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The Physiological Relevance of Interactions between cMyBP-C and Actin Studied in a Transgenic Mouse Model

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Cardiac myosin binding protein C (cMyBP-C) is a sarcomeric protein that plays an essential role in the regulation of cardiac contraction. Until recently, cMyBP C was thought to hamper cross bridge cycling trough interaction of its N-terminus with the heads of myosin. However, the N-terminal domains of cMyBP-C can also bind with actin and this interaction could well explain the currently known functions of cMyBP-C. For instance, by forming a connection between the thin and the thick filament cMyBP-C could pose a load on the sarcomere and alter its visco-elastic properties. Additionally, cMyBP-C could alter the activation state of the thin filament by intervening with the position of tropomyosin.

To study the physiological relevance of an interaction between cMyBP-C and actin, we created a transgenic mouse with a point mutation (L348P) that increases the binding affinity between cMyBP-C and actin in vitro. We hypothesized that stronger binding between cMyBP-C and actin will cause- through afore mentioned mechanisms, which have been demonstrated in different in vitro studies- stiffer cardiomyocytes which would ultimately lead to diastolic dysfunction in the hearts of L348P-Tg mice.

Results show that the left atria of L348P-Tg mice are significantly enlarged. Echocardiograms in 12 week old animals demonstrated misshapen E and A waves, making diastolic function difficult to determine. Interestingly, pulse wave and tissue Doppler images were more defined in 24 weeks old mice. Preliminary data show decreased early blood flow over the mitral valve (E) and mitral valve movement (E') in L348PTg mice, indicative of a stiffer left ventricle. In correspondence with that, isovolumetric relaxation time was prolonged. At neither age we observed systolic dysfunction. Additional in-depth hemodynamic studies to characterize the mechanisms of cardiac function longitudinally in L348P-Tg mice are ongoing.

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The HCM Mutation L348P in cMyBP-C Enhances Thin Filament Activation through Tropomyosin Shift

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Cardiac myosin binding protein C (cMyBP-C) is a thick filament protein that plays an important role in modulating contraction of the heart. Mutations in cMyBP-C are a major cause of inherited hypertrophic cardiomyopathy (HCM). In addition to binding to myosin, cMyBP-C also interacts with thin filaments via its N-terminal region, mainly the C1 and M-domains, enhancing thin filament Ca^{2+}-sensitivity. L348P is an HCM-causing mutation that occurs in a conserved sequence in the M-domain, enhancing cMyBP-C binding to actin and thin filament Ca^{2+}-sensitization (Bezold et al., JBC 2013). Our goal was to elucidate the structural basis of L348P's gain of function. By EM 3D reconstruction we previously showed that binding of N-terminal cMyBP-C fragments to actin causes displacement of tropomyosin from its blocked (low Ca^{2+}) position to its closed (high Ca^{2+}) position (Mun et al., PNAS 2014), consistent with the increase in thin filament Ca^{2+}-sensitivity. Phosphorylation of the M-domain resulted in a smaller movement of tropomyosin. Here we have investigated the impact of the L348P mutation on tropomyosin shift. Thin filaments were decorated with wild type C1C2 (containing the C1, C2 and M-domains) and with C1C2 having the L348P mutation. F-actin reconstructions showed additional density on actin subdomain 1 with both fragments, but the density was larger with L348P. When thin filaments at low Ca^{2+} were decorated with C1C2, tropomyosin moved from the blocked to the closed position, as found previously. In contrast, C1C2-L348P showed a significantly larger tropomyosin shift, to approximately the open position, consistent with L348P's greater Ca^{2+}-sensitization of motility and enhanced Ca^{2+} sensitivity of tension in cardiac myocytes. Preliminary studies showed a smaller shift with phosphorylated than with unphosphorylated C1C2, while phosphorylated C1C2-L348P showed a larger shift, comparable to L348P.

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Crossbridge Arrangement in Cardiac Thick Filaments Isolated from cMyBP-C Phosphomimetic Mice

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Although cardiac myosin binding protein C (cMyBP-C) has a key regulatory role in cardiac contraction, the mechanism by which changes in phosphorylation of cMyBP-C affect crossbridge kinetics remains unknown. cMyBP-C mediated changes in thick filament structure, binding of cMyBP-C to the thin filament, or both remain viable alternatives. In this study, we isolated thick filaments from the hearts of phosphomimetic mice in which the three phosphorylatable serine residues (Ser273, Ser282 and Ser302) in the m-domain of cMyBP-C were replaced by either alanine or aspartic acid to mimic the fully non-phosphorylated (CT-SA mice) or fully phosphorylated (CT-SD mice) state of cMyBP-C, respectively. Thus, the state of phosphorylation of cMyBP-C of every filament was known in this transgenic system. We found that the thick filaments from the CT-SA mouse hearts corresponding to the non-phosphorylated state of cMyBP-C had highly ordered crossbridge arrays, while the filaments from the CT-SD mouse hearts showed a strong tendency towards disorder of the myosin array. These results are consistent with our previous studies of filaments from cMyBP-C knockout mouse hearts. The results support the hypothesis that dephosphorylation of cMyBP-C promotes or stabilizes the relaxed quasi-helical ordering of the myosin heads on the backbone consistent with slower crossbridge kinetics; while filaments with phosphorylated cMyBP-C show a greater tendency towards disorder of the heads consistent with increased flexibility of the myosin heads, extension of the heads from the backbone, and increased potential interaction with the actin thin filament. Work supported by NIH grants SC1 HL096017 (RWK), AR034711 (RC), R37 HL82900 and P01 HL094291 (RLM). RC also received funding through PPG grant P01 HL059408 (David Warshaw).