthetic thick filaments resemble native thick filaments in their thickness, length, bare zone, and distribution of myosin heads.^{4,5} MyBP-C binds in a highly regular fashion to myosin and titin. Second, MyBP-C is present in all striated muscles except cardiac Purkinje fibers, which contain a significant amount of MyBP-H.²⁹ MyBP-C appears relatively late and is first detected as nonstriated myofibrils⁴⁹ or nascent myofibrils (the stage between premyofibrils located at the cell periphery and native myofibrils⁵⁰) evolve into mature myofibrils. MyBP-C reduces the critical concentration required for polymerization of myosin.⁸ MyBP-C appears only at its characteristic location within the myofibril and is absent from nonstriated myofibrils.⁵¹ Third, in myotubes expressing a significant amount of mutant MyBP-C lacking the CX module, cross-striations were missing in 97% of cells,²⁰ and in cardiac muscle cells with truncated MyBP-C deficient in myosin binding site, there is a significant degree of disorder and myofibrillar disarray.³⁹ The 1 exception to the otherwise uniform association of MyBP-C with striated myofibrils is the cardiac Purkinje cells, but these cells, although capable of contraction, are primarily conducting cells. They also contain MyBP-H, another myosin binding protein that contains the 4 C-terminal modules, in which the myosin binding site exists.¹⁴

ATPase Activity

The effect of the presence of MyBP-C in myosin filaments on actomyosin ATPase activity depends on ionic strength.^{52–54}

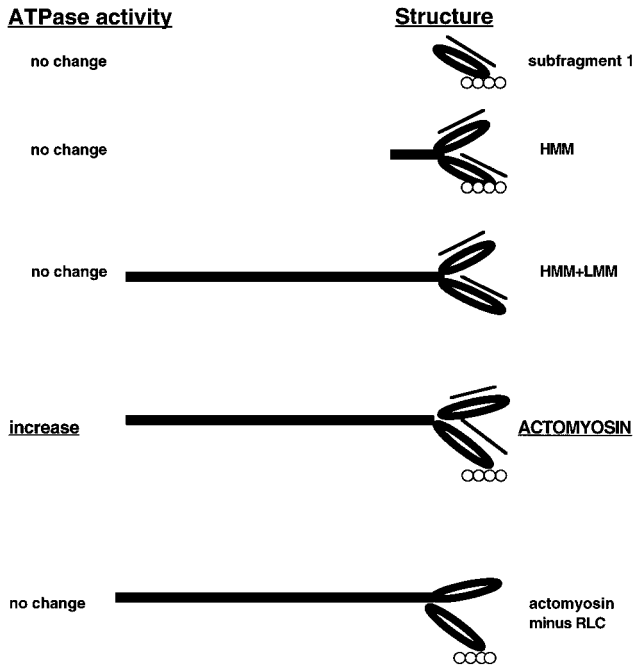


Figure 3. Diagram showing the effect of addition of MyBP-C on the ATPase activity of various preparations of myosin and its subfragments. RLC indicates regulatory light chain.

At low ionic strength there is a decrease, and at normal ionic strength a small increase, in the rate of ATP hydrolysis by skeletal actomyosin. On the other hand, when the isoform of myosin is cardiac, addition of MyBP-C increases ATPase activity at all ionic strengths, both low and normal. The effect of MyBP-C on the ATPase activity depends on the isoform of myosin and not on the isoform of MyBP-C. Cardiac MyBP-C added to skeletal myosin lowered ATPase activity at low ionic strengths, whereas skeletal MyBP-C raised the ATPase activity of cardiac myosin at all ionic strengths.⁵¹

To produce any change in ATPase activity of actomyosin, the entire myosin molecule must be present (Figure 3).^{53–55} Modification of the enzymatic activity by MyBP-C does not occur unless the entire MHC including the rod portion that forms the backbone of the normal thick filament and both light chains are present. There is no significant change in the rate of ATP hydrolysis in the absence of actin.⁵⁴ These results, together with those of the effect of MyBP-C on the thickness of myosin filament, suggest that the ATPase of actomyosin is influenced by the environment of the rod portion of the molecule and is not exclusively regulated by the crossbridge or heavy meromyosin (HMM) portion of the molecule. The increase in ATPase activity of cardiac actomyosin produced by MyBP-C is prevented by removing the regulatory light chain of myosin (LC2) from the cardiac muscle, and the increase is restored when LC2 is restored.⁵⁵ This suggests that MyBP-C and LC2 may work in concert on ATPase activity of actomyosin. In support of this interpretation is the fact that removal of MyBP-C, truncation of MyBP-C, and phosphorylation of LC2 all have the same effect on calcium sensitivity of the contraction of cardiac myocytes. The phosphorylation of either protein results individually in a change in structure of the thick filament with

an extension of the crossbridges, but the effects on the degree of order of the crossbridges are different.

Phosphorylation of C Protein

Based on amino acid sequence of the human cardiac isoform, 4 phosphorylation sites (designated A-D by Gautel et al¹⁵) appear to exist within residues 157 to 259 in the MyBP-C motif. Putative site D is, however, not phosphorylated by protein kinase A (PKA) or calmodulin kinase (CAMK), presumably because the site is inaccessible because of folding of the molecule. PKA can phosphorylate sites A, B, and C, and site B can be phosphorylated by CAMK, a kinase that is normally present in thick filaments and can be removed from the filament by special effort.^{56,57} Besides their relative sensitivity to CAMK, phosphorylation sites A, B, and C are not equivalent in other ways. If site B is mutated to prevent phosphorylation, the ability of PKA to phosphorylate sites A and C is markedly reduced. It appears as if there is a preferential sequence of phosphorylation: site B phosphorylation facilitates the phosphorylation of sites A and C. In chicken ventricle, there is also a CAMK bound to the MyBP-C after extraction, and it remains after partial purification.^{56,57} However, 3 phosphates are added per molecule of MyBP-C by CAMK and only 2 by PKA, and the combination produces phosphorylation of 4 sites.

Effect of MyBP-C on Contractile Function

Much work has been done over the past 2 decades to try to determine the function of MyBP-C. The general approach has been to compare the structure and ATPase activity of myosin filaments with and without added MyBP-C.^{7,46,52–54} As regards structure, the results have been inconsistent, primarily because the effect of MyBP-C on the formation of thick filaments depends on the temperature, ionic strength, and relative concentration of MyBP-C with respect to myosin. In no case, however, have thick filaments with a normal physiological appearance been produced without MyBP-C present.

The physiological function of MyBP-C and its phosphorylation in cardiac myocytes have been hard to evaluate because of the inability to remove and replace >70% of MyBP-C in preparations with an intact filament lattice.^{40,41,58} In skinned rabbit skeletal muscle maximally activated by calcium, removal of 40% to 70% of MyBP-C has no effect on the maximum velocity of unloaded shortening. At submaximal activation, in which 2 phases of unloaded shortening velocity occur, partial extraction of MyBP-C increases V_{\max} during the low-velocity phase without altering the high-velocity phase. From these data, Hofmann et al^{40,41} suggest that MyBP-C contributes to an internal load, possibly by increasing the stiffness of the S2 portion of myosin.

Partial extraction of MyBP-C in skinned cardiac muscle produces an increase in force at submaximal activation by calcium without changing the maximum force generated at optimal concentration of calcium.^{41,58} The slope of the calcium-force curve is reduced. Restoration of MyBP-C reverses these changes. The direction of the change in force in this muscle model, which contains an intact filament lattice, is the opposite of that observed in ATPase activity in isolated

actin and myosin, in which addition of MyBP-C increases actomyosin ATPase activity at normal ionic strength. However, the rate-limiting steps in the crossbridge cycle for generation of force and hydrolysis of ATP are probably not the same. In view of the requirement for LC2 in order to see an effect of MyBP-C on actomyosin ATPase, it is of interest that (1) phosphorylation or removal of LC2 has the same effect on the calcium-tension curve as removal of MyBP-C and (2) phosphorylation of LC2 modifies crossbridge order and flexibility.⁵⁹

Function of Phosphorylation of Cardiac MyBP-C

Elucidation of the function of phosphorylation of MyBP-C in cardiac muscle with intact structure has been difficult, because it has not yet been possible to produce phosphorylation of MyBP-C in the absence and in the presence of specific phosphorylation of other myofilament proteins, except for the special case of the neonatal (3-day-old) rat,⁶⁰ in which some cardiac regulatory systems are absent or very poorly developed,⁶¹ and some of the isoforms of myofibrillar proteins are still fetal. How phosphorylation of 1 myofilament protein may affect the change produced by phosphorylation of another is not a trivial consideration. In isolated unrestrained myofibrils, PKA, which phosphorylates TNI and MyBP-C, changes the calcium sensitivity of actomyosin ATPase activity without changing the maximum value. On the other hand, PKC, which phosphorylates TNI at different sites and MyBP-C in the same peptide fragment and, in addition, phosphorylates LC2, can cause a decrease in maximum ATPase activity without a shift in the Ca-ATPase activity relation.⁶² The effect of PKC-mediated phosphorylation on ATPase activity depends on the isoform of PKC and the specific sites within the inhibitory subunit of TNI that are phosphorylated.⁶³ These results point out the need to evaluate the effects of phosphorylation of a given site under the several different physiological conditions that can occur.

Greater control over phosphorylation can be exercised in reconstituted actomyosin systems. Using this approach, Garvey et al⁶⁴ have generated strong evidence against a major role for phosphorylation of MyBP-C in modulating contractility. After removal of unphosphorylated MyBP-C from reconstituted regulated actomyosin systems and replacement with phosphorylated MyBP-C, no change in the calcium activation of Mg-ATPase activity occurs.⁶⁴ There are no physical restraints on the contractile system in this model of contraction, however, and a change in crossbridge kinetics that is sensitive to stress or force on the crossbridge, such as the detachment step, may not be detected.

Cryostatic sections of quickly frozen cardiac muscle retain the contractile filament lattice, and both actomyosin ATPase activities and phosphorylation of all the contractile proteins can be measured after treatment of the frozen sections with cAMP-activated PKA, β -adrenergic agonists, or α -adrenergic agonists.^{65,66} In this preparation, PKA increased actomyosin ATPase activity as well as phosphorylating MyBP-C and TNI.⁴⁰ In the presence of blockade of α -adrenergic activity, PKA lowered ATPase activity and decreased phosphorylation of MyBP-C. Of all of the phosphorylations of the contractile

and regulatory proteins, only phosphorylation of MyBP-C showed a consistent correlation with the level of ATPase activity under all conditions examined.⁶⁴ Phosphorylation of TNI changed in the same direction as ATPase, but the relation between the 2 parameters was not simple. This does not, however, preclude the possibility that the changes in ATPase activity could be due to the phosphorylation of TNI alone or that the phosphorylation of both proteins is necessary.⁶⁷

Five major or potentially major regulators of contractility, α - and β -adrenergic activation, cholinergic stimulation, calcium, and endothelin lead to changes in phosphorylation of MyBP-C, but other myofibrillar proteins may also be phosphorylated. The accompanying phosphorylations introduce a major difficulty in evaluating the role of phosphorylation of MyBP-C in the regulation of contraction because of the inability to examine its effects in the intact heart specifically. Isoproterenol and carbachol each induce changes in relaxation time that correlate well with the degree and time course of phosphorylation of MyBP-C, but phosphorylation of other proteins that could alter the kinetics of relaxation have not been simultaneously examined.⁶⁸ The effects of PKA-induced phosphorylation of TNI and MyBP-C on isometric force and unloaded shortening velocity have been examined in skinned isolated cardiac myocytes. A decrease in calcium sensitivity but no change in either maximum force or unloaded shortening velocity were observed.⁶⁹ The decrease in calcium sensitivity is due to phosphorylation of TNI,⁶⁷ but the absence of a change in unloaded shortening velocity is surprising inasmuch as an increase in velocity and apparent rate of crossbridge cycling from PKA activation or β -adrenergic stimulation has been demonstrated in intact cardiac cells.⁷⁰⁻⁷³

These results also point out the importance of evaluating the contractile response to a given intervention by measuring several parameters of contraction, including force, velocity of shortening, and ATPase activity. The preparation itself is important particularly as regards the state of the filament lattice, filament overlap, and filament separation.⁷⁴ In fact, the absence of an effect of phosphorylation of MyBP-C in reconstituted systems and the apparent relation of the degree of phosphorylation with crossbridge cycling in the presence of the filament lattice need not be contradictory. Both sets of results can be interpreted as the consequence of an effect of MyBP-C phosphorylation on steps in the crossbridge cycle that are sensitive to stress or to the separation of thick and thin filaments (Figure 4). In reconstituted systems or actomyosin preparations using only myosin heads (HMM or subfragment 1), little stress can be developed as the myosin heads cycle, whereas crossbridges cycling in the intact filament lattice develop stress. If lattice integrity is required to see the effects of MyBP-C phosphorylation on crossbridge cycling, maximum velocity of shortening, ATPase activity, and the economy of energy transduction are the parameters most likely to be affected. Detachment of crossbridges, the rate-limiting step for shortening, is believed to be sensitive to stress or strain.⁷⁵ Economy of energy transduction will be sensitive to the fraction of the time the cycling crossbridges are attached. The rate of hydrolysis of ATP will be influenced by the rate of attachment bridges to actin, a step apparently sensitive to

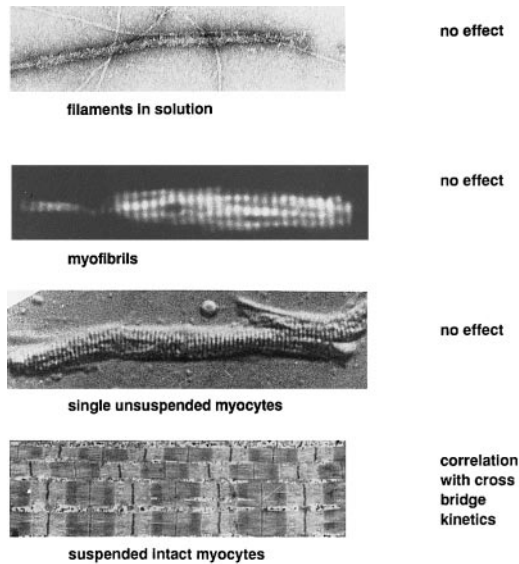


Figure 4. Diagram showing the response of different contractile models of cardiac muscle to the phosphorylation of MyBP-C.

the distance between thick and thin filaments,⁷⁴ and the steric arrangement of the crossbridge with respect to its binding site on actin.

Interference from phosphorylation of other myofilament proteins can be eliminated by working with isolated natural thick filaments in the presence of inhibitors of myosin light chain kinase.^{76–78} With these preparations, changes in the dimensions of the filaments and in the degree of order of the crossbridges can be detected by electron microscopy and optical diffraction. Cardiac thick filaments isolated from dissected rat hearts do not have uniform structure. On the basis of thickness and crossbridge order, the filaments fall into 3 groups. The filaments with intermediate thickness have a low degree of order, and the thickest filaments have the highest degree of order of crossbridges.

After selective phosphorylation of the MyBP-C in thick filaments, the heterogeneity disappears, and all filaments have the same dimensions and crossbridge order. Two detectable changes in thick filament structure have been produced by the phosphorylation of MyBP-C, as follows: (1) an expansion of the backbone of the filament including an outward movement of the ends and the centers of mass of the crossbridges of ≈ 3 nm, bringing them to what would be the surface of a thin filament in the intact filament lattice,⁷⁸ and (2) an apparent decrease in the flexibility of the crossbridges.⁷⁸ Selective phosphorylation of LC2 extends the crossbridges without expanding the backbone of the filament, and it decreases the order (and increases the flexibility) of the crossbridges.⁷⁶ Therefore, the 3 groups probably represent unphosphorylated filaments, filaments with LC2 phosphorylated, and filaments with MyBP-C phosphorylated.

A combination of the structural and the sequence data about cMyBP-C suggests a mechanism for the increase in the thickness of the thick filament produced by phosphorylation of cMyBP-C. cMyBP-C is ≈ 44 nm long and binds to the rod portion of myosin. Three molecules of cMyBP-C appear to be bound to the filament surface every 43 nm in the C zone,

probably oriented perpendicularly to the filament axis. In the absence of phosphorylation, 3 cMyBP-C molecules perpendicularly oriented would overlap (Figure 5) so that the last 3 modules of 1 molecule could interact with the first 3 modules of the adjacent molecule, as suggested by the adhesive tendency of immunoglobulin and fibronectin modules and the apparent capacity for dimer formation of cMyBP-C. The 3 overlapping molecules of cMyBP-C should maintain tight packing of myosin rods in the backbone of the thick filament and therefore restrict the myosin molecules. With phosphorylation of cMyBP-C, the thickness of the filament in the C zone increases from 30 to 37 nm, and the circumference should increase by ≈ 22 nm or ≈ 7 to 8 nm per cMyBP-C molecule (Figure 5). By adding a large amount of negative charge at sites in the link between modules I and II, phosphorylation of cMyBP-C should disrupt the interaction of modules I and II with modules IX and X of adjacent cMyBP-C molecules, resulting in a shift of module 0 to an interaction with module X. This type of structural change would explain why cMyBP-C has an additional immunoglobulin module at the N terminus that is unnecessary in skeletal muscle. In skeletal muscle, force development is varied by the number of cells activated, whereas in cardiac muscle contractility is modulated in each myocyte. The increase in the circumference of the ring of cMyBP-C allows the filament to increase in thickness by decreasing the packing of myosin rods and decreasing the restriction of myosin, particularly the HMM portion of myosin.

In the absence of phosphorylation of cMyBP-C, the smaller ring of encircling molecules enhances the stability of the thick filament, making it quite resistant to disruption by force, but the myosin molecules are more restricted, which limits their ability to adjust to changes in the myofilament proteins such as might be produced by phosphorylation of LC2 or TNI. In the presence of phosphorylation of cMyBP-C, the total length of the 3 interacting molecules of cMyBP-C increases by $\approx 20\%$, producing less restriction of myosin and facilitating regulated changes in the actin-myosin interaction. Given that phosphorylation of cMyBP-C should reduce the interaction between adjacent molecules of cMyBP-C from 3 to 1 modules, the stability of the thick filament should also be decreased.

This structural change would explain why every (or almost every) physiological positive or negative modulation of contractility is accompanied by the appropriate change in the extent of phosphorylation of cMyBP-C. It also can explain how changes in phosphorylation of cMyBP-C can occur without a change in contractility. In the absence of MyBP-C^{7,8,20} or when the myosin binding domain is absent,³⁹ the thick filament would be inherently less stable and more easily disrupted over time, particularly by high inotropic states. Later appearance of sarcomere disorganization and associated clinical symptoms would be expected with FHC from truncated cMyBP-C than with lesions in force-producing proteins.

Phosphorylation of cMyBP-C also produces a change in flexibility of the crossbridges, probably by changes in the hinge region between the head and the rod portions of the myosin molecule. It is not yet clear how this occurs, but 1 of the 2 cMyBP-C binding sites on myosin is in this region. Changes in flexibility of the crossbridges could modulate

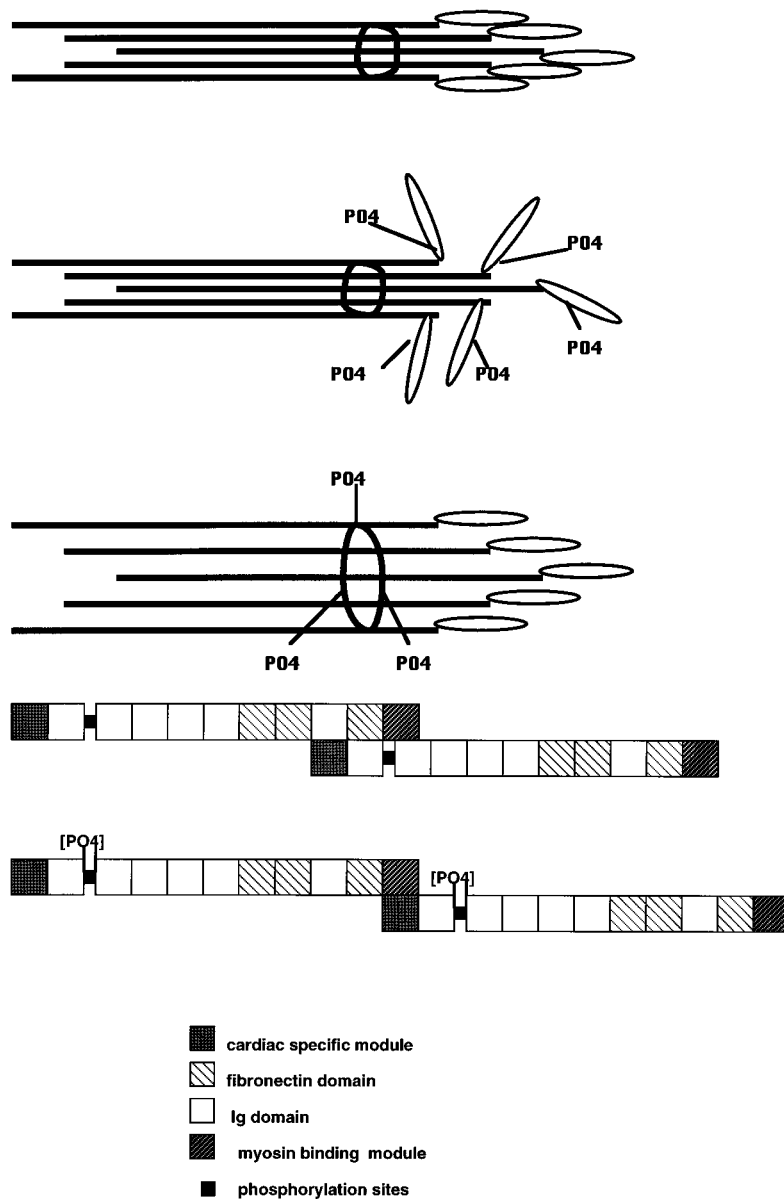


Figure 5. Upper group, Diagram showing the effect of phosphorylation of thick filament proteins on the structure of the filament. Top, Absence of phosphorylation. Middle, Phosphorylation of the regulatory light chain of myosin (LC2). Bottom, Phosphorylation of cMyBP-C. Phosphorylation of LC2 extends the cross-bridges and decreases their order,⁷⁴ whereas phosphorylation of cMyBP-C loosens the packing of the rod portion of the myosin molecule.⁷⁶ Lower group, Diagram showing the proposed influence of phosphorylation of cMyBP-C on the interaction between cMyBP-C molecules in the thick filament. The phosphorylation inhibits interaction between modules 0 to 2 with 8 to 10 on the 2 molecules because of the added charge from the phosphate groups. The interaction takes place between the 0 module that is specific to the cardiac isoform and module X, the myosin binding module. PO4 indicates phosphorylation.

crossbridge detachment rate, and changes in distance from its actin binding site as a result of altered packing of myosin rods modulate the attachment rate (Figure 6).

The effect of PKA-induced phosphorylation of MyBP-C on cardiac muscle depends on the isoform of myosin just as the effect of MyBP-C addition on actomyosin ATPase activity does. With myosin containing α -MHC, the phosphorylation leads to a decrease in the flexibility of the crossbridges, looser packing of the backbone of the thick filament, and extension of the crossbridge. In contrast, the flexibility of the crossbridges containing β -MHC is greater with MyBP-C unphosphorylated than those with α -MHC, and treatment with PKA does not extend the crossbridges or change their flexibility.

The smaller separation of the crossbridge from the thin filament should increase the probability of attachment of the crossbridge to actin, resulting in a faster rise in force and a greater ATPase activity during activation.⁷⁴ The duration and possibly the range of the force transient could be decreased

because of the decreased flexibility of the crossbridge, which would result in a greater stress for a given strain of the crossbridge at the end of the cycle and a more rapid detachment that leads to a greater velocity of shortening and rate of ATP hydrolysis. This type of model for regulation of crossbridge cycling can explain why PKA-mediated phosphorylation of MyBP-C increases ATPase activity of crossbridges with α -MHC but not with β -MHC. It can also account for the more rapid rate of relaxation with the phosphorylation⁶⁸ and the apparent decrease in internal load on the contraction when MyBP-C is partially extracted from the filament.^{41,42}

Future Directions

It is likely that there are multiple functions of cMyBP-C in heart muscle. Existing data favor a developmental role in the formation of normal thick filaments and the ordered sarcomere pattern in myofibrils and a modulatory role in the cycling of crossbridges. In neither case are the specifics of the roles defined.

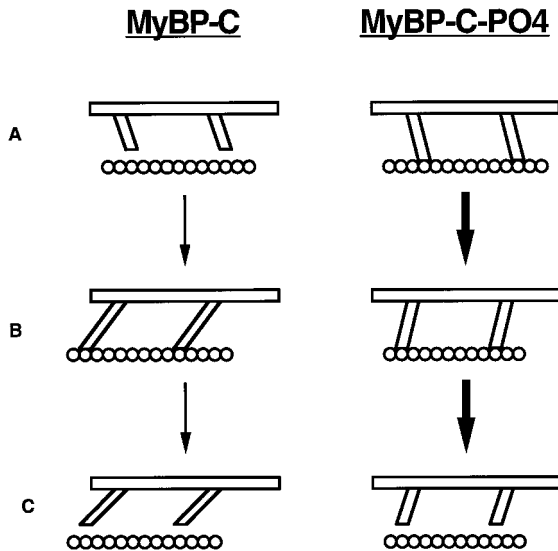


Figure 6. Diagram of a model to explain the effects of phosphorylation of MyBP-C on the cycling of crossbridges in cardiac muscle. The 2 major proposals are that (1) the flexibility of the crossbridge modulates its rate of detachment from the actin filament and (2) the distance between the thick and thin filaments modulates the rate of attachment of the crossbridges. Flexibility of the crossbridges is modified by phosphorylation of MyBP-C and is different in crossbridges with different isoforms of MHC. Phosphorylation of MyBP-C and change in sarcomere length can alter the distance between thick and thin filaments.

With the development of new mutants, not only of cMyBP-C but also other myofilament proteins, it will be possible to examine the structural changes and the biochemical and biophysical consequences of alteration of the phosphorylation sites and protein-interactive sites more precisely. It will be important to understand the relationship of calcium regulated to PKA-induced phosphorylation of cMyBP-C. This information will shed light on the molecular mechanisms involved in the functions of all of the myofilament proteins. Functional data from biochemical and biophysical studies indicate that contraction has a more finely tuned mechanism than can be provided by modulation only of excitation-contraction coupling. It will be important to understand the extent to which the different parameters of contraction, such as force, velocity, power, and efficiency can be regulated independently.

References

- Offer G, Moos C, Starr R. A new protein of the thick filaments of vertebrate skeletal myofibrils: extraction, purification and characterization. *J Mol Biol.* 1973;74:653–676.
- Bennett P, Craig R, Starr R, Offer G. The ultrastructural location of C-protein, X-protein and H-protein in rabbit muscle. *J Muscle Res Cell Motil.* 1986;7:550–567.
- Sjostrom M, Squire J. Fine structure of the A-band in cryo-sections. *J Mol Biol.* 1977;109:49–68.
- Huxley HE. Electron microscope studies of the structure of natural and synthetic protein filaments from striated muscle. *J Mol Biol.* 1963;7:281–308.
- Maw MC, Rowe AJ. The reconstruction of myosin filaments in rabbit psoas muscle from solubilized myosin. *J Muscle Res Cell Motil.* 1986;7:97–109.
- Craig R, Offer G. The location of C-protein in rabbit skeletal muscle. *Proc R Soc Lond B Biol Sci.* 1976;192:451–461.
- Koretz JF. Effects of C-protein on synthetic myosin filament structure. *Biophys J.* 1979;27:433–446.
- Davis J. Interaction of C-protein with pH 8.0 synthetic thick filaments prepared from the myosin of vertebrate skeletal muscle. *J Muscle Res Cell Motil.* 1988;9:174–183.
- Weber FE, Vaughan KT, Reinach FC, Fischman DA. Complete sequence of human fast-type and slow-type muscle myosin binding protein C (MyBP-C): differential expression, conserved domain structure and chromosome assignment. *Eur J Biochem.* 1993;216:661–669.
- Yasuda M, Koshida S, Sato N, Obinata T. Complete primary structure of chicken cardiac C-protein (MyBP-C) and its expression in developing striated muscle. *J Mol Cell Cardiol.* 1995;27:2275–2286.
- Alyonycheva TN, Mikawa T, Reinach FC, Fischman DA. Isoform-specific interaction of the myosin-binding proteins (MyBPs) with skeletal and cardiac myosin is a property of the C-terminal immunoglobulin domain. *J Biol Chem.* 1997;272:20866–20872.
- Furst P, Vinkemeier U, Weber K. Mammalian skeletal muscle C-protein: purification from bovine muscle, binding to titin and the characterization of a full length human cDNA. *J Cell Sci.* 1992;102:769–778.
- Vaughan KT, Weber FE, Einheber S, Fischman DA. Molecular cloning of chicken myosin binding protein (MyBP) H (86-kDa protein) reveals extensive homology with MyBP-C (C-protein) with conserved immunoglobulin C2 and fibronectin type III motifs. *J Biol Chem.* 1993;268:3670–3676.
- Vaughan KT, Weber FE, Ried T, Ward DC, Reinach FC, Fischman DA. Human myosin-binding protein H (MyBP-H): complete primary sequence, genomic organization, and chromosomal localization. *Genomics.* 1993;16:34–40.
- Gautel M, Zuffardi O, Freiberg A, Labeit S. Phosphorylation switches specific for the cardiac isoforms of myosin binding protein C: a modulator of cardiac contraction. *EMBO J.* 1995;14:1952–1960.
- Carrier L, Bonne G, Bahrend E, Yu B, Richard P, Niel F, Hainque B, Cruard C, Gary F, Labiet S, Bouhours J-B, Dubourg O, Desnos M, Hagege A, Trent RJ, Komajda M, Fiszman M, Schwartz K. Organization and sequence of human cardiac myosin binding protein C gene (MyBP-C3) and identification of the mutations predicted to produce truncated proteins in familial hypertrophic cardiomyopathy. *Circ Res.* 1997;80:427–434.
- Okagaki T, Weber FE, Fischman DA, Vaughan KT, Mikawa T, Reinach FC. The major myosin binding domain of skeletal muscle MyBP-C (C protein) resides in the COOH-terminal, immunoglobulin C2 repeat. *J Cell Biol.* 1993;123:619–626.
- Hartzell C, Sale WC. Structure of C-protein purified from cardiac muscle. *J Cell Biol.* 1985;100:208–215.
- Swan RC, Fischman DA. Electron microscopy of c-protein molecules from chicken skeletal muscle. *J Muscle Res Cell Motil.* 1986;7:160–166.
- Gilbert R, Kelly MG, Mikawa T, Fischman DA. The carboxyl terminus of myosin binding protein-C (MyBP-C, C-protein) specifies incorporation into the A-band of striated muscle. *J Cell Sci.* 1996;109:101–111.
- Freiburg A, Gautel M. A molecular map of the interactions between titin and myosin-binding protein C. *Eur J Biochem.* 1996;235:317–323.
- Labeit S, Kolmer B. Titins: giant proteins in charge of muscle ultrastructure and elasticity. *Science.* 1995;270:293–296.
- Yamamoto K. The binding of skeletal C-protein to regulated actin. *FEBS Lett.* 1986;208:123–127.
- Moos C, Mason CM, Besterman JM, Feng IM, Dubin JH. The binding of skeletal muscle C-protein to F-actin, and its relation to the interaction of actin with myosin subfragment-1. *J Mol Biol.* 1978;124:571–586.
- Starr R, Offer G. H-protein and X-protein: two new components of the thick filaments of vertebrate skeletal muscle. *J Mol Biol.* 1983;170:675–698.
- Starr R, Almond G, Offer G. Location of C protein, H protein and X protein in rabbit skeletal muscle fiber types. *J Muscle Res Cell Motil.* 1985;6:227–256.
- Yamamoto K, Moos C. The C-proteins of rabbit red, white, and cardiac muscles. *J Biol Chem.* 1983;258:8395–8401.
- Bahler M, Eppenberger HM, Walliman T. Novel thick filament protein of chicken pectoralis muscle: the 86 kd protein, I: purification and characterization. *J Mol Biol.* 1985;186:381–391.
- Alyonycheva T, Cohen-Gould L, Siewert C, Fischman DA, Mikawa T. Skeletal muscle-specific myosin binding protein-H is expressed in Purkinje fibers of the cardiac conduction system. *Circ Res.* 1997;80:665–672.
- Yamamoto K. Characterization of H-protein, a component of skeletal muscle myofibrils. *J Biol Chem.* 1984;259:7163–7168.
- Bonne G, Carrier L, Bercovici J, Cruard C, Richard P, Hainque M, Gautel M, Labeit S, Jones M, Beckman J, Vosberg H-P, Komajda M, Schwartz K. Cardiac myosin-binding protein C gene splice is associated with familial hypertrophic cardiomyopathy. *Nat Genet.* 1995;11:438–440.

32. Watkins H, Conner D, Thierfelder L, Jarcho JA, MacCrea C, McKenna WJ, Maron BJ, Seidman JG, Seidman CE. Mutations in the cardiac myosin-binding protein-C on chromosome 11 cause familial hypertrophic cardiomyopathy. *Nat Genet.* 1995;11:433–438.
33. Rottbauer W, Gautel M, Zehelein M, Labeit S, Franz WM, Fischer C, Vollrath B, Mall G, Dietz R, Kubler W, Katus HA. Novel splice donor site mutation in the cardiac myosin-binding protein C gene in familial hypertrophic cardiomyopathy: characterization of cardiac transcript and protein. *J Clin Invest.* 1997;100:475–482.
34. Niimura H, Bachinski LL, Sangwatanaroj S, Watkins H, Chudley AE, McKenna W, Kristinsonn A, Roberts R, Maron BJ, Seidman JG, Seidman CE. Mutations in the gene for cardiac myosin-binding protein C and late-onset familial hypertrophic cardiomyopathy. *N Engl J Med.* 1998;338:1248–1257.
35. Yu B, French JA, Carrier L, Jeremy RW, McTaggart DR, Nicholson MR, Hambly B, Semsarian C, Richmond DR, Schwartz K, Trent RJ. Molecular pathology of familial hypertrophic cardiomyopathy caused by mutations in myosin binding protein C. *J Med Genet.* 1998;35:205–210.
36. Charron P, Dubourg O, Desnos M, Bannaceur M, Carrier L, Camproux A-C, Isnard R, Hagege A, Langlard JM, Bonne G, Richard P, Hainque B, Bouhour J-B, Schwartz K, Komajda M. Clinical features and prognostic implications of familial hypertrophic cardiomyopathy related to the cardiac myosin-binding protein C gene. *Circulation.* 1998;97:2230–2236.
37. Fougerousse F, Delzoid A-L, Fiszman MY, Schwartz K, Beckmann JS, Carrier L. Cardiac myosin binding protein C is specifically expressed in heart during murine and human development. *Circ Res.* 1998;82:130–133.
38. Gautel M, Furst DO, Cocco A, Schiaffino S. Isoform transitions of the mouse binding protein C family in developing human and mouse muscles: lack of isoform transcomplementation in cardiac muscle. *Circ Res.* 1998;82:124–129.
39. Yang Q, Sanbe AJ, Osinska H, Hewett TE, Klevitsky R, Robbins J. A mouse model of myosin binding protein C human familial hypertrophic cardiomyopathy. *Clin Invest.* 1998;102:1292–1300.
40. Hofmann P, Hartzell C, Moss R. Alterations in Ca sensitive tension due to partial extraction of C protein from rat skinned cardiac myocytes and rabbit skeletal muscle fibers. *J Gen Physiol.* 1991;97:1141–1163.
41. Hofmann P, Greaser M, Moss R. C-protein limits shortening velocity of rabbit skeletal muscle fibers at low levels of Ca activation. *J Physiol (Lond).* 1991;439:701–715.
42. Squire JM, Luther PK, Morris EP. Organization and properties of the striated-muscle sarcomere. In: Squire JM, ed. *Molecular Mechanisms in Muscle Contraction*. Boca Raton, Fla: CRC Press; 1990:1–48. Topics in Molecular and Structural Biology Series.
43. Reinach FC, Masaki T, Fischman DA. Characterization of the C-protein from posterior latissimus dorsi muscle of the adult chicken: heterogeneity within a single sarcomere. *J Cell Biol.* 1983;96:297–300.
44. Dennis JE, Shimizu T, Reinach FC, Fischman DA. Localization of C-protein isoforms in chicken skeletal muscle: ultrastructural detection using monoclonal antibodies. *J Cell Biol.* 1984;98:1514–1522.
45. Starr R, Offer G. The interaction of C-protein with heavy meromyosin and subfragment-2. *Biochem J.* 1978;171:813–816.
46. Moos C, Offer G, Starr R, Bennett P. Interaction of C-protein with myosin, myosin rod and light meromyosin. *J Mol Biol.* 1975;97:1–9.
47. Huxley HE, Brown W. The low-angle x-ray diagram of vertebrate striated muscle and its behaviour during contraction and relaxation. *J Mol Biol.* 1967;30:383–434.
48. Seiler SH, Fischman DA, Leinwand LA. Modulation of myosin filament organization by C protein family members. *Mol Biol Cell.* 1996;7:113–127.
49. Lin Z, Lu MH, Schultheiss T, Choi J, Holtzer S, DiLuulo C, Fischman DA, Holtzer H. Sequential appearance of muscle-specific proteins in myoblasts as a function of time after cell division: evidence for a conserved myoblast differentiation program in skeletal muscle. *Cell Motil Cytoskeleton.* 1994;29:1–19.
50. Rhee D, Sanger JM, Sanger JW. The premyofibril: evidence for its role in myofibrillogenesis. *Cell Motil Cytoskeleton.* 1994;28:1–24.
51. Schultheiss T, Lin ZX, Lu MH, Fischman DA, Weber K, Masaki T, Imamura M, Holtzer H. Differential distribution of subsets of myofibrillar proteins in cardiac nonstriated and striated myofibrils. *J Cell Biol.* 1990;110:1159–1172.
52. Yamamoto K, Moos C. The C-proteins of rabbit red, white, and cardiac muscles. *J Biol Chem.* 1983;258:8395–8401.
53. Moos C, Feng IM. Effect of C protein on actomyosin ATPase. *Biochim Biophys Acta.* 1980;632:141–149.
54. Hartzell HC. Effects of phosphorylated and unphosphorylated C-protein on cardiac actomyosin ATPase. *J Mol Biol.* 1985;186:185–195.
55. Margossian SK. Reversible dissociation of dog cardiac myosin regulatory light chain 2 and its influence on ATP hydrolysis. *J Biol Chem.* 1985;260:13747–13754.
56. Schlender K, Bean L. Phosphorylation of chicken cardiac C protein by calcium calmodulin-dependent protein kinase II. *J Biol Chem.* 1991;266:2811–2817.
57. Hartzell C, Glass D. Phosphorylation of purified cardiac muscle protein by purified cAMP-dependent and endogenous Ca-calmodulin-dependent protein kinases. *J Biol Chem.* 1984;259:15587–15596.
58. Moss RL. Ca²⁺ regulation of mechanical properties of striated muscle: mechanistic studies using extraction and replacement of regulatory proteins. *Circ Res.* 1992;70:865–884.
59. Levine RJC, Kensler R, Yang Z, Stull JT, Sweeney HL. Myosin light chain phosphorylation affects the structure of rabbit muscle thick filaments. *Biophys J.* 1996;71:898–907.
60. Bartel S, Karczewski P, Morano I, Krause E-G. Function of C-protein and troponin I phosphorylation in the heart. *Ann NY Acad Sci.* 1995;752:43–245.
61. Horowitz R, Winegrad S. cAMP regulation of myosin ATPase activity in the maturing rat heart. *Circ Res.* 1987;61:914–924.
62. Venema RC, Kuo J. Protein kinase C mediated phosphorylation of troponin I and C protein in isolated myocardial cells is associated with inhibition of actomyosin Mg ATPase. *J Biol Chem.* 1993;268:2705–2711.
63. Noland TA, Raynor RA, Jideama NM, Guo X, Kazanietz MG, Blumberg PM, Solaro RJ, Kuo JF. Differential regulation of cardiac actomyosin S-1 MgATPase by protein kinase C isozyme-specific phosphorylation of specific sites in cardiac troponin I and its phosphorylation site mutants. *Biochemistry.* 1996;35:14923–14931.
64. Garvey L, Kranias E, Solaro J. Phosphorylation of C protein, troponin I and phospholamban in isolated rabbit hearts. *Biochem J.* 1988;249:709–714.
65. Winegrad S, Weisberg A. Isozyme specific modification of myosin ATPase by cAMP in rat heart. *Circ Res.* 1987;60:384–392.
66. McClellan G, Weisberg A, Winegrad S. Cyclic AMP can raise or lower cardiac actomyosin ATPase activity depending on alpha adrenergic activity. *Am J Physiol* 1994;267:H431–H442.
67. Solaro RJ, Van Eyk J. Altered interactions among thin filament proteins modulate cardiac function. *J Mol Cell Cardiol.* 1996;28:217–230.
68. Hartzell C. Phosphorylation of C protein in intact amphibian cardiac muscle: correlation between ³²P incorporation and twitch relaxation. *J Gen Physiol.* 1984;186:563–588.
69. Hofmann PA, Lange JH III. Effects of phosphorylation of troponin I and C protein on isometric tension and velocity of unloaded shortening in skinned single cardiac myocytes from rats. *Circ Res.* 1994;74:718–726.
70. Strang KT, Moss RL. β -Adrenergic receptor stimulation increases unloaded shortening velocity in skinned single ventricular myocytes from rats. *Circ Res.* 1994;74:542–549.
71. Hasenfuss G, Mulieri LA, Leavitt BJ, Alpert NR. Influence of isoproterenol on contractile protein function, excitation-contraction coupling, and energy turnover of isolated nonfailing human heart. *J Mol Cell Cardiol.* 1994;26:1461–1469.
72. Hoh JF, Rossmanith GH, Kwan LJ, Hamilton AM. Adrenaline increases the rate of cycling of cross bridges in rat cardiac muscle as measured by pseudo-random binary noise-modulated perturbation analysis. *Circ Res.* 1988;62:452–461.
73. Saeki Y, Kobayashi T, Takigiku K, Sugi H. Influence of protein kinase A on actin-myosin interaction and energy consumption of skinned cardiac muscles. In: Sugi H, Pollack G, eds. *Mechanism of Work Production and Work Absorption in Muscle*. New York, NY: Plenum Publishing; 1998.
74. McDonald K, Moss RL. Osmotic compression of single cardiac myocytes eliminates the reduction in Ca²⁺ sensitivity of tension at short sarcomere length. *Circ Res.* 1995;77:199–205.
75. Huxley AF. Muscle structure and theories of contraction. *Prog Biophys.* 1957;7:255–318.
76. Levine RJC, Yang Z, Epstein ND, Fananapazir L, Stull JT, Sweeney HL. Structural and functional responses of mammalian thick filaments to alterations in myosin regulatory light chains. *J Struct Biol.* 1998;122:149–161.
77. Weisberg A, Winegrad S. Alteration in myosin cross bridges by phosphorylation of myosin-binding protein C in cardiac muscle. *Proc Natl Acad Sci U S A.* 1996;93:8999–9003.
78. Weisberg A, Winegrad S. Relation between crossbridge structure and actomyosin ATPase activity in rat heart. *Circ Res.* 1998;83:60–72.