Myocardial force generation is initiated on the release of calcium ions from the sarcoplasmic reticulum and subsequent activation of the contractile apparatus. A schematic of the cardiac sarcomere and some of its component proteins is shown in the Figure. Contractile activation involves binding calcium to Tn-C, redistribution of the affinity of Tn-I for Tn-C away from Tn-T, and movement of tropomyosin on the thin filament, thereby exposing binding sites on actin. Binding of the myosin S1 domain to actin leads to cross-bridge force development. However, this simple two state model of muscle contraction is not sufficient to explain many features of muscle contraction. For example, the rate of force development critically depends on the activation state of the thin filament. Also, binding of a cross-bridge cycling rate apart from its impact on cooperative interaction with S1 and force generation (bottom). One explanation for such behavior is that the thin filament must have at least three states: a blocked state (top left), where tropomyosin sterically blocks S1 binding; a closed state, induced by calcium binding to Tn-C, where tropomyosin has shifted away somewhat allowing weak interaction with the S1 domain; and an open state, where tropomyosin has moved away fully allowing for a strong interaction with S1 and force generation (bottom). One interesting aspect of this theory is that the binding of myosin to actin by itself induces further activation of the thin filament, thus facilitating S1 domain binding along the filament; an elegant, yet simple molecular model of cooperative activation. Cardiac muscle activation is highly cooperative, even though calcium binding to isolated cardiac troponin-C itself is not cooperative. However, it is less clear how modulation of cross-bridge cycling rate could be accomplished by such a theory. To further complicate matters, phosphorylation of contractile proteins, in particular troponin-I and troponin-T, leads to marked alterations in cross-bridge cycling rate apart from its impact on cooperative activation. Thus, modulation of thin filament activation, either directly by varying the level of thin filament activation or indirectly by posttranslational modification of regulatory proteins, appears to modulate the kinetics of the actin–myosin interaction. Data presented by Herron et al in their issue suggest that myosin binding protein C (MyBP-C) now needs to be added to this list.

MyBP-C, or C-protein, was first identified by Starr and Offer in myosin extracts from muscle. The protein was named on the basis of mobility on an SDS-gel (the third protein, ie, C-protein after the first and second proteins on the gel). MyBP-C is a large protein, consisting of 10 domains: 7 immunoglobulin motifs and 3 fibronectin motifs. MyBP-C exists in a ~1:8 molar ratio with myosin, and there are several muscle type specific isoforms. Notably, as is the case for troponin-I, the cardiac isoform of MyBP-C is larger and contains several phosphorylation sites. The protein has been located in the sarcomere by immunohistochemistry to ~8 10-nm-wide transverse stripes, at 43 nm intervals along the thick filament in two zones located on either side of the M-line. It has been speculated that 3 MyBP-C proteins join to form a “collar structure” surrounding the thick filament. It is important to note that because of the stoichiometry and this “collar,” only ~1 of 7 to 8 myosin molecules in the sarcomere are actually exposed to MyBP-C. The precise function of MyBP-C has been somewhat of a mystery. It has been suggested that MyBP-C is important as a molecular “ruler” (as has the giant protein titin) to regulate the length of the thick filament during assembly of the sarcomere. Recent data support this notion, albeit in a slightly modified way; the role of MyBP-C here is proposed to be a binding partner to a LIM scaffold protein involved in the regulation of myosin filament formation and sarcomere assembly. However, murine knockout models of MyBP-C do not show a lack of sarcomere formation, a result that either implies a minor role for MyBP-C in sarcomere assembly or that this important function has been taken over by some other as yet unidentified mechanism. Second, MyBP-C may play a role in the regulation of muscle contraction. Indeed, early studies in which MyBP-C was chemically extracted from skinned myocardium showed an increase in calcium sensitivity and velocity, consistent with recent results obtained in the MyBP-C knockout models. Cardiac MyBP-C is a substrate for PKA; stimulation of beta-adrenergic receptors in the heart leads to marked phosphorylation of both MyBP-C and Tn-I with a similar time course. Earlier work by the Winegrad group suggested that PKA-mediated phosphorylation allowed myosin heads to move away from the backbone of the thick filament. Overall, these results paint a picture for a molecular model where MyBP-C plays a critical role in the activation process of the cardiac sarcomere by restraining or limiting the reach of myosin heads toward the thin filament. An interesting twist to this theory was recently provided by Cazorla et al, who showed that PKA mediated phosphorylation of Tn-I in a MyBP-C knockout model failed to affect myofilament calcium sensitivity, similar to earlier work using a slow skeletal muscle...
Tn-I replacement transgenic animal model. The implication of these two studies is that MyBP-C needs to be phosphorylated, or at least be present, for the PKA signal to be effective in modulating contractile function in the heart.

How MyBP-C functions in the heart, and which are the domains within MyBP-C that are responsible for its action, are still actively being investigated. The current study reveals some new secrets of MyBP-C. Application of various amounts of recombinant MyBP-C to skinned myocardium induced force production in the absence of calcium. The effect was localized to the C0-C1 N amino terminus domain of cardiac MyBP-C (Figure). These results imply that the normal role of MyBP-C (left panels) is to limit binding of S1 to inactivated thin filaments; competitive binding of exogenously applied MyBP-C Co-C1 (right panels) relieves this restriction. In addition, Herron et al clearly demonstrated that MyBP-C-C0-C1 induced cross-bridges behave quite like regular activated cross-bridges in terms of the kinetics of force development. A problem with the model presented in the Figure is that the total absence of MyBP-C, as is the case in the various knockout models, should lead to substantial contractile activation in diastole incompatible with life. Yet this is not what was observed. A likely explanation for this conundrum may lie in the fact that MyBP-C normally exists in a ∼1:8 stoichiometry to myosin. Hence, application of a saturating concentration of MyBP-C fragment will likely lead to binding of the protein to all remaining sites on myosin along the filament. Thus, the results may simply be attributable to the induction of an unphysiological state where every myosin molecule in the sarcomere is no longer “limited” by being tethered to MyBP-C. This is a limitation, as acknowledged by Herron et al of any study where MyBP-C fragments are applied to skinned muscle. To address this potential pitfall, Herron and colleagues also studied the impact of a much lower concentration of MyBP-C fragment, that is, a concentration where there was no observable impact on inactivated myofilament function. Surprisingly, this low concentration of MyBP-C-C0-C1 resulted in marked increases in cross-bridge cycling kinetics at sub-maximal activation levels and at saturating levels of calcium activation. In addition, the impact of sarcomere length on myofilament calcium sensitivity, a property regularly referred to as myofilament length dependent activation, was completely abolished in the presence of a low concentration of the protein fragment. Although the molecular mechanisms that underlie myofilament length dependent activation are not known at present, these data strongly suggest that MyBP-C plays a major role in this important physiological regulatory process. MyBP-C may be well positioned within the contractile apparatus to perform such a role (Figure). It is possible that a change in sarcomere length indirectly affects the interaction between myosin and MyBP-C via changes in inter-filament spacing. Alternatively, sarcomere length changes may be transmitted directly to MyBP-C via the giant molecule titin. Regardless of the underlying mechanisms, however, the data by Herron et al demonstrate a prominent role for MyBP-C in the regulation of myofilament force development, calcium sensitivity, cross-bridge cycling rate, and length dependent activation.

It is now recognized that mutations in the MyBP-C gene underlie a large proportion of familial hypertrophic cardiomyopathy (FHC) cases. Many of these mutations involve the loss of critical binding sites for myosin from MyBP-C, thus often causing reduced levels of MyBP-C in the sarcomeres of the individuals affected by the mutation. As in the murine knockout models, loss of MyBP-C in humans does not lead to
complete impairment of sarcomere assembly nor its function. Nevertheless, lack of functional MyBP-C clearly sets the stage for severe cardiac dysfunction, consistent with the notion that MyBP-C is an important regulatory protein in the cardiac sarcomere. Unraveling the precise molecular mechanisms will require future studies, using all of the tools that modern biology can offer, such as transgenic animals (including knock-out and knock-in models), isolated human myocardium studies, viral transfection studies, and studies using recombinant contractile proteins applied to skinned myocardium.19

Acknowledgments
This work was supported in part by National Institutes of Health grants HL-62426, HL75494, and HL77195.

References

Key Words: myosin accessory proteins  sarcomere  cross-bridge cycling  regulation
Myosin Binding Protein C in the Heart
Pieter P. de Tombe

Circ Res. 2006;98:1234-1236
doi: 10.1161/01.RES.0000225873.63162.c4

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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