Published in final edited form as:
Pflugers Arch. 2014 February ; 466(2): 195–200. doi:10.1007/s00424-013-1396-8.

Cardiac myosin binding protein-C as a central target of cardiac sarcomere signaling: a special mini-review series

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Abstract

Cardiac myosin binding protein-C (cMyBP-C) is a cardiac-specific thick filament assembly, accessory and regulatory protein. Physiologically, it is a key regulator of cardiac contractility. With more than two hundred mutations in the cMyBP-C gene directly linked to the development of cardiomyopathy and heart failure, cMyBP-C clearly plays a critical role in heart function. At baseline, cMyBP-C is highly phosphorylated, a condition required for normal cardiac function. However, the level of cMyBP-C phosphorylation is significantly decreased during heart failure, indicating that the level of cMyBP-C phosphorylation is directly linked to signaling and cardiac function. Early studies indicated that cMyBP-C interacts with myosin and titin, whereas studies now show that it also interacts with thin filament proteins. However, the exact role(s) of cMyBP-C in the heart remain(s) to be elucidated. As such, we invited experts in the field of cardiac muscle to identify and address key issues related to cMyBP-C by contributing a mini-review on such topics as structure, function, regulation, cardiomyopathy, proteolysis, and gene therapy. Starting from this issue, *Pflügers Archiv European Journal of Physiology* will publish two invited mini-review articles each month to discuss the most recent advances in the study of cMyBP-C.

Keywords

MYBPC3; cMyBP-C; Cardiac myosin binding protein-C; Cardiomyopathy; Cardiac biomarker; Heart failure; Phosphorylation; Sarcomeric proteins

Discovery of cMyBP-C

Similar to a piston in a car engine, cardiac sarcomeres are composed of long, fibrous proteins that slide past each other when the muscles contract and relax. With every heartbeat, the sarcomere relaxes during diastole to fill the heart with blood in both ventricles and then contracts during systole to pump out blood to the lungs and the rest of the body. This is a cycle that never stops until death. It is estimated that the human heart pumps about 7,570 liters and beats approximately 100,000 times a day in this systematic manner. The pump rate also responds to various conditions, such as physiological exercise, medications, diet and age, indicating that the cardiac sarcomere responds to these signals and, correspondingly, regulates cardiac output. Cardiac myofibrils contain distinct and repeated

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sarcomeres that are surrounded by mitochondria and cytoplasm in the cardiomyocyte cell. A single sarcomere is defined as the region between two Z-lines ranging from 1.6 to 2.4 μm in sarcomere length (SL). Each sarcomere is made of thick and thin filament proteins, myosin and actin, respectively. Interaction between myosin and actin results in force development and SL shortening. To facilitate myosin and actin interactions, other accessory proteins are present in the thick and thin filaments (Figure 1A). Thin filament proteins are composed of actin, α-tropomyosin and the troponin complex, including C, I and T units while thick filament proteins consist of titin, myosin and cardiac myosin binding protein-C (cMyBP-C). cMyBP-C is a thick filament accessory protein that regulates muscle contraction. Recently, cMyBP-C has received increased attention among cardiovascular scientists who are investigating the exact role of this protein in muscle contraction [46]. G. Offer et al. initially discovered MyBP-C of the cardiac isoform in 1973 as an impurity in skeletal muscle myosin preparations [44]. cMyBP-C was later discovered in cardiac myosin preparations in 1976 [41] and characterized in 1983 [70]. Mathias Gautel and Saul Winegrad opened up the cMyBP-C field by discovering the structural and functional roles of cMyBP-C in cardiac muscle. Their earlier contributions are the building blocks for the latest discoveries (reviewed in the early 2000s [65–68,4]). Following this, several mini-reviews appeared in mid 2000s [15,43,42,9,47,21,60], and over the last five years, many more reviews have reported on the roles of cMyBP-C in the heart [37,51,3,48,12,25,5,52,45,32,30,36,27,1,38,8]. Today, cMyBP-C has been studied in various aspects, including hypertrophic cardiomyopathy, gene therapy, posttranslational modifications, and sarcomere structure and function in both health and disease. It is also a biomarker of myocardial infarction [20] and autoimmune-induced myocarditis [29]. The cMyBP-C field has advanced using the latest technologies, which have raised many new questions. Accordingly, this special series of *Pflügers Archiv European Journal of Physiology* aims to publish each month two invited mini-review articles from experts in the cMyBP-C field.

Arrangement of cMyBP-C in the sarcomere

cMyBP-C is a large 150 kDa molecular weight protein that belongs in the intracellular immunoglobulin (Ig) superfamily. It is composed of eight Ig and three fibronectin type-3 repeating domains (Figure 1B). cMyBP-C differs from its two skeletal isoforms by having a proline-alanine-rich (Pro-Ala) region located between domains C0 and C1, a phosphorylatable motif (M-motif), which lies between the C1 and C2 domains in the N-terminal region as well as a C0 domain and a small insertion within the C5 domain. It is exclusively expressed in the heart, not in skeletal muscle [34]. Electron micrographs determined that cMyBP-C specifically localizes in the C-zone of the A-band in the sarcomere on both sides of the M-line. cMyBP-C is arranged in the C-zone to run vertically through thick and thin filaments as stripes. There are 9 stripes in each C-zone in the cardiac sarcomere. Importantly, a ratio of 1 cMyBP-C to 3 myosins in the C-zone is present. The literature describes three different models of cMyBP-C arrangement, indicating a lack of scientific consensus on this issue. Some X-ray diffraction and electron tomography studies on cMyBP-C arrangement and interactions are available in the literature. **Roger Craig, Pradeep P. Luther and their groups** have extensively focused on studying the structural arrangement of cMyBP-C in the sarcomere and its binding to thin filaments. In this mini-review series, they will discuss key features of cMyBP-C structure, its 3D organization in the sarcomere, and its interaction with the thin filaments.

Structure of cMyBP-C

No crystal structures are available for cMyBP-C, which is a major weakness in the cMyBP-C field. However, some secondary structures of its N′ region are available by nuclear

magnetic resonance (NMR) studies using C0, C1, M and C2 domains. Atomic force microscopy studies are also available to determine the order of N-terminal domain arrangement. In the mini-series, **Natosha L. Finely** will review the current literature reporting on the structure of cMyBP-C and its dynamic changes in relation to posttranslational modifications (PTM). This area of research should be promoted in future studies to provide a complete understanding of the structure and, hence, regulation of cMyBP-C.

MYBPC3 and Genetic Diseases

Mutations in the cMyBP-C gene (MYBPC3) cause hypertrophic and dilated cardiomyopathy [62,7]. Thus far, more than 200 mutations in MYBPC3 have been identified as causes of sarcomeric disease [25]. Among them, a polymorphic 25-bp deletion mutation was reported to be highly prevalent and associated with cardiomyopathies and heart failure [33]. This report revealed the alarming discovery that this particular mutation is inherited in more than 60 million South Asian descendants worldwide, underscoring the necessity of studying this specific mutation. In the MYBPC3 mini-series, effects of MYBPC3 gene mutations on sarcomere structure and function (**Jolanda van der Velden**), diagnostic and cellular functions (**Sakthivel Sadayappan**), gene corrections (**Lucie Carrier**) and gene therapy (**Julian E. Stelzer**) are reviewed.

Physiological role of cMyBP-C in the heart

cMyBP-C expression is not essential for heart formation, but essential for normal function. cMyBP-C knockout in mouse models results in altered sarcomere structure, altered contractile function, cardiac hypertrophy and severe heart failure, indicating the importance of cMyBP-C for baseline cardiac function [24,59,39]. However, the role of cMyBP-C in sarcomere function still remains unclear. Richard L. Moss and his former trainees have spent many years determining the exact role of cMyBP-C in muscle physiology. cMyBP-C was originally identified as one of the myosin binding proteins. However, it was then discovered that it not only binds with myosin, but also titin, actin, regulatory light chain and now α-tropomyosin (Sadayappan S, unpublished data). In this mini-review series, **Richard L. Moss, Samantha P. Harris** and **Carl W. Tong** discuss the role of cMyBP-C in cardiac function. Recently, **David M. Warshaw's** group has determined that cMyBP-C acts as a tether to control myosin-actin interaction, which was published in *Science* [46]. Dr. Warshaw and his team are contributing a mini-review discussing the molecular modulation of actomyosin function by cMyBP-C.

Posttranslational modifications of cMyBP-C

Heart failure (HF) afflicts about five million people in the U.S. each year at an estimated cost of $30 billion [56]. In HF patients, increased sympathetic nervous activity compensates for reduced cardiac function. Acute β-AR stimulation leads to positive inotropic and lusitropic responses, an increase in chronotropic response and a decrease in blood pressure. β-AR antagonists, such as β-blockers, are often used to treat β-AR activation to reduce the positive inotropic and lusitropic responses during early onset of β-AR activation. Cardiac contractility is regulated by the β-AR system, and the activation of these receptors induces chronotropic, lusitropic and inotropic effects. Activated β-AR transduces its signal by activating adenylyl cyclase and, subsequently, cyclic AMP-mediated protein kinase, which promotes maximal myocardial contractility. However, prolonged sympathetic stimulation can lead to HF. Compared to the skeletal forms of MyBP-C, cardiac MyBP-C has unique multiple phosphorylation sites within the M-domain, such as Ser-273, Ser-282, Ser-302 and Ser-307 (Mouse, Uniprot ID O70468). The multiple phosphorylation sites are targeted by

protein kinase A [18,40], protein kinase C [40,69], CaMKII [18,49], protein kinase D [2] and ribosomal s6 kinase [14], affecting sarcomere structure and function. Recently, it was further shown that GSK3β phosphorylates cMyBP-C at Ser-133[54], which is positioned in the proline-alanine-rich region and increases kinetics of force development. cMyBP-C also undergoes other posttranslational modifications in addition to phosphorylation, including acetylation [61], glutathionylation [55,35,28], citrullination [6] and O-GlcNAcylation [58], suggesting that cMyBP-C is a central downstream target of signaling in the sarcomere. The N-terminus (C1-M-C2 domains) also binds to the S2 segment of myosin [17,22,63,65,66], close to the lever arm domain. As such, interaction can be dynamically regulated by phosphorylation and dephosphorylation of cMyBP-C [23]. Phosphorylation of cMyBP-C at Ser-273, Ser-282 and Ser-302 in the M-motif is essential for normal cardiac function [50,57]. However, dephosphorylation at these sites is directly associated with heart failure [50,3] and cMyBP-C degradation [10,11,20]. Physiologically, cMyBP-C phosphorylation plays a major role in regulating myosin-actin interactions, which is dynamically regulated in an on-off fashion by phosphorylation within the M domain. Phosphorylation by PKA inhibits the ability of the N-terminal region of cMyBP-C to bind actin, bind myosin-S2, or alter myofilament Ca^{2+} sensitivity [23,26,53]. However, it is still unclear whether phosphorylation of Ser-273, Ser-282 and Ser-302 is necessary and sufficient to regulate cardiac contractility. In the mini-review series, **Jeffrey Robbins** will provide an extensive discussion of cMyBP-C posttranslational modifications and their regulation in the heart.

Prognostic and diagnostic values of cMyBP-C release in the blood

Following myocardial infarction, total phosphorylation of cMyBP-C is decreased, most notably in the tri-phosphorylated species (Ser-273, Ser-282, Ser-302), which is accompanied by contractile dysfunction and increased cleavage of cMyBP-C [10,16]. Previously, studies showed that during myocardial injury in mice, rats, and humans, a predominant N-terminal 40 kDa fragment of cMyBP-C is readily detectable in the circulatory system and is strongly correlated with contractile dysfunction [51,20]. Mass spectrometry analyses show that the 40 kDa protein is generated by cleaving cMyBP-C at 272-TSLAGAGRRTS, near a conserved phosphorylation motif [51]. The resulting 40 kDa N′-fragment contains the C0 and C1 domains, plus the first 17 residues of the M-domain (C0C1f). Because the 40 kDa fragment (C0-C1-17 residues of the M domain) retains strong interaction with actin, but lacks the phosphorylation sites necessary for phosphorylation-dependent on-off interaction with myosin and actin, the presence of the 40 kDa N′-fragment may alter actin-myosin interaction by constitutively interacting with actin, in turn having detrimental consequences on sarcomeric function [64]. Furthermore, recent studies demonstrated that plasma cMyBP-C levels were elevated significantly in a rat MI model and patients with MI [20], suggesting that plasma cMyBP-C level could be a potential diagnostic biomarker of MI [48] and prognostic marker of heart failure. **Sakthivel Sadayappan** and his lab are actively engaged in determining the pathological properties of cMyBP-C degradation and the potential use of cMyBP-C in the blood as a biomarker of myocardial infarction [48,20,13,19,31].

In conclusion, the special series of *Pflügers Archiv - European Journal of Physiology* provides readers with up-to-date information in the cMyBP-C field and suggests areas for future investigation, thus encouraging collaborations among those primarily engaged in the field. The ultimate goal of cardiovascular science today is complete prevention or rescue of HF in patients. Aiming toward this goal, we propose that determining the structure, arrangement, regulation and function of cMyBP-C is critically important and will have more emphasis in future studies.

Acknowledgments

The authors were supported by National Institutes of Health grants R01HL105826 and K02HL114749 (SS), HL101297, HL75494 and HL62426 (PPdT).

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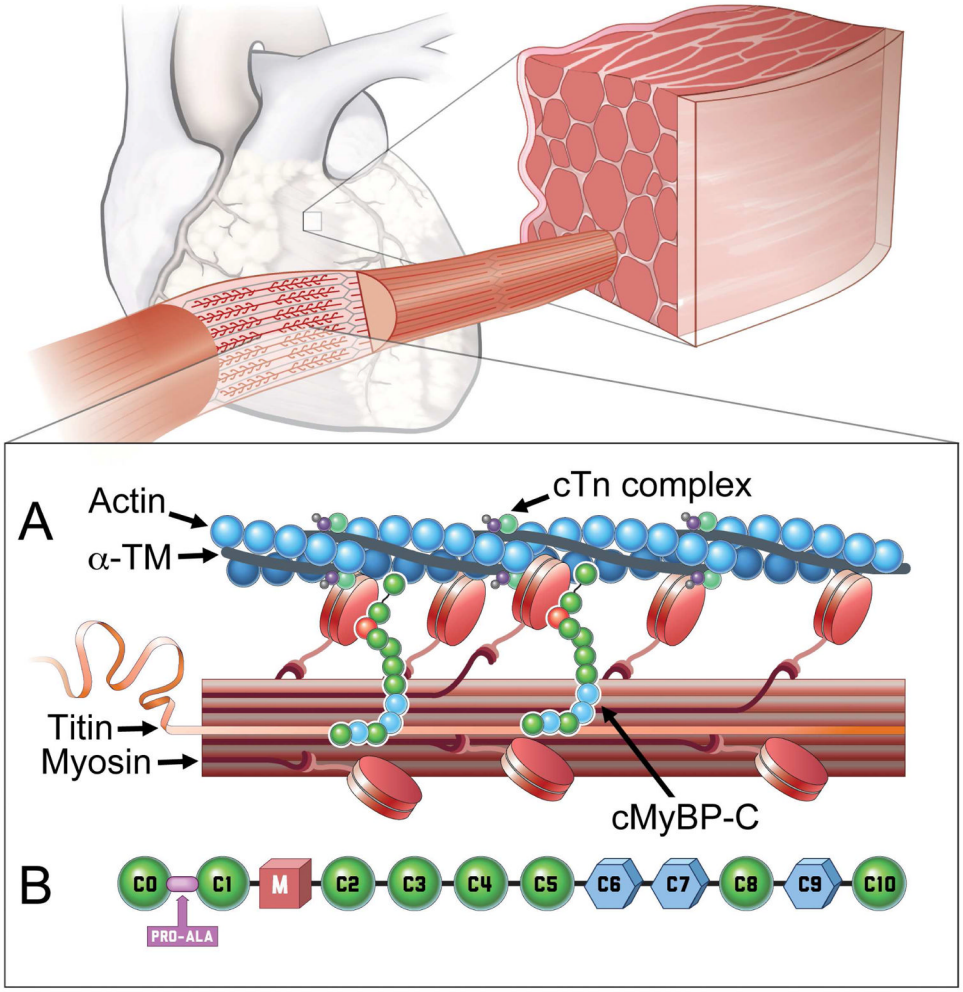

Figure 1. Schematic diagram of cMyBP-C localization in the heart

Cardiac striated muscle in the myocardium is predominantly made up of myofibrils. (**A**) The sarcomere, which is the functional unit of myofibrils, consists of thick and thin contractile filament proteins. The thick filament proteins are titin, myosin and cMyBP-C, whereas the thin filament proteins are actin, α-TM, cTnT, cTnI and cTnC. The cMyBP-C protein is exclusively expressed in the heart, and it transversely connects both thick and thin filament proteins. (**B**) cMyBP-C is comprised of 8 immunoglobulin (Ig) and 3 fibronectin type III domains, numbered from the N-terminus as Motifs 0 to 10. In addition, a proline-alanine-rich region is found between the C0 and C1 domains, as well as a phosphorylation motif (M domain), which is found between the C1 and C2 domains. N-terminal regions (C0-C2 domains) interact with thin filament proteins such as actin and thick filament proteins such as myosin S2. C-terminal regions (C8-C10 domains) interact with the thick filament protein titin and the LMM hinge regions.