Familial hypertrophic cardiomyopathy (HCM) is an autosomal dominant disorder of heart muscle, occurring with a frequency of 1:500 in the general population. Mutations in genes encoding sarcomeric proteins are found in approximately 60% of cases. Amongst the mutations identified, about 45% are accounted for by mutated genes encoding myosin heavy or light chains, a further 35% by myosin-binding C protein (cMyBP-C) mutations, and the remainder by mutant forms of tropomyosin, troponin or titin. While some patients may be asymptomatic or mildly symptomatic, others exhibit more severe medical conditions such as dyspnea, arrhythmia, angina and even sudden cardiac death (hypertrophic cardiomyopathy is the main cause of sudden cardiac death amongst persons under 30 years of age). Although the functional consequences of these mutations for cardiac performance and anatomy are well documented, their effects on the fundamental processes governing the regulation and generation of mechanical work are not well understood, nor has a firm link been established between such effects and the deterioration in cardiac performance of the cardiomyopathic heart.

Studies of the physiological role of cMyBP-C have mainly focussed on extraction of the protein or on the development of murine models in which cMyBP-C is expressed in a truncated form. More recently, a knockout murine line has been developed in which the transcription initiation site has been removed, producing a null allele and preventing not only the occurrence of cMyBP-C protein, but also the production of cMyBP-C mRNA. By using this null allele to produce heterozygous cMyBP-C+/– and homozygous cMyBP-C−/− phenotypes, it was found that heterozygous cMyBP-C+/– hearts replicated many of the anatomical features of HCM, suggesting that haploinsufficiency may be the cause of the condition, while homozygous cMyBP-C−/− hearts exhibited anatomical features resembling dilated cardiomyopathy (DCM). Unlike other HCM inducing mutations, which are fatal in the first weeks after birth, cMyBP-C homozygous knockout murine models can survive for more than 1 year without functioning cMyBP-C, making the knockout mouse an excellent subject for the investigation of HCM. The role of cMyBP-C in myocardium remains uncertain because many effects of its extraction or ablation are contradictory. The protein was originally thought to play an important part in structural integrity and development of the sarcomere, but cMyBP-C knockout mice are viable and have well-ordered sarcomeric architecture and thick filament structure.

MyBP-C is a large (140-150kDa) protein found only in the A-band of striated muscles. It is composed of 11 modules, consisting of eight immunoglobulin-C2 type domains, three fibronectin type 3 domains plus a MyBP-C motif close to the N-terminus containing three phosphorylation sites. Unlike the skeletal muscle MyBP-C, cMyBP-C is an excellent substrate for cyclic AMP-dependent protein kinase A (PKA), and phosphorylation of cMyBP-C has been proposed to reduce myosin-MyBP-C motif interaction, allowing greater mobility of the myosin subfragment 1 (S1) moiety. The C-terminal domains 8, 9 and 10 are known interact with titin, domain 10 binds to light meromyosin, and the MyBP-C motif binds to myosin subfragment 2 (S2). N-terminal domain 0 contains an actin binding site. In vivo, cMyBP-C is bound to the thick filament at its C-terminal end in groups of 3 monomers at axial intervals of 43nm.

Growing awareness of the importance of structural parameters in contraction, plus the development of synchrotron X-ray sources and advances in detector technology, have promoted interest in the application of X-ray techniques to cardiac muscle. X-ray studies of earlier murine models of cMyBP-C ablation have led to the proposal that the N-terminal portion of cMyBP-C interacts with myosin to restrict the mobility of S1 heads, tethering them close to the surface of the thick filament. Phosphorylation or ablation of cMyBP-C causes changes in the equatorial pattern of myocardium consistent with an increased radial displacement of S1 from the surface of the thick filament.
Using the null allele murine model of cMyBP-C ablation, we examined chemically skinned murine left ventricular myocardium. Papillary and trabecular muscle tissue was treated with 1% Triton X100 for 10 hours to remove the sarcolemma, allowing direct control and manipulation of the chemical environment of the myofilaments. In agreement with previous studies, we found no change in lattice spacing (centre to centre myosin filament spacing: control 53.7±1.3nm (n=6); knockout 53.7±0.3nm (n=4)), but we also found no significant change in relaxed I11/I10 (control 0.83±0.02nm (n=6); knockout 0.87±0.12nm (n=4)), an index of the displacement of S1 from the thick filament. Upon calcium activation (pCa 4.6), knockout tissue produced only 41% of the force generated by control. This reduced tension was not a result of impairment of force transmission, since the equatorial reflections also showed smaller changes on activation of the knockout line.

We also examined the behaviour of intact myocardium from control and knockout lines. Experiments were conducted as blind trials, in which the nature of the tissue was not known to the experimenter at the time of testing. Tyrode’s solution was gassed with 100% oxygen throughout the experiment, and contractions were elicited to field stimulation (typically a pulse of 0.5ms duration, field intensity 3kV.m⁻²). Temperature was 24°C. In the relaxed state, lattice spacing (centre to centre distance between thick filaments) was slightly higher in knockout tissue (46.3±0.5nm compared to 44.9±0.4nm). In all tissues, I11/I10 (expected to be about 0.5 in the relaxed state) was greater than 1, which suggests rigor or partial calcium activation may have been present in intact tissue samples. This was more pronounced in knockout tissue, and was insensitive to temperature and to 10mM BDM (a muscle relaxant). Twitch force from knockout muscle was only ca. 1% of control tissue. Since this force reduction is much greater than we observed from direct application of calcium ions to skinned myocardium, we suspect that knockout tissue might suffer from additional effects of the mutation on electrical excitability or calcium fluxes. These findings call into question the proposed role of cMBP-C in restriction of S1 mobility and suggest possible additional effects of cMyBP-C ablation beyond those associated with the contractile apparatus.