CARDIAC MYOSIN BINDING PROTEIN C

Cardiac myosin binding protein C, adrenergic stimulation and cardiac contractility

Amsha Ramburan, Lundi Korkie and Johanna C. Moolman-Smook

Dept Biomedical Sciences, University of Stellenbosch Health Sciences Faculty, Tygerberg

Address for correspondence:
J.C. Moolman-Smook
Dept Biomedical Sciences
University of Stellenbosch Health Sciences
PO Box 19063
Tygerberg
7505
South Africa

Email:
hm@sun.ac.za

ABSTRACT

Myosin binding protein C remained a perplexing although integral component of the sarcomeric thick filament until the discovery that genetic defects in its corresponding gene is a frequent cause of hypertrophic cardiomyopathy. Basic science investigation subsequently revealed that it is one of the most potent regulators of cardiac contractility. Phosphorylation of its N-terminus upon adrenergic stimulation, causes increased order in myosin heads as well as increased ATPase activity, F_{max} and Ca^{2+}-sensitivity of contraction, while its dephosphorylation upon cholinergic stimulation or during low flow ischaemia leads to changes in the sarcomeric thick filament that diminish interaction between myosin heads and actin. This dynamic flux in phosphorylation upon adrenergic stimulation is not only crucial to normal cardiac function and structure, but also vital for protection against ischaemic injury. Genetically-driven deficiency or inadequacy in cMyBPC leads to severe cardiac dysfunction and structural changes, including cardiac hypertrophy and dilation, and particularly attenuates the adaptive increase in left ventricular contractility that follows on β-adrenergic stimulation or pressure overload, resulting in decreased systolic function, and reduced cardiac output. SAHeart 2010; 7:38-47

Cardiac contractility is largely determined by nervous input to the heart and circulating chemical messengers, but mechanisms intrinsic to the myocardium also contribute to these control systems.[31] These systems are multilayered and complex, and are ultimately regulated by the parameters that affect cross-bridge cycling within the sarcomere, that precisely organised repeating contractile unit found within all striated muscle, at the molecular level. These include the flow of Ca^{2+} through compartments of the cell as well as the interactions between components of the thick and thin filaments of the sarcomere. Upon β-adrenergic stimulation, particular proteins within the cardiomyocyte, including cardiac troponin I and cardiac myosin binding protein C (cMyBPC), undergo orchestrated functional changes that together comprise the positive inotropic and lusitropic effects caused by β-adrenoreceptor activation. These effects serve to enhance cardiac output: the positive inotropic effect boosts myocyte contractility by optimising Ca^{2+} homeostasis, mainly via phosphorylation of Ca^{2+}-handling proteins, while the positive lusitropic effect accelerates myocyte relaxation, thereby facilitating an increase in cardiac output via elevation of heart rate.[21,31,51,58]

In the cardiac muscle thick filament, the primary regulator of contractility is cMyBPC. This integral thick filament protein was discovered in the early 1970's,[53] yet more than 30 years later its structural and functional roles are still being solved. Such investigation is clinically relevant, as genetically encoded defects in cMyBPC are the most common cause of familial hypertrophic cardiomyopathy (HCM).

cMyBPC is located in the C-zone of the A-band in a structurally regular pattern of 7 to 9, 43nm-apart, transverse stripes.[8] The 43nm spacing of these stripes dictates that only every third level of myosin heads in the C-zone is associated with a cMyBPC molecule. This and the number of myosin heads that fall outside the C-zone, limit the number of myosin heads that can interact directly with cMyBPC.[37] Each stripe is composed of 2 to 4

cMyBPC molecules and represents about 2 percent of the protein mass in the myofibril.

STRUCTURE

Three isoforms of myosin binding protein C, encoded by three distinct genes, viz. fast skeletal (fsMyBPC; ~128 kDa), slow skeletal (ssMyBPC; originally described as MyBPX; ~128 kDa), and cardiac muscle isoforms (cMyBPC; ~137 kDa) have been identified.[8] Myocardial cells were thought to contain only the cardiac isoform throughout development and in adulthood,[12] but ssMyBPC has been shown to be present in moderate amounts in the right atrium and interatrial septum of adult human heart muscle.[5] Although the three isoforms share a similar structure of immunoglobulin (Ig) and fibronectin type domains, the cardiac isoform has 3 distinguishing structural differences from the skeletal isoforms, viz. an extra Ig domain at the N-terminal (C0), a proline-rich insert in the central C5 domain and three functional phosphorylation sites located in the MyBPC motif, a compact domain located between the N-terminal domains C1 and C2 (Figure 1).[38] These sites have relative sensitivities to the kinases that are present in the cardiac cell, as well as a hierarchy of phosphorylation. A Ca^{2+}/calmodulin-dependent kinase (CamK), which co-purifies with cMyBPC, phosphorylates only the middle site;[8,60] this step is a prerequisite for protein kinase A (PKA) phosphorylation of the remaining sites upon β-adrenergic stimulation.[11] Since the middle site is phosphorylated by CamK-II, Ca^{2+} plays an important role in cMyBPC phosphorylation. McClellan and colleagues[29] speculated that the phosphorylation of this site was possibly constitutive in vivo, as it may be tied to the normal dynamic flux of Ca^{2+} in the myocytes.

Dephosphorylation of cMyBPC occurs in response to cholinergic agonists such as acetylcholine.[15] In vitro studies in chicken have shown that this occurs predominantly via protein phosphatase-2A,[47] which is involved in the dephosphorylation of other sarcomeric proteins and has been shown to co-purify with cMyBPC.[48] It is not known whether there is a specific order for dephosphorylation of cMyBPC.

SARCOMERIC ARRANGEMENT

We have previously proposed that cMyBPC is arranged around the backbone of the thick filament in a "trimeric collar", in which 3 staggered cMyBPC molecules form a ring/collar around the thick filament (Figure 2), which is thought to be stabilised by specific interactions that have been demonstrated between domains C5 and C8, and domains C7 and C10,[33] as well as the constitutive interaction between domain C10 and the myosin rod.[39] This model predicts that domains C0C4, forming the N-terminal of MyBPC, has sufficient length to reach out from the thick filament to take part in interactions previously suggested

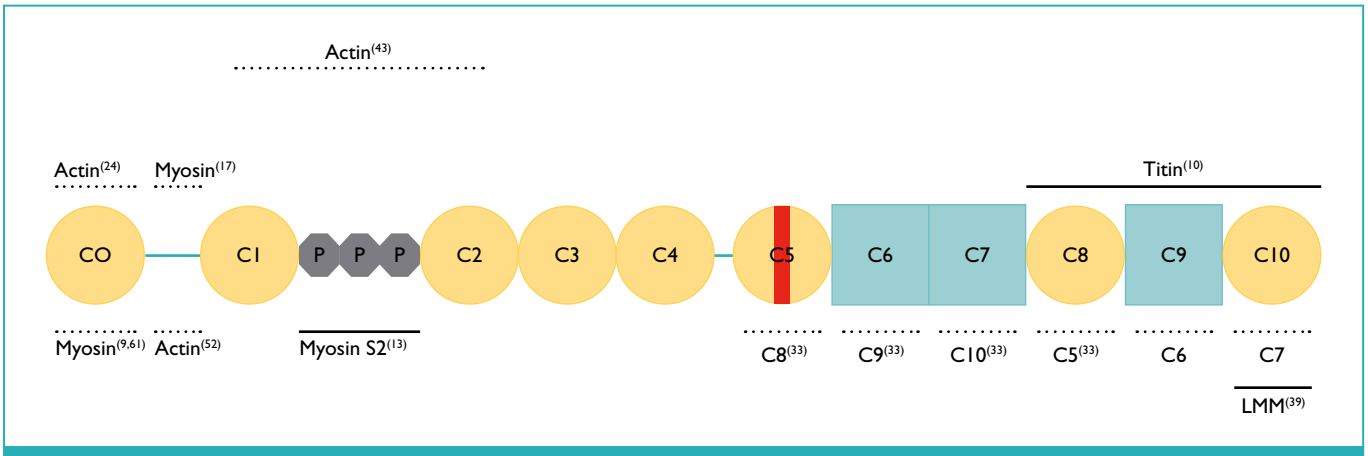

FIGURE 1: Schematic representation showing the modular organisation of cMyBPC into distinct domains. Some domains have been shown to direct the binding of cMyBPC to other proteins within the sarcomere (represented by solid black lines), while other domains have been implicated in protein interactions but not yet confirmed (shown in broken black lines). cMyBPC is made up of two particular types of protein domains, viz. immunoglobulin domains (shown in yellow circles) and fibronectin domains (shown in blue squares), which are sometimes connected by linker sequences (shown in solid blue lines), such as those between domains C0 and C1, and between domains C4 and C5. The cardiac version of MyBPC also differs from the skeletal muscle forms of MyBPC by the presence of amino-acid insertions, viz. the cardiac-specific insert in domain C5 (shown as a red rectangle) and the phosphorylation sites between domains C1 and C2 (shown as grey octagons within the MyBPC motif).

FIGURE 2: Based on protein interaction studies, we have proposed a trimeric collar model to explain the arrangement of cMyBPC within the thick filament of the sarcomere. In this model, three cMyBPC molecules form a belt or collar around the thick filament (shown as a yellow rod), with domains C5-C7 of one molecule interacting with domains C8-C10 of the next in an overlapping, parallel arrangement.[33] In this model, we also propose that the N-terminal domains (C0-C4) extend into the interfilament space between thick filaments, where the cMyBPC motif would be available to interact with the region of myosin that supports the myosin head, viz. the myosin S2 region (myosin and additional thick filaments not shown). [Image taken from Moolman-Smook[33]]

to occur with the myosin crossbridge[9,17,61] and thin filaments.[24,43,52] It is through these interactions, mediated via its N-terminal, that cMyBPC regulates muscle contractility.

INTERACTIONS WITH MYOSIN AND ACTIN

Firstly, cMyBPC binds to the S2 region of myosin in a reversible fashion that is dependent on the phosphorylation status of the MyBPC motif. When dephosphorylated, the C1C2 region binds the proximal 126 residues of myosin S2, but upon phosphorylation of the MyBPC motif, the binding is abrogated.[13] Residues within domain C2[1] and Ser-273/Ser-282 within the MyBPC motif of cMyBPC act synergistically to form an extended myosin S2 "interaction interface".[20] At activating Ca^{2+} concentrations, when cMyBPC is fully phosphorylated, increased maximum force has been observed.[29] These results suggest that cMyBPC phosphorylation, regulated by intracellular Ca^{2+} levels (to activate CamK-II) and β-adrenergic stimulation (to activate PKA), determines the state of interaction of cMyBPC with myosin S2 and the regulation of cross-bridge cycling,[29] possibly by implementing a tethering-effect on the myosin heads.

However, it is not only via its interaction with myosin S2 that cMyBPC regulates contractility. Early studies have shown that cMyBPC interacted with actin in both regulated and unregulated filaments,[35,62] producing links between actin and myosin and increasing actin-activated myosin-ATPase activity in vitro.[22,35,36] It has been suggested that this interaction involves a region between domains C0 and C1.[24] Furthermore, Razumova and colleagues[43] showed that the C1C2 region of cMyBPC also interacts with actin to affect cross-bridge kinetics (reducing actin filament velocity at high Ca^{2+} concentrations), without a requirement for myosin S1 or S2.[50] Interestingly, Herron and colleagues[17] showed that N-terminal fragments of cMyBPC containing the pro-ala-rich linker sequence between C0 and C1 was able to switch on the thin filament by a mechanism different from that of Ca^{2+}. The precise mechanism for this Ca^{2+}-independent activating effect of N-terminal cMyBPC on force production and crossbridge cycling remains to be established.

Thus, N-terminal domains of cMyBPC interact with both thick and thin filaments and can therefore modulate actomyosin contacts through these interactions directly.[50] It has been suggested that cMyBPC could give rise to an internal load by tethering myosin S2 to the thick filament, thereby limiting myosin head position and/or mobility, and limiting cross-bridge formation,[33] or via its interaction with the thin filament when cMyBPC is phosphorylated.[23] Such an internal load would serve a useful function if cMyBPC stores some of the energy generated during systole to assist in the filling of the heart during diastole.[23] The effect of cMyBPC to slow cross-bridge formation may appear to be deleterious to contractile function; however slowed rates of cross-bridge cycling may be beneficial in vivo by prolonging systolic ejection and increasing contractile efficiency by minimising ATP utilisation, as well as promoting diastolic filling rates.[24]

Removal of the constraint either by MyBPC deficiency, such as occurs in some forms of cardiomyopathy, or its phosphorylation under adrenergic stimulation, would bring the myosin head in

closer proximity to actin, thereby enhancing the probability of cross-bridge formation and increasing power output,[16] contractility,[42] and V_{max},[18] as well as lowering viscous load.[42]

FUNCTION OF CMYBPC

Thick filament formation

In vitro evidence suggests that cMyBPC plays an important role in the formation of myofibrils in skeletal and cardiac muscle.[8] Purified myosin can self-assemble into thick filaments in the absence of cMyBPC; however, only in the presence of the normal content of cMyBPC do synthetic thick filaments resemble native thick filaments in their thickness, length, bare-zone and distribution of myosin heads.[8,19,28,49] However, while in vitro experiments indicated that cMyBPC may play a role in thick filament formation, murine knockout models of cMyBPC did not show a lack of sarcomere formation.[14,30] Rather, transgenic mice with no cMyBPC expression are viable and have regular sarcomere striations such as the Z-line, A-band and M-line, but these sarcomeric features are frequently misaligned.[14]

Thick filament stability

Moreover, evidence suggests that cMyBPC phosphorylation provides thick filament stability[25] showed that cMyBPC exists in 2 different forms that produced either stable or unstable thick filaments.[25] The stable form had well-ordered myosin heads and required cMyBPC phosphorylation, while the unstable form was associated with disordered myosin heads and unphosphorylated cMyBPC. Unphosphorylated cMyBPC was more easily proteolysed even within intact cardiac cells, causing cMyBPC and myosin to be released, and myofilaments deficient in cMyBPC were fragmented by shear force that is well tolerated by the stable form.[25]

cMyBPC and cardiac function

While the presence of cMyBPC is not absolutely required for sarcomere formation, the lack of cMyBPC[14,40,54,55,56] and decreased levels of cMyBPC phosphorylation[45] in the myocardium have been implicated in both systolic and diastolic dysfunction.

Further animal studies showed that homozygous and heterozygous cMyBPC-null mice exhibited different cardiac disorders.[3] Heterozygous mice developed asymmetric septal hypertrophy associated with fibrosis at 10 to 11 months of age, without impairment of left ventricular function. This type of hypertrophy, although without myofiber disarray, is similar to human HCM. On the other hand, homozygous mice expressing a C8C10-truncated cMyBPC exhibited neonatal onset of dilated cardiomyopathy with prominent histopathology of myocyte hypertrophy, myofibrillar disarray, fibrosis, dystrophic calcification, left ventricular (LV) dilation and reduced contractile function, which manifested in depressed systolic contractility with diastolic dysfunction, as well as reduced systolic LV chamber stiffness.[30,40] The reduced stiffness is attibuted to the complete lack of cMyBPC, which, if present, would have provided structural support via its strong interactions with myosin and the giant sarcomeric support protein, titin. The structural integrity afforded by these interactions may be responsible for as much as 50% of the normal longitudinal stiffness between crossbridges and the sarcomeric M-line. Functionally, the overly compliant cMyBPC-deficient myocardium of these transgenic mice may not be able to provide the requisite transmural wall stress at normal LV chamber dimensions to accommodate normal LV pressure and would therefore be expected to dilate.[41]

In addition, Palmer and colleagues[41] showed that cMyBPC appeared less important for initiating force development than for sustaining force and muscle stiffening so that systole can extend normally throughout ejection. Without it, chambers are capable of only a very short eject period: time to peak elastance is reduced, with premature relaxation starting shortly after ejection and resulting in reduced cardiac output. This reduced elastance appeared to be fairly specific to cMyBPC-deficient hearts, as it was not mimicked in other models of cardiac dysfunction such as autoimmune myocarditis and myocardial stunning. These findings[40,41] suggested that cMyBPC provided mechanical stability to the myofilament lattice in such a manner as to significantly influence the transmission of force across the sarcomere and sustain systolic stiffening.[41]

In a long-term follow-up study of transgenic mice expressing truncated cMyBPC that lacked C-terminal domains, cardiac hypertrophy was observed in adults older than one year.[65] When these were stressed either by physical exercise or chronic β-adrenergic stimulation, bradycardia and sudden death was observed, while unstressed transgenic animals had a normal life span. This suggests

that the unstressed heart was able to compensate effectively for the intrinsic mechanical and kinetic deficits resulting from cMyBPC defects, but that functional deficits presented at the whole organ level upon exposure to stress. These findings are similar to what has been observed in human subjects, where mutations in the cMyBPC-encoding gene, MYBPC3, are often, but not always, associated with late onset and benign phenotype.[65]

Phosphorylation patterns of cMyBPC have also been shown to have a significant effect on the whole-heart function and cardiac haemodynamics.[45] In various mouse models of cardiac stress, cMyBPC was extensively phosphorylated under basal conditions and became dephosphorylated during the development of heart failure or pathological hypertrophy, with the trisphosphorylated form largely or completely absent in the advanced stages of heart failure.[45] This phenomenon appeared to be independent of the type of cardiac stress, as pressure overload, ischaemic-reperfusion injury, and various genetic alterations in the cardiac machinery all resulted in significantly decreased phosphorylation. In mice engineered to express a non-phosphorylatable form of cMyBPC, hearts- appeared overtly normal with no cardiac hypertrophy and/or dilation. However, sarcomeric patterns were ill-defined with altered H-zones and M-lines, and genes associated with a hypertrophic response (such as atrial-natriuretic factor, β-MHC, brain natriuretic peptide, and skeletal β-actin) were up-regulated. Moreover, contraction and relaxation were significantly decreased, indicating that the capacity for cMyBPC phosphorylation is essential for normal cardiac function.[45] Reduced cMyBPC phosphorylation has been reported in human atrial fibrillation[6] and in failing human hearts,[7] which forms part of the picture of attenuated β-adrenergic responsiveness (desensitisation) during heart failure. This may be mainly as a result of decreased β1-adrenoreceptor number and function and thus attenuated PKA activity[27,57] but likely contributes to the downwards spiral of contractile dysfunction.

While dephosphorylated cMyBPC may be associated with disease either as cause or consequence, cMyBPC phosphorylation can actually protect the myocardium from ischaemic injury: the hearts of transgenic mice engineered to express a constitutively phosphorylated form of cMyBPC were resistant to ischaemia-reperfusion injury.[46] Taken together, these studies show that, while cMyBPC is not absolutely essential for sarcomere formation, it is necessary for the integrity of sarcomere structure and its phosphorylation in response to adrenergic stimulation is crucial for normal systolic and diastolic cardiac function.

PATHOPHYSIOLOGICAL MECHANISM OF CMYBPC DEFECTS

The mechanism by which mutant cMyBPC protein causes dysfunction, either via haploinsufficiency (too little protein) or "poison" peptide (a dysfunctional protein), has also been investigated, but have yielded contradictory results[64] showed that mutant cMyBPC (lacking only the C-terminal myosin-binding domain C10) was stably expressed and, although it was incorporated into the sarcomere, it was not restricted to its normal position within the A-band, while mutant cMyBPC protein lacking both the myosin and titin-binding domains (domains C8 through C10) was not detectable.[30,40,63] The absence of cMyBPC protein in some of these models precludes the possibility that the truncated MyBPC always acts as a "poison" peptide and the cardiomyopathy observed must therefore be due to the mechanical consequences of the absence or insufficient levels of cMyBPC.[40]

What may these mechanical consequences be? It has been shown that structural changes occur in the thick filament upon cMyBPC phosphorylation, with different degrees of cMyBPC phosphorylation resulting in distinct changes in the appearance of isolated thick filaments. In the absence of cMyBPC phosphorylation, the thick filament had a disordered structure (myosin heads extending at different angles from the backbone). The addition of the first phosphate to cMyBPC induced a change from the disordered structure to a tight structure (myosin heads lying along the backbone). With the addition of the second and third phosphates, by PKA, greater order of myosin heads and looser packing of myosin, concomitant increase in thick filament diameter in the region of the sarcomere which houses cMyBPC, was observed.[26,59] In the fully phosphorylated state, the myosin heads appeared extended from the thick filament backbone, closer to the thin filament surface, favouring interaction between the thick and thin filament.[26]

Thus, the packing of myosin filaments and their heads are intimately linked to the state of cMyBPC phosphorylation, where the extent of phosphorylation correlates with an increased ability of

myosin heads to interact with the thin filament. Phosphorylation of cMyBPC also produces a change in flexibility of the cross-bridges. It is not yet clear how this occurs, but changes in flexibility of the crossbridges could modulate cross-bridge detachment rate, and changes in the distance from its actin-binding sites as a result of altered packing of myosin rods modulate the attachment rate.[60] Disordering of the arrangement of surface myosin heads and declining contractility are coincident with dephosphorylation of cMyBPC, while rephosphorylation leads to recovery of both cross-bridge order and contractility.[26]

HCM-causing mutations

We had previously found that domain C5 of a cMyBPC molecule bound to domain C8 of another cMyBPC molecule (K_a=1×10^5 M^{-1}), and similarly, that domain C7 bound to domain C10 (K^a=1×10^5 M^{-1}), supporting the trimeric collar model for the quaternary arrangement of monophosphorylated cMyBPC, and consistent with the dimensions of the thick filament backbone in the corresponding narrow conformation. However, as the thick filament backbone is known to expand upon adrenergic stimulation and maximal phosphorylation of cMyBPC, a trimeric cMyBPC collar would have to accommodate this change by abrogating intermolecular interactions between domains C5:C8, C6:C9 and C7:C10 and either completely breaking the collar apart or assuming a looser conformation in this activatory condition. We therefore explored the theoretical effect of cMyBPC phosphorylation on collar dimensions.[26] Based on thick filament backbone dimensions in the "loose" conformation, and with domain C10 firmly anchored to myosin and possibly titin, interaction would shift from existing between central cMyBPC domains and extreme C-terminal domains (C9 and/or C10), to between the latter domains and N-terminal domains of cMyBPC in the vicinity of the C1C2 region.[4] When we tested for the possibility of such interactions using yeast two-hybrid interaction assays, we showed that domain C3 interacted with domain C10 (Figure 3), suggesting that conditions exist under which domain C10 can interact with either C7 or C3. This data, coupled with the observed changes in the thick filament back bone diameter upon cMyBPC phosphorylation, led us to propose a "loose" collar conformation for cMyBPC around the thick filament backbone during conditions of adrenergic stimulation, depicted in Figure 4. Upon PKA-dependent phosphorylation of cMyBPC, the "loose" collar would allow the thick filament to expand and adopt the loose structure which has been associated with increased crossbridge cycling.[26,29]

FIGURE 3: Yeast two-hybrid reporter gene activation by prey cMyBPC domains paired with bait domain C7. Growth of auxotrophic diploid yeast on selective medium indicates interaction of C7 with a prey domain. A representative direct protein-protein interaction assay demonstrates that only the occurance of domain C10 activates the reporter genes in the presence of domain C7, as monitored by growth of the yeast.

In support of this hypothesis, we also found, using yeast two-hybrid library screens, that a "bait" representing the native cMyBPC region of domains C1 through C2 (C1C2), and a bait representing the fully phosphorylated form of this region (C1P$_1$P$_2$P$_3$C2) identified 3 cMyBPC clones (P213, C412 and P62) as putative interactors of domains C1 through C2. The clone encoding the largest peptide (C412) corresponded to a fragment representing from about midway through domain C5 to domain C10, the smallest clone (P62) represented an even more C-terminal region, encompassing midway through domain C8 through to domain C10, while P213 was intermediate in size, encoding region C7 through C10. Co-immunoprecipitation experiments using these N- and C-terminal fragments of cMyBPC showed that the trisphosphorylated bait C1P$_1$P$_2$P$_3$C2 had a greater affinity for the C-terminal cMyBPC clones C412 and P62 than did the unphosphorylated bait C1C2 (Figure 5). These results could be interpreted as follows: when the cMyBPC motif is phosphorylated, the C1 through C2 N-terminal domains make direct contact with C-terminal domains (observed as C1P$_1$P$_2$P$_3$C2 interacting strongly with C412 and P62, Figure 5), while, when the cMyBPC motif is unphosphorylated, an interaction between these domains and C-terminal domains is not favoured (observed as weak interactions of C1C2 with C412 and P62, Figure 5). Although domain C3 was not part of the constructs used in this assay, the interaction observed between domains C3 and C10 in direct interaction assays by De Lange suggests that interactions of phosphorylated N-terminal domains with C-terminal domains are not perfectly juxtaposed; thus, the

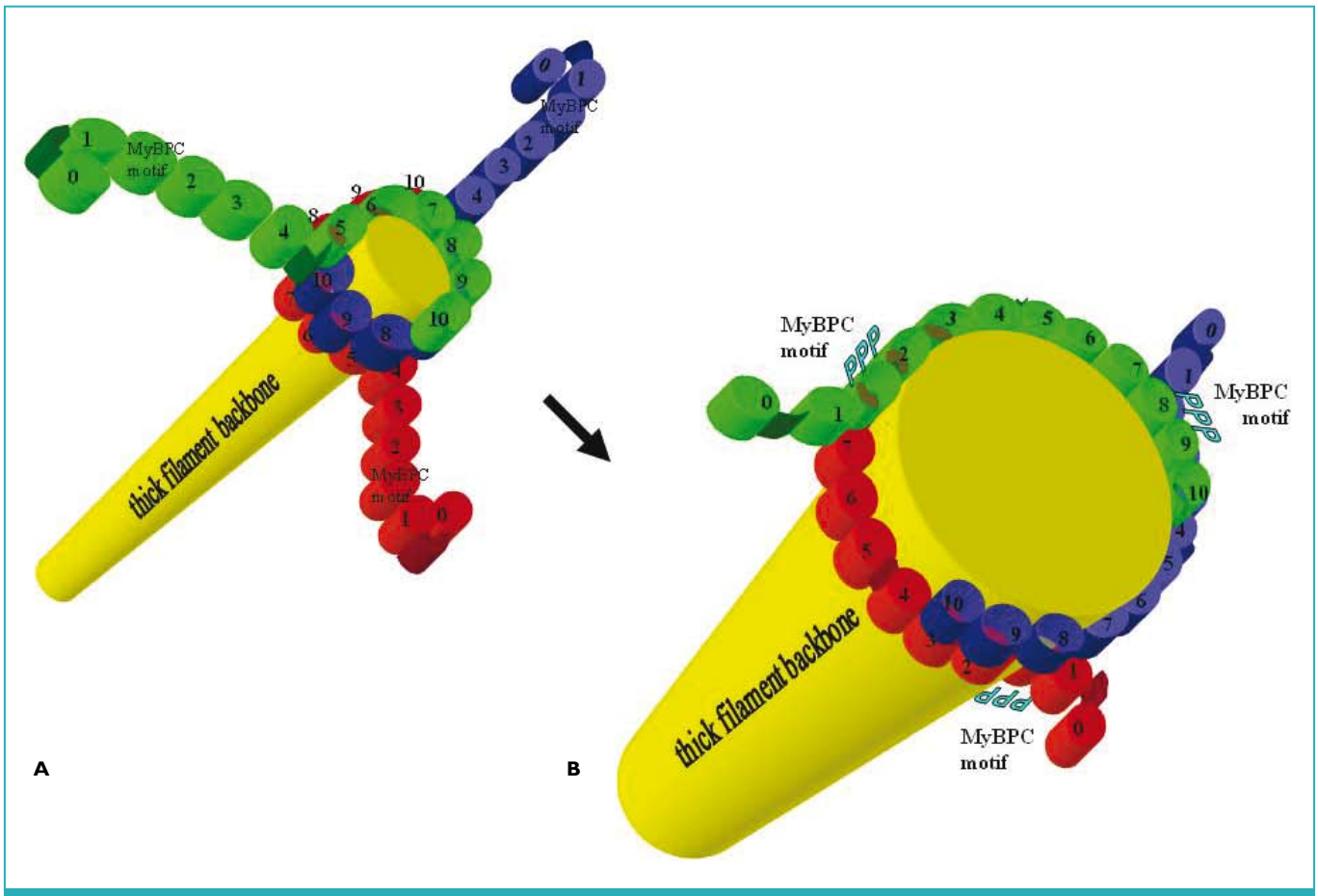

FIGURE 4: The hypothesised transition of the thick filament from the tight (A) to loose (B) structure upon β-adrenergic stimulation. Under non-stimulated conditions, the myosin rods in the thick filament (shown in yellow) are tightly wound and in this tight structure, three cMyBPC molecules wrap around the thick filament in such a way that domains C5:C8, C6:C9 and C7:C10 of successive cMyBPC molecules interact according to the collar model. Upon β-adrenergic stimulation and trisphosphorylation of the cMyBPC motif, the myosin rods of the thick filament adopts a loose structure with a wider diameter which is proposed to be facilitated by the widening of the cMyBPC collar. Thus, domains C2 or C3 may now interact with domain C10. Image A taken from Moolman-Smook[33] and image B from de Lange.[4]

region of interaction with domain C10 may be staggered and include part of domain C2. This differential affinity of the N-terminal domains of cMyBPC for C-terminal domains (depending on the phosphorylation status of the cMyBPC motif) lent support for a hypothesised transition from the "tight" to "loose" collar conformation upon PKA-dependent phosphorylation to accommodate changes in the thick filament. When it is in this loose conformation, the myosin heads enter into a weakly bound state with actin, which in turn determines how many crossbridges will enter into the crossbridge cycle. Thus, maximum phosphorylation of MyBPC leads to enhanced contractility and increased Fmax.

We have previously shown that the HCM-causing missense mutations Arg654His and Asp755Lys in domain C5 weaken the C5:C8 interaction, suggesting that the tight MyBPC collar may be unstable in their presence.[33] Similarly, we found that the Val894Met amino acid variation in C7 weakens its binding with C10 (Figure 6); this variant occurs in unaffected individuals too, but appears to exascerbate hypertrophy in individuals carrying another HCM-causing mutation.[34] Thus, it would appear that hypertrophy-associated variations in regions of cMyBPC that are involved in forming a tight trimeric collar may act by destabilising this collar formation, presumably promoting formation of the loose collar arrangement and promoting thick filament expansion. In this conformation, the formation of cross-bridges would be enhanced and the rate of ATP consumption in mutant sarcomeres increased. This therefore suggests that cMyBPC mutations, both those causing loss of the protein as well as those leading to defective proteins, fit into the same class of pathophysiological mechanism as HCM mutations in other genes that appear to act by increasing the cost

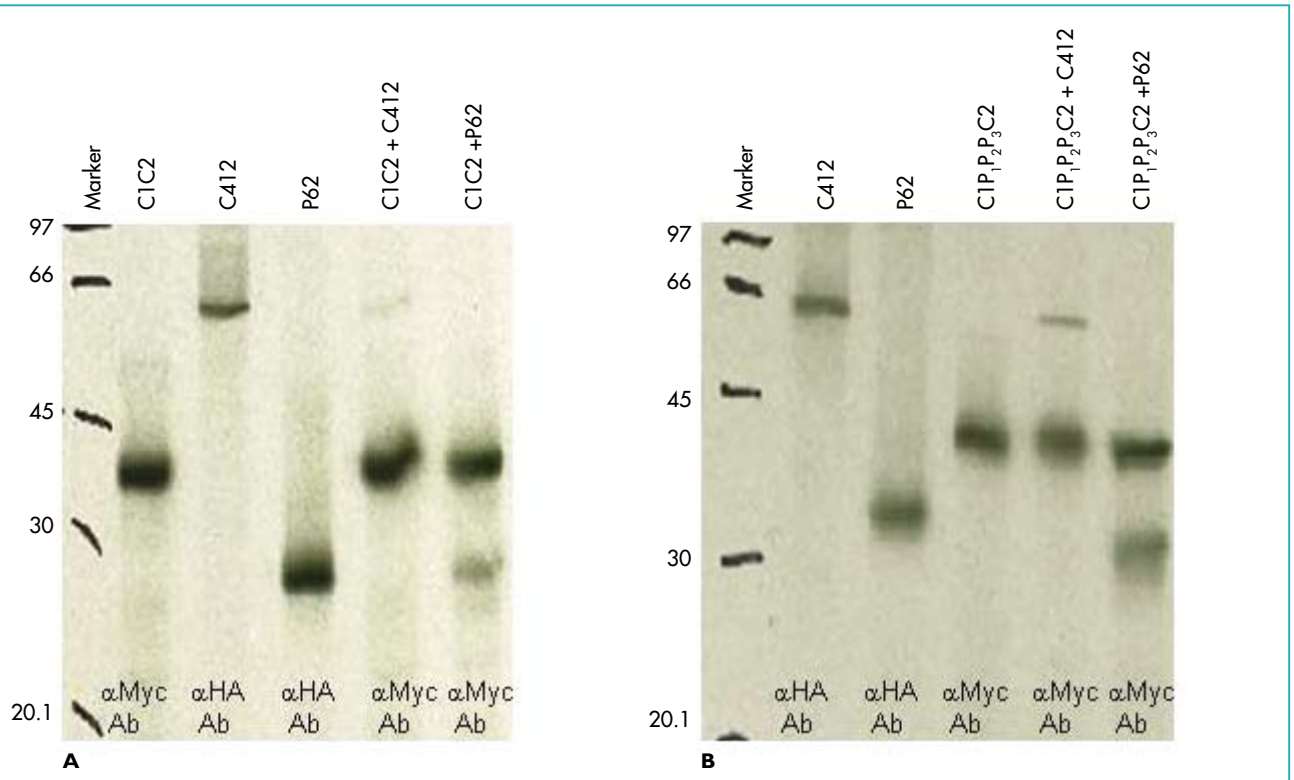

FIGURE 5: SDS-PAGE gel showing co-immuno precipitation reactions between cMyBPC clones (C412 and P62) and baits (C1C2 and $C1P_1P_2P_3C2$). Gels A and B show that cMyBPC clones interact much stronger with $C1P_1P_2P_3C2$ bait than the C1C2 bait. The antibody used for immunoprecipitation is shown at the bottom of the lane.

of force production, suggesting that an inability to maintain energy homeostasis may underly many, if not all, forms of HCM.[32,44]

In summary, closer investigation of this little understood protein of the sarcomere, driven by its role in HCM, has revealed one of the most potent regulators of cardiac contractility. Phosphorylation of its N-terminus upon adrenergic stimulation, causes increased order in myosin heads as well as increased ATPase activity, F_{max} and Ca^{2+} sensitivity of contraction,[38] while its dephosphorylation upon cholinergic stimulation or during low flow ischaemia leads to changes in the sarcomeric thick filament that diminish interaction between myosin heads and actin. This dynamic flux in phosphorylation upon adrenergic stimulation is not only crucial to normal cardiac function and structure, but also vital for protection against ischaemic injury.[46] Genetically-driven deficiency or inadequacy in cMyBPC leads to severe cardiac dysfunction and structural changes, including cardiac hypertrophy and dilation, and particularly attenuates the adaptive increase in left ventricular contractility that follows on B-adrenergic stimulation or pressure overload,[2] resulting in decreased systolic function, and reduced cardiac output.

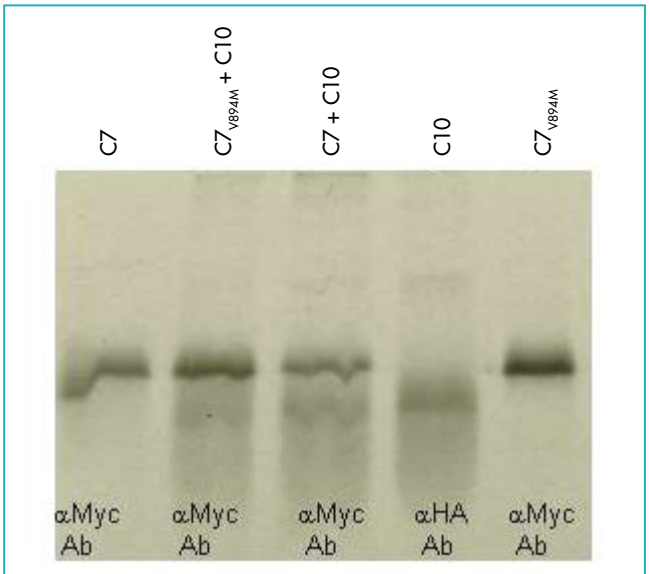

FIGURE 6: SDS-PAGE gel showing co-immunoprecipitation reactions between cMyBPC domains C7 and C10, as well as the effect of the Val894Met variant in C7 on this interaction. The Val894Met variant appears to reduce the affinity of these two domains for each other, as evidenced by the lighter C10 band. The antibody used for immunoprecipitation is shown at the bottom of the lane.

REFERENCES

1. Ababou A, Zhou L, Gautel M, et al. Sequence specific assignment of domain C1 of the N-terminal myosin-binding site of human cardiac myosin binding protein C (MyBP-C). J Biomol NMR. 2004;29:431-432.
2. Brickson S, Fitzsimons DP, Pereira L, et al. In vivo left ventricular functional capacity is compromised in cMyBPC-null mice. Am J Physiol Heart Circ Physiol. 2007;292:1747-1754.
3. Carrier L, Knöll R, Vignier N, et al. Asymmetric septal hypertrophy in heterozygous cMyBP-C null mice. Cardiovasc Res. 2004;63:293-304.
4. de Lange WJ. An investigation of myosin binding protein C mutations in South Africa and a search for ligands binding to myosin binding protein C. PhD Thesis. 2004;University of Stellenbosch.
5. Dhoot GK, Perry SV. Expression of slow skeletal myosin binding C-protein in normal adult mammalian heart. J Muscle Res Cell Motil. 2005;26:143-148.
6. El-Armouche A, Boknik P, Eschenhagen T, et al. Molecular determinants of altered Ca^{2+} handling in human chronic atrial fibrillation. Circulation. 2006;114:670-680.
7. El-Armouche A, Pohlmann L, Schlossarek S, et al. Decreased phosphorylation levels of cardiac myosin-binding protein-C in human and experimental heart failure. J Mol Cell Cardiol. 2007;43:223-229.
8. Flashman E, Redwood C, Moolman-Smook J, et al. Cardiac myosin binding protein C. Its role in physiology and disease. Circ Res. 2004;94:1279-1289.
9. Flavigny J, Souchet M, Sebillon P, et al. COOH-terminal truncated cardiac myosin-binding protein C mutants resulting from familial hypertrophic cardiomyopathy mutations exhibit altered expression and/or incorporation in fetal rat cardiomyocytes. J Mol Biol. 1999;294:443-456.
10. Freiburg A, Gautel M. A molecular map of the interactions between titin and myosin binding protein C. Implications for sarcomeric assembly in familial hypertrophic cardiomyopathy. Eur J Biochem. 1996;235:317-323.
11. Gautel M, Zuffard O, Freiburg A, et al. Phosphorylation switches specific for the cardiac isoform of myosin binding protein-C: a modulator of cardiac contraction? EMBO J. 1995;14:1952-1960.
12. Gautel M, Fürst DO, Cocco, A et al. Isoform transitions of the myosin binding protein C family in developing human and mouse muscles. Circ Res. 1998;82: 124-129.
13. Gruen M, Prinz H, Gautel M. cAPK-phosphorylation controls the interaction of the regulatory domain of cardiac myosin binding protein C with myosin-S2 in an on-off fashion. FEBS Lett. 1999;453:254-259.
14. Harris SP, Bartley CR, Hacker TA, et al. Hypertrophic cardiomyopathy in cardiac myosin binding protein-C knockout mice. Circ Res. 2002;90:594-601.
15. Hartzell HC, Titus L. Effects of cholinergic and adrenergic agonists on phosphorylation of a 165, 000-dalton myofibrillar protein in intact cardiac muscle. J Biol Chem. 1982;257:2111-2120.
16. Herron TJ, Korte FS, McDonald KS. Power output is increased after phosphorylation of myofibrillar proteins in rat skinned cardiac myocytes. Circ Res. 2001;89:1184-1190.
17. Herron TJ, Rostkova E, Kunst G, et al. Activation of myocardial contraction by the N-terminal domains of myosin binding protein-C. Circ Res. 2006;98:1290-1298.
18. Hofmann PA, Greaser M, Moss RL. C-protein limits shortening velocity of rabbit skeletal muscle fibres at low levels of Ca^{2+} activation. J Physiol. 1991;439:701-715.
19. Huxley HE. Electron microscope studies on the structure of natural and synthetic protein filaments from striated muscle. J Mol Biol. 1963;7:281-308
20. Jeffries CM, Whitten AE, Harris SP, et al. Small-angle X-ray scattering reveals the N-terminal domain organisation of cardiac myosin binding protein C. J Mol Biol. 2008;377:1186-1199.
21. Kögler H. The role of cardiac myosin binding protein-C as a regulator of myofilament Ca^{2+} sensitivity. Cardiovasc Res. 2006;69:304-306.
22. Korte FS, McDonald KS, Harris SP, et al. Loaded shortening, power output, and rate of force redevelopment are increased with knockout of cardiac myosin binding protein-C. Circ Res. 2003;93:752-758.
23. Kulikovskaya I, McClellan G, Flavigny J, et al. Effect of MyBP-C binding to actin on contractility in heart muscle. J Gen Physiol. 2003a;122:761-774.
24, Kulikovskaya I, McClellan G, Levine R, et al. Effect of extraction of myosin binding protein C on contractility of rat heart. Am J Physiol Heart Circ Physiol. 2003b;285: H857-H865.
25. Kulikovskaya I, McClellan G, Levine R, et al. Multiple forms of cardiac myosin-binding protein C exist and can regulate thick filament stability. J Gen Physiol. 2007;129:419-428.
26. Levine R, Weisberg A, Kulikovskaya I, et al. Multiple structures of thick filaments in resting cardiac muscle and their influence on cross-bridge interactions. Biophys J. 2001;81:1070-1082.
27. Lohse MJ, Engelhardt S, Eschenhagen T. What is the role of beta-adrenergic signaling in heart failure? Circ Res. 2003;93:896-906.
28. Maw MC, Rowe AJ. The reconstruction of myosin filaments in rabbit psoas muscle from solubilised myosin. J Muscle Res Cell Motil. 1986;7:97-109.
29. McClellan G, Kulikovskaya I, Winegrad S. Changes in cardiac contractility related to calcium-mediated changes in phosphorylation of myosin-binding protein C. Biophys J. 2001;81:1083-1092.
30. McConnell BK, Jones KA, Fatkin D, et al. Dilated cardiomyopathy in homozygous myosin-binding protein-C mutant mice. J Clin Invest. 1999;104:1235-1244.
31. Metzger JM, Westfall MV. Covalent and noncovalent modification of thin filament action. The essential role of troponin in cardiac muscle regulation. Circ Res. 2004;94:146-158.
32. Montgomery DE, Tardiff JC, Chandra M. Cardiac troponin T mutations: correlation between the type of mutation and the nature of myofilament dysfunction in transgenic mice. J Physiol 2001; 536:583-592.
33. Moolman-Smook JC, Flashman E, de Lange W, et al. Identification of novel interactions between domains of myosin binding protein-C that are modulated by hypertrophic cardiomyopathy missense mutations. Circ Res. 2002;91:704-711.
34. Moolman-Smook JC, Mayosi B, Brink P, et al. Identification of a new missense mutation in MyBP-C associated with hypertrophic cardiomyopathy. J Med Genet. 1998;35:253-254.
35. Moos C, Mason CM, Besterman JM, et al. 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.

36. Moos C, Feng IN. Effect of C-protein on actomyosin ATPase. Biochem Biophys Acta. 1980;632:141-149.
37. Oakley CE, Hambly BD, Curmi PM, et al. Myosin binding protein C: structural abnormalities in familial hypertrophic cardiomyopathy. Cell Res. 2004;14:95-110.
38. Oakley CE, Chamoun J, Brown LJ, et al. Myosin binding protein-C: enigmatic regulator of cardiac contraction. Int J Biochem Cell Biol. 2007;39:2161-2166.
39. Okagaki T, Weber FE, Fischman DA, et al. The major myosin-binding domain of skeletal muscle MyBP-C (C-protein) resides in the COOH-terminal, immunoglobulin C2 motif. J Cell Biol. 1993;123:619-626.
40. Palmer BM, McConnell BK, Li GH, et al. Reduced cross-bridge dependent stiffness of skinned myocardium from mice lacking cardiac myosin binding protein-C. Mol Cell Biochem. 2004a;263:73-80.
41. Palmer BM, Georgakopoulos D, Janssen PM, et al. Role of cardiac myosin binding protein C in sustaining left ventricular systolic stiffening. Circ Res. 2004b;94:1249-1255.
42. Palmer BM, Noguchi T, Wang Y, et al. Effect of cardiac myosin binding protein-C on mechanoenergetics in mouse myocardium. Circ Res. 2004c;94:1615-1622.
43. Razumova MV, Shaffer JF, Tu A, et al. Effects of the N-terminal domains of myosin binding protein-C in an in vitro motility assay. J Biol Chem. 2006;281:35846-35854.
44. Redwood C, Moolman-Smook J, Watkins H. Properties of mutant contractile proteins that cause hypertrophic cardiomyopathy. Cardiovasc Res. 1999;44:20-36.
45. Sadayappan S, Gulick J, Osinka H, et al. Cardiac myosin-binding protein-C phosphorylation and cardiac function. Circ Res. 2005;97:1156-1163.
46. Sadayappan S, Osinka H, Klevitsky R, et al. Cardiac myosin binding protein C phosphorylation is cardioprotective. Proc Natl Acad Sci USA. 2006;103:16918-16923.
47. Schlender KK, Hegazy MG, Thysseril TJ. Dephosphorylation of cardiac myofibril C-protein by protein phosphatase 1 and protein phosphatase 2A. Biochim Biophys Acta. 1987;928:312-319.
48. Schlender KK, Bean LJ. Phosphorylation of chicken cardiac C-protein by calcium/calmodulin-dependent protein kinase II. J Biol Chem. 1990;266:2811-2817.
49. Sébillon P, Bonne G, Flavigny J, et al. COOH-terminal truncated human cardiac MyBP-C alters myosin filament organisation. C R Acad Sci III. 2001;324:251-260.
50. Shaffer JF, Razumova MV, Tu A et al. Myosin S2 is not required for effects on myosin binding protein-C on motility. FEBS Lett. 2007;581:1501-1504.
51. Solaro J, Van Eyk J. Altered interaction among thin filament proteins modulate cardiac function. J Mol Cell Cardiol. 1996;28:217-230.
52. Squire JM, Luther PK, Knupp C. Structural evidence for the interaction of C-protein (MyBP-C) with actin and sequence identification of a possible actin-binding domain. J Mol Biol. 2003;331:713-724.
53. Starr R, Offer G. Polypeptide chains of intermediate molecular weight in myosin preparations. FEBS Lett. 1971;15:40-44.
54. Stelzer JE, Fitzsimons DP, Moss RL. Ablation of myosin-binding protein-C accelerates force development in mouse myocardium. Biophys J. 2006a;90:4119-4127.
55. Stelzer JE, Dunning SB, Moss RL. Ablation of cardiac myosin-binding protein-C accelerates stretch activation in murine skinned myocardium. Circ Res. 2006b;98:1212-1218.
56. Stelzer JE, Patel JR, Walker JW, et al. Differential roles of cardiac myosin-binding protein C and cardiac troponin I in the myofibrillar force responses to protein kinase A phosphorylation. Circ Res. 2007;101:503-511.
57. Vatner DE, Sato N, Ishikawa Y, et al. Beta-adrenoreceptor desensitisation during the development of canine pacing-induced heart failure. Clin Exp Pharmacol Physiol. 1996;23:668-692.
58. Venema RC, Kuo JF. Protein kinase C-mediated phosphorylation of troponin I and C-protein in isolated myocardial cells is associated with inhibition of myofibrillar actomyosin MgATPase. J Biol Chem. 1993;4:2705-2711.
59. Weisberg A, Winegrad S. Relation between crossbridge structure and actomyosin ATPase activity in rat heart. Circ Res. 1998;83:60-72.
60. Winegrad S. Cardiac myosin binding protein C. Circ Res. 1999;84:1117-1126.
61. Witt CC, Gerull B, Davies MJ, et al. Hypercontractile properties of cardiac muscle fibers in a knock-in mouse model of cardiac myosin-binding protein-C. J Biol Chem. 2001;276:5353-5359.
62. Yamamoto K. The binding of skeletal muscle C-protein to regulated actin. FEBS Lett. 1986;208:123-127.
63. Yang Q, Sanbe A, Osinka H, et al. A mouse model of myosin binding protein C human familial hypertrophic cardiomyopathy. J Clin Invest. 1998;102:1292-1300.
64. Yang Q, Sanbe A, Osinka H, et al. In vivo modeling of myosin binding protein C familial hypertrophic cardiomyopathy. Circ Res. 1999;85:841-847.
65. Yang Q, Osinka H, Klevitsky R, et al. Phenotypic deficits in mice expressing a myosin binding protein C lacking the titin and myosin binding domains. J Mol Cell Cardiol. 2001;33:1649-1658.