Structure-Function Relationships in Myosin Binding Protein-C

Taking Off the Blinders and Collaring Hypertrophic Cardiomyopathy

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Myosin binding protein-C (MyBP-C), a major component of the thick filament, binds to both the myosin (thick) and titin filament systems through defined domains and is believed to make an important contribution to sarcomere structure. Discovered over 27 years ago, interest in the protein's role(s) intensified after familial hypertrophic cardiomyopathy (FHC) was linked to chromosome 11p13-q13, where cardiac MyBP-C (MYBPC3) is encoded. Reports of MYBPC3 mutations in FHC appeared a short time later. Over 30 heterozygous MYBPC3 mutations have been identified in subsequent studies; less than half have been missense mutations, whereas the remainder are nonsense mutations predicted to result in truncated polypeptides. Age-dependent penetrance is a characteristic feature of the MyBP-C phenotype. For example, hypertrophy is not evident in nearly 50% of mutant carriers before the age of 40 years, whereas by 60 years, hypertrophy occurs in more than 90%. These patients generally have favorable survival when compared to individuals with mutations in other genes causing FHC, but the MyBP-C mutations can result in sudden cardiac-related death.

MyBP-C is localized in the inner two-thirds of the A band, the so-called C zone (Figure). Negative staining and optical diffraction techniques reveal 11 transverse bands that are approximately 60 nm wide. Antibody staining shows that MyBP-C is restricted to the outer 7 to 9 axial bands, which are spaced approximately 43 nm apart in each half-sarcomere. This particular organization implies some functional sequelae: MyBP-C can only interact with certain myosin molecules and these, in turn, will interact with the thin filament only when the sarcomere is shortened. Thus, (one of) the role(s) of MyBP-C may come into play only when the central part of the sarcomere undergoes thick-thin filament interaction: that is during contraction, when the myosin-actin interaction actually extends into the central core of the A band.

As noted above, multiple mutations, both missense but more commonly, nonsense mutations that result in truncated proteins have been found in patients suffering from FHC, and both gain- and loss-of-function studies performed in mice have shown these mutations are causative. The animal models, although not exactly mimicking the human pathology, have shed light on the resultant fates of the different mutated proteins, and both altered incorporation of mutant protein and the lack of appreciable amounts of truncated MyBP-C polypeptides have been observed.

Despite our knowledge of the gene and protein structure, its abundance as a sarcomeric protein, and the commonality of mutations within the gene causing significant heart disease, the function or functions of MyBP-C are still obscure. Like other myosin-binding proteins and titin, MyBP-C belongs to the intracellular immunoglobulin (Ig) superfamily and is composed of repeated Ig and fibronectin domains (Figure). As is the case for many sarcomeric proteins, multiple genes are present and these encode isoform-specific protein species: for MyBP-C, three unique genes encode the fast and slow skeletal proteins, as well as the cardiac isoform. In vitro modeling, fiber reconstitutions, and cell transfections all have yielded data consistent with the importance of MyBP-C in assembling and maintaining the overall architecture of the sarcomere. For example, Winegrad and coworkers showed that phosphorylation of MyBP-C can change the overall extension of the thick filament, and has the net effect of increased order. This in turn may change the orientation or flexibility of the cross bridges and affect the intrinsic rate constants that govern the on/off rates of the actomyosin complex. Additional data show that MyBP-C phosphorylation can change both the orientation and contractile mechanics of filaments. Interestingly, the cardiac isoform is phosphorylated by both PKA as well as CaM kinase. In the cardiac isoform specifically, the MyBP-C motif (Figure) can be phosphorylated at three residues, and in vitro biochemical studies show that phosphorylation abolishes the [MyBP-C]-[myosin S2] interaction, subsequently modulating contractility. As cardiac MyBP-C can be phosphorylated in response to β-adrenergic stimulation and this is accompanied by increases in systolic tension development, perhaps MyBP-C plays a critical role in the overall probability of the initial cross-bridge attachment to thin filaments (weak attachment). This in turn could lead to major changes in the numbers of cross bridges that actually generate force (strong attachment) during activation of contraction.

A logical point of departure for understanding how mutations at particular residues might cause disease is to explore the roles the altered protein or domain might play in protein-protein interactions. In this issue of Circulation Research,
Moolman-Smook et al. focus on the C5 domain (one of 14 well-defined domains in the molecule, see Figure) for a number of very good reasons: (1) it is a relative “hot-spot” for the subclass of FHC-causing MyBP-C missense mutations, so that the results of a single amino acid change, rather than a truncation of multiple domains could be studied; (2) it contains a 28–amino acid insert that is unique to the cardiac isoform; and (3) this cardiac-specific region is flanked by mutations that result in FHC, implying its general importance in the function(s) of the molecule. The authors thus used the C5 domain as “bait” in a yeast two-hybrid assay and found that they frequently “caught” fragments corresponding to the C-terminal 608 amino acids of MyBP-C itself. Deletion mapping quickly linked the responsible sequence to C8, another immunoglobulin-like domain. Model building on the basis of the C5-C8 interaction, as well as a reading of the literature, prompted the authors to hypothesize that the C7 domain of one MyBP-C might interact with the C10 domain of another and a subsequent yeast two-hybrid experiment with C7 confirmed the specificity of this interaction as well.

Flanking the cardiac-specific insertion in C5 are two missense mutations, R654H and N755K, which have been linked to FHC (Figure). Using surface plasmon resonance to measure affinity constants in vitro, the authors found that although C5 bound to C8 with a $K_a \approx 1 \times 10^5 \text{ mol/L}^{-1}$, C5R654H almost halved the apparent affinity of the two domains, whereas C5N755K reduced it by 90%. The authors then used their data set to construct a model consisting of a staggered parallel arrangement of cardiac MyBP-C multimers. The model is consistent with their domain interactions, as well as with preexisting sets of biochemical and structural data. In essence, they build upon the previous concept of a trimeric MyBP-C collar that is wrapped around the thick filament. Consistent with early considerations of stoichiometry, their model invokes a two-molecule–thick collar with overlaps of C5-C7 of MyBP-C[1] interacting with C8-C10 of MyBP-C[2], C5-C7 of MyBP-C[2] interacting with C8-C10 of MyBP-C[3], and C5-C7 of MyBP-C[3] interacting with C8-C10 of MyBP-C[1], forming an integral circle perpendicular to the thick filament bundle in that region of the sarcomere.

The authors note that the observed $K_a$s are quite modest, implying relatively weak binding, although the values are compatible with in vivo interactions. Detailed structural data on MyBP-C, in either its monomeric or multimeric forms, are lacking. Solving the molecule’s structure, or even the structure of relevant subdomains would lead to major advances in our understanding of the molecule’s interactions, both with itself and with the thick and titin filament systems of the contractile apparatus. A spectroscopic analysis of a FHC mutation in domain 10 (the carboxyl terminus; Figure) was relatively uninformative. Data that bear more directly on the correctness and potential functional implications of the model come from optical diffraction studies obtained from negatively stained thick filaments in which the phosphorylation
state of MyBP-C was varied. These studies support the idea that the thick filament can transition between a number of different packaging states, termed tight, loose, or disordered. Sequential phosphorylation of MyBP-C converted the thick filaments almost entirely from the disordered to the tight structure and then to the loose structure, which can result in increased cross-bridge attachment. Thus, posttranslational modification of the proposed collar resulted in significantly altering thick filament packing, as might be expected if the MyBP-C trimer encircles the intact thick filament.

In spite of our ability to stratify FHC by genotype, many questions remain regarding the pathogenesis of specific mutations, both within and among the different sarcomeric proteins that can be affected. The collar model is certainly a step forward but without more structural data, it will be difficult to test the model’s correctness directly. We already know through loss-of-function experiments that MyBP-C is not essential for sarcomere assembly or maintenance, at least in the short term. This, coupled with the relatively benign disease course in many human patients could be due to the protein exerting only subtle changes in myofibril viability or function. Alternatively, the full protein complement of the C-zone transverse bands may not be defined. Indeed, optical diffraction techniques reveal a subset of transverse bands in this region that apparently do not contain MyBP-C. There may be enough redundancy in these structures to partially conserve the role(s) they play, even with altered MyBP-C being present (or not). It would be informative to see if and how the C-zone bands are altered in the different MyBP-C knock-in, knock-out, and transgenic animal models as in at least one of these models, the domains postulated for collar formation are missing. To date, the animal models have been used mainly to give us a snapshot of the pathology and it remains difficult to conceptualize how, in human patients, a mutation that takes 50 to 60 years to result in a hypertrophy can, in a brief moment, cause a lethal cardiac event. The value of the collar model lies in its potential to help us to explain how, over a prolonged period of time, the structural and functional changes that result in FHC and sudden cardiac death occur.

References


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