REVIEW

Myosin binding protein C: implications for signal-transduction

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Abstract Myosin binding protein C (MYBPC) is a crucial component of the sarcomere and an important regulator of muscle function. While mutations in different myosin binding protein C (*MYBPC*) genes are well known causes of various human diseases, such as hypertrophic (HCM) and dilated (DCM) forms of cardiomyopathy as well as skeletal muscular disorders, the underlying molecular mechanisms remain not well understood. A variety of *MYBPC3* (cardiac isoform) mutations have been studied in great detail and several corresponding genetically altered mouse models have been generated. Most *MYBPC3* mutations may cause haploinsufficiency and with it they may cause a primary increase in calcium sensitivity which is potentially able to explain major features observed in HCM patients such as the hypercontractile phenotype and the well known secondary effects such as myofibrillar disarray, fibrosis, myocardial hypertrophy and remodelling including arrhythmogenesis. However the presence of poison peptides in some cases cannot be fully excluded and most probably other mechanisms are also at play. Here we shall discuss MYBPC interacting proteins and possible pathways linked to cardiomyopathy and heart failure.

Keywords Myosin binding protein C ·
Myocardial function

Abbreviations

DCM	Dilated cardiomyopathy
HCM	Hypertrophic cardiomyopathy
LMM	Light meromyosin
MYBPC	Myosin binding protein C (in italics: gene)
MYBPC1	Slow twitch isoform (in italics: gene)
MYBPC2	Fast twitch isoform (in italics: gene)
MYBPC3	Cardiac isoform (in italics: gene)
cMyBP-C	Cardiac myosin binding protein C
PKA	Protein kinase A
RLC	Regulatory light chain
TnC, I, T	Troponin C, I, T

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For in that profession there is no such thing as coincidence.

(John le Carré)

Introduction—domain structure and binding partners

Myosin binding protein C (also known as C-protein: MyBP-C; cardiac isoform abbreviated: cMyBP-C) was discovered in 1973 (Offer et al. 1973) and is located on 7–9 stripes of 43 nm spacing in each half of the A-band (cross bridge bearing zone, C-region) of the sarcomere in skeletal and cardiac muscles (Fig. 1). To date three different MYBPC proteins are known, each encoded by different genes (*MYBPC 1–3*), where slow (MYBPC1, 1141 aa) and fast (MYBPC2 1141 aa) twitch muscle isoforms are restricted to skeletal muscle tissues and whereas MYBPC3 (1273 aa) is expressed exclusively in cardiac myocytes (Furst et al. 1992; Gautel et al. 1995; Weber et al. 1993). All MYBPC molecules share common architectural features: they are composed of seven immunoglobulin domains (Ig) and three fibronectin type III domains (FnIII) called C1–10 where a 105 amino acid myosin binding protein motif is localized between C1 and C2. In addition, a proline and alanine rich

(PA) domain is localized near the aminoterminus (Fig. 2). Domains C9–C10 interact with titin and C10 interacts with light meromyosin (LMM) (Freiburg and Gautel 1996). The actin and S2 (head region) myosin heavy chain binding regions are located at the aminoterminus (Fig. 2). Skeletal muscle MYBPC also interacts with nebulin, but this interaction is not well studied (Jin and Wang 1991) (for a review (Kontrogianni-Konstantopoulos et al. 2009)). Also the MYBPC1-variant 1 interacts with the giant protein obscurin (Ackermann et al. 2009). Another novel interaction between the cardiac specific C0 domain and the regulatory light chain (RLC) has been analyzed by Ratti et al. (2011).

In addition, domains in the mid-region (C5–C8) have been hypothesized to interact with one another, forming a "trimeric collar" that constrains the thick filament (Moolman-Smook et al. 2002). Also, a slightly different model of MYBPC organisation within the sarcomere has been proposed by Squire et al. (2003). These authors due to structural considerations and because domains C7–C10 are also

reported to interact with titin, which is unlikely to run other than axially along the myosin filament, propose a model whereby the carboxyterminal end of MYBPC is aligned axially, which contrasts the collar alignment structure (Fig. 3).

Fig. 3 Summarizes the major differences between the MYBPC models proposed by (Moolman-Smook et al. 2002) (a) and (Squire et al. 2003) (b). Please note in a MYBPC forms a "trimeric collar" that constrains the thick filament (Moolman-Smook et al. 2002). Top: schematic diagram showing a single MYBPC protein interacting with myosin ("top view"), bottom: side view. Whereas in b the carboxy-terminal end of MYBPC is aligned axially (Squire et al. 2003). Top view: a thick filament is represented along with six surrounding actin filaments (in striated muscle), the broken circle represents the C0 domain in the MYBPC3 isoform. Bottom: side view, domains C7–C10 running axially along the myosin filament backbone. A few myosin heads are drawn as "transparent" ghosts to indicate their position (grey circles: MYBPC3 with green indicating the P/A rich domain; yellow/brown: myosin, dark grey: actin; figures are from: (Squire et al. 2003) with kind permission from the authors and the publisher (Journal of Molecular Biology))
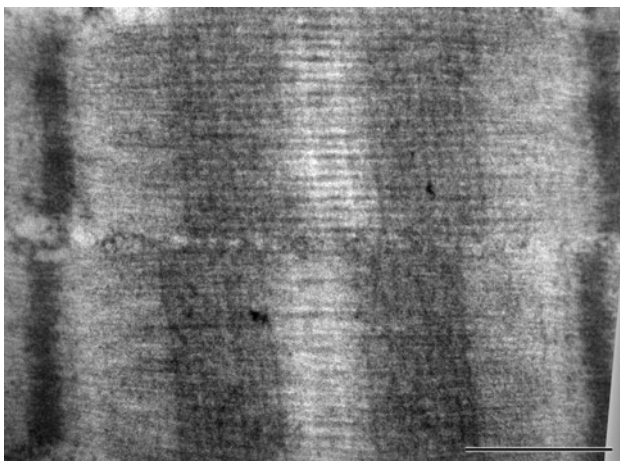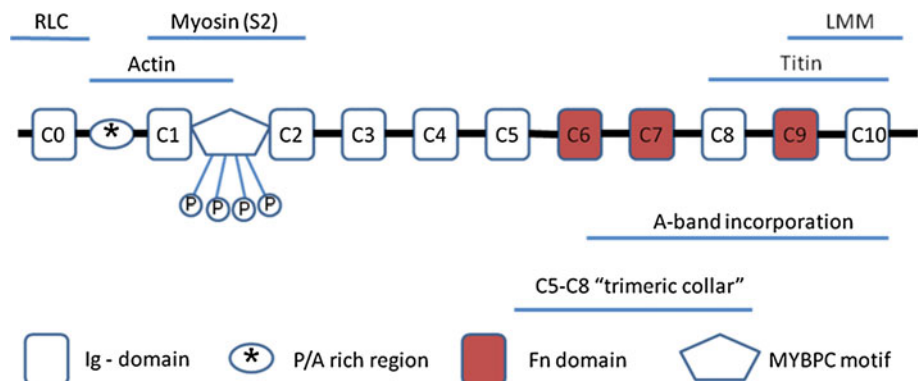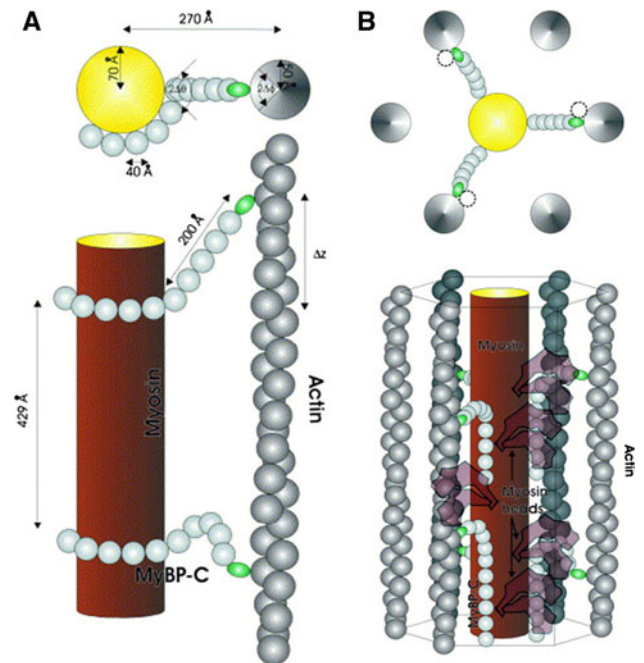

Fig. 1 Electron micrograph of rat cardiac muscle labelled with a MYBPC antibody. It shows that MYBPC in cardiac muscle occurs over 9 stripes. The scale bar is 500 nm (Luther et al. 2008) (with Dr Pradeep Luther's kind permission)

Fig. 2 Depicts the structure of MYBPC3 as well as highlights important structural domains and indicates MYBPC3 interacting proteins. Please note: the number of phosphorylation sites localized in the MYBPC motif is species dependent. Ig immunoglobulin, P/A proline/alanine, Fn fibronectin)

However three major differences exist between the cardiac and skeletal muscle isoforms: MYBPC3 consists of an additional immunoglobulin domain (C0) at its amino-terminus, the domain C5 contains a proline rich 25 residue insertion and several phosphorylation sites are species dependent localized in the myosin binding motif (Gautel et al. 1995; Mohamed et al. 1998).

Implication in human disease

MYBPC3 mutations were first reported in 1995, which was an important discovery (Bonne et al. 1995; Watkins et al. 1995a). Indeed HCM is a frequent disease, affecting 1:500 individuals (Maron et al. 2006) and depending on the population analyzed, MYBPC3 mutations are found in up to 40–50% of the genotyped HCM patients (Richard et al. 2003). To date about 200 different MYBPC3 mutations have been reported (Marston 2011; Schlossarek et al. 2011). In general, MYBPC3 mutations are associated with a slightly lower penetrance, later onset of disease and with milder forms of disease progression in comparison to other HCM causing mutations located for example in the beta myosin heavy chain (MYH7) gene (Niimura et al. 1998; Watkins et al. 1995b). However this general statement may not necessarily be true for all MYBPC3 mutations and indeed some MYBPC3 mutations are associated with a poor prognosis. For example—a recently discovered deletion in intron 32 in MYBPC3 (nt 21348-21372, accession no. U91629), leading to a pre-terminal stop codon, has been found at a frequency of about 4% in India and South East Asia, with significant impact on human heart failure (Dhandapany et al. 2009; Waldmuller et al. 2003). While this mutation has been initially described in HCM patients, it is important to note that this mutation is also associated with other types of cardiomyopathy such as DCM. Other MYBPC3 mutations primarily found in DCM patients have also been reported, for example the Asn948Thr missense mutation (Daehmlow et al. 2002).

In addition the MYBPC3 Arg502Trp mutation with a frequency of about 2.4% is the most common HCM-causing mutation among individuals of European descent in the USA (Saltzman et al. 2010). However other mutations may be prevalent in different European populations, such as in the Netherlands, where three different founder mutations are present: (i) the c.2373_2374insG MYBPC3 which is present in the great majority of HCM patients (up to 25%) and where (ii) the c.2864_2865delCT and (iii) the c.2827C > T mutations occur in about 5% of HCM patients each (Alders et al. 2003; Christiaans et al. 2010).

Beside the important mutations in MYBPC3, MYBPC1 mutations are a cause of distal arthrogryposis type 1 (DA1), a disease characterized by congenital contractures of the hands and feet (Gurnett et al. 2010).

Although by now MYBPC3 mutations are well known causes of HCM and DCM including associated heart failure, the underlying molecular mechanisms are not well defined, some of which will be discussed in the next chapters.

Animal models

To gain more insight into the underlying molecular mechanisms, Mybpc3 has been deleted in genetically altered mouse models by two independent groups. Loss of this protein is not associated with any embryonic lethality and MYBPC3 also is not essential for sarcomere formation but its absence results in profound eccentric hypertrophy in the homozygous animals (Carrier et al. 2004; Harris et al. 2002). Hemodynamic analysis in the mice generated by the Carrier group, where exons 1 and 2 have been deleted, revealed the presence of normal contractility but severe diastolic defects. In addition heterozyogous animals develop septal hypertrophy, a hallmark of HCM (Carrier et al. 2004). However these animals were engineered so that they harbour a complete ablation of the gene and therefore are useful to identify basic mechanisms, but the overwhelming majority of human mutation carriers express mutant mRNAs and probably proteins, which makes it difficult to relate these data directly to the situation in patients.

Therefore, in addition to the pure knockout models, wildtype Mybpc3 and different Mybpc3 mutants have been overexpressed in various models such as an amino-terminal truncated MYBPC3, which mimics a certain type of human mutations which lead to the loss of the carboxyterminal domains including the titin and myosin binding sites. Overexpression of the mutant, but not the wildtype protein, caused major features of HCM including hypertrophy and an increase in calcium sensitivity (Yang et al. 1998).

Whereas above mentioned transgenes were well expressed at the mRNA and protein levels, overexpression of a Mybpc3 mutant lacking only the myosin binding domain resulted in the expression of only very modest levels of mutant protein (i.e. about 5%) which led to a mild hypertrophy and heart failure phenotype (Yang et al. 1999).

Two additional knock in mouse models have been engineered to carry mutations found in patients and which affect titin and myosin binding. Interestingly animals homozygous for these mutations develop a DCM like phenotype with depressed contractility and hypertrophy (McConnell et al. 1999). Another knock in mouse model was generated such that the mutant protein did not contain the aminoterminal myosin binding domain. The mutant

protein was readily integrated into the sarcomeres of heterozygous and homozygous animals, was PKA phosphorylatable and major structural defects could not be detected. However this mutation was associated with a significant increase in calcium sensitivity (Witt et al. 2001). An additional mouse knock in model, based on a G > A transition located on the last nucleotide of exon 6 and which was found in a patient with HCM, has been generated by Lucie Carrier's group. Interestingly this mutation gives rise to three different mRNAs: (i) missense mutation (ii) nonsense due to exon skipping, frameshift and premature stop codon and (iii) deletion/insertion as nonsense but with additional partial retention of a downstream intron which restores the reading frame and which leads to an almost full length protein. Homozygous animals develop hypertrophy, interstitial fibrosis and decreased myocardial function whereas heterozygous animals do not have any obvious phenotype (Vignier et al. 2009).

Additional genetically altered mouse models have been generated to study MYBPC3 phosphorylation, which will be discussed in the next chapter.

Phosphorylation

Human MYBPC3 contains at least four phosphorylation sites which are localized inside the myosin binding motif (serines 275, 284, 304, with an additional phosphorylation site not unequivocally identified) (Copeland et al. 2010)), the mouse myosin binding motif contains three to four sites (serines 273, 282, 302, (305)) and five sites have been identified in the canine genome as well as three sites in the rat genome (Jia et al. 2010; Yuan et al. 2006, 2008). These phosphorylation sites are targets of protein kinase A (PKA; serines 273, 282, 302) (Gautel et al. 1995), protein kinase C (PKC; serines 273, 302) (Mohamed et al. 1998), calmodulin dependent kinase II (CamKII; serine 302) (Gautel et al. 1995), protein kinase D (PKD; serine 302) (Bardswell et al. 2010) and ribosomal S6 kinase (RSK; serine 282) (Cuello et al. 2011).

MYBPC3 phosphorylation also has been studied in various transgenic mouse models, for example lines have been established where the phosphorylation sites (Ser273, Ser282, and Ser302), along with two adjacent sites that could be potentially phosphorylated (Thr272, Thr281), were converted to alanines. While overexpression of wildtype *MYBPC3* was able to rescue the *MYBPC3* null phenotype, the non-phosphorylatable *MYBPC3* was not (Sadayappan et al. 2005). Also a similar approach has been used to analyze MYBPC3 phosphorylation by generating animals whereby only the three known phosphorylation sites were converted into alanines (t3SA). Again these animals showed, when crossed into the *Mybpc3* null

background, in addition to hypertrophy systolic and diastolic defects, further supporting the notion that PKA phosphorylation of MYBPC3 is important for myocardial function (Tong et al. 2008). Another type of transgenic animals, where the phosphorylation motif "LAG-AGRRTS" was completely deleted, where also generated. These animals showed an increase in contractility and relaxation of about 22 and 25%, respectively. An increase in the phosphorylation of the remaining MYBPC3 and other proteins such as troponin I and phospholamban was also observed (Yang et al. 2001).

The reverse experiment, i.e. overexpression of a phosphomimetic MYBPC3 in a *Mybpc3* null background, has also been performed, which resulted in subtle changes in sarcomeric ultrastructure characterized by increased distances between the thick filaments which may indicate that phosphomimetic MYBPC3 affects thick–thin filament relationship. The phosphomimetic MYBPC3 also prevented interaction with myosin heavy chain in vitro as analyzed by yeast two hybrid and pull down assays. Moreover these transgenic animals were also resistant to ischemia reperfusion injury. This effect is difficult to explain, but it may well be that phosphomimetic MYBPC3 prevents an increase of thick filament packing density which is associated with reduced calcium activated force generation. An alternative explanation could be that phosphorylated MYBPC3 is protected from calpain (see also later) mediated proteolysis (Sadayappan et al. 2006, 2009). In this context it is probably important to mention, that loss of MYBPC3 phosphorylation also has been observed in failing human hearts and strategies to increase its phosphorylation may have cardio-protective effects (Copeland et al. 2010; Sadayappan et al. 2006).

Above mentioned studies have been intensified by generating mouse models overexpressing various single or double phosphomimetic or non phosphorylatable serines in the *Mybpc3* knockout background (i.e. the cMyBP-C$^{SAS(t/t)}$, cMyBP-C$^{ADA(t/t)}$, cMyBP-C$^{DAD(t/t)}$; on position S-273, S-282 and S-302, respectively). One major conclusion of these studies is that S-282 has a unique regulatory role in that its phosphorylation is critical for the subsequent phosphorylation of S-302, but that all residues play a role in regulating the contractile response to β-agonist stimulation (Sadayappan et al. 2011).

Although there is now compelling evidence supporting the notion that MYBPC3 phosphorylation modulates contractility by controlling the proximity of the myosin heads to actin, however the precise molecular mechanism remains unclear (reviewed in (Barefield and Sadayappan 2010; Schlossarek et al. 2011)). In particular when using in vitro systems it was shown that MYBPC3 phosphorylation can abolish the ability of MYBPC3 to interact with the S2 region of the myosin heavy chain but may enhance

MYBPC3 interactions with the thin filament. Vice versa—dephosphorylation results in strong binding of MYBPC3 to the myosin head, probably preventing its force generating strong interaction with actin (Kulikovskaya et al. 2003a; Sadayappan et al. 2006). However recent data also indicate that MYBPC3 may act synergistically with the myosin RLC to enhance cross-bridge formation by altering the interaction of the myosin head with actin. It may well be that this interaction depends on phosphorylation of either MYBPC3 or RLC and may well be able to provide another mechanism how phosphorylation of sarcomeric proteins may affect protein/protein interactions and kinetics of force development (Colson et al. 2010). Although it is well known that the distance between thick and thin filaments is a major determinant of calcium sensitivity and that loss of MYBPC3 is associated with increased calcium sensitivity, the precise molecular mechanism of how MYBPC3 phosphorylation affects this system is unknown. Part of this problem is that PKA phosphorylation decreases calcium sensitivity via troponin I phosphorylation which leads to a decrease in tropinin C calcium sensitivity. However MYBPC3 phosphorylation, which decreases calcium sensitivity, has no effect on interfilament spacing (Colson et al. 2010) (for more details on calcium sensitivity see also next chapter). Future studies, based on various transgenic animals, will certainly help to answer this question.

MYBPC3 mutations and molecular mechanisms leading to myocardial dysfunction

In general two different molecular mechanisms may account for the observed pathologic effects of any mutation: (i) poison peptide (functional integration of a mutant protein) (ii) haploinsufficiency (loss of one allele).

However, any mutation may also cause disturbances in the degradation of such a product, including its mRNA. For example, the mRNA might be degraded via nonsense mediated mRNA decay or the protein may be degraded via the ubiquitin proteasome system (UPS) with implications for protein degradation in general, including autophagy.

Most of the more than 600 known HCM causing mutations located in other sarcomeric protein genes are point mutations or single amino acid deletions (Van Driest et al. 2004), hence the poison peptide mechanism seems to be the likely mechanism for these mutations. In contrast, the most frequent type of MYBPC3 mutation affects splice acceptor and donor sites or are deletions or insertions predicted to result in truncated MYBPC3 proteins (Van Driest et al. 2004).

However, multiple studies were unable to detect or document the presence of the predicted truncated MYBPC3 molecules. For example van Dijk and colleagues analyzed the c.2373dupG and the c.2864_2865delCT mutations and found a significant decrease in mutant MYBPC3 mRNA. Also they detected a significant loss of MYBPC3 protein but were unable to detect the truncated proteins (van Dijk et al. 2009). Marston and colleagues studied MYBPC3 protein expression in two patients carrying the Glu258Lys and the Arg502Trp missense mutations in addition to seven other mutations which are predicted to lead to premature terminations and truncated proteins. However no truncated protein was detectable and in all samples studied significant lower amounts of MYBPC3 protein was detected (Marston et al. 2009). Another complex mutation where a T to A transition occurred on position 2604 together with a deletion of C at 2605 was predicted to lead to a truncated protein (1-868 + 13 nonsense amino acids with a premature stop codon in C7) was also studied in great detail, but no truncated MYBPC3 protein could be detected (Jacques et al. 2008). In summary, several different studies failed to detect truncated MYBPC3 proteins in MYBPC3 mutation carriers, which makes the poison peptide mechanism unlikely to occur in the studied mutations/individuals. Also these studies documented loss of MYBPC3 protein in mutations carriers, with only 67–75% of the normal protein present, which indicates haploinsufficiency may contribute to the phenotype.

MYBPC3 plays a modulatory role in the regulation of actin–myosin interaction and by binding to both proteins it may directly affect calcium sensitivity. By employing skinned myocytes from patients with HCM mutations, a variety of studies showed that MYBPC3 mutations are associated with higher calcium sensitivity. However, these data may also result from low levels of troponin I (TnI) phosphorylation present in the myectomy tissue rather than the mutation itself (Hoskins et al. 2010; Jacques et al. 2008; van Dijk et al. 2009). Nevertheless an increase in calcium sensitivity in myocardial samples obtained from patients affected by MYBPC3 mutations is likely because removal of MYBPC3 from myocardial samples under experimental conditions is associated with an increase in calcium sensitivity (Hofmann et al. 1991a, b; Kulikovskaya et al. 2003b) and an increase in calcium sensitivity has also been shown in Mybpc3 deficient myocardium (Rybakova et al. 2011). Calcium sensitivity depends on the rate of calcium binding to or calcium release from troponin C and kinetic studies suggest that for HCM causing mutations the rate of calcium binding to TnC is increased, but it remains to be elucidated whether this is a general phenomenon or whether other determinants of calcium sensitivity such as the TnC–calcium dissociation rate may also be affected and whether this holds true for MYBPC3 mutations (Dong et al. 2008).

Therefore, MYBPC3 mutations may primarily increase calcium sensitivity (gain of function) which may cause

faster cross bridge cycling (hypercontractility), but which may also cause incomplete relaxation, a cause of diastolic dysfunction frequently observed in HCM. This concept also is supported by hemodynamic data obtained from *Mybpc3* deficient animals, where no systolic but severe diastolic dysfunction has been observed (Carrier et al. 2004).

It might well be that *MYBPC3* mutations are primarily linked to an increase in calcium sensitivity, but it is difficult to link this event directly to the secondary effects such as myocardial hypertrophy including myofibrillar disarray, fatal arrhythmias and sudden cardiac death (Fig. 4). One hypothesis proposes energy deficiency due to reduced thermal efficiency in HCM or another hypothesis states that troponin C functions as an intracellular calcium buffer or calcium store (Bers 2008). An increase in calcium sensitivity could affect calcium transients, which indeed have been found to be increased in Myh6 Arg403Gln knock in animals (Gao et al. 1999). An increase in calcium transients may also have effects on calcium dependent enzymes such as calcineurin, calmodulin dependent kinase, and protein kinase C all of which have been shown to be important for

Fig. 4 Summary of possible pathologic events caused by a primary increase in calcium sensitivity, which has been shown to be present in various *Mybpc3* transgenic animal models as well as in patients affected by mutations in the *MYBPC3* gene. *CamKII* calmodulin dependent kinase II, *PKC* protein kinase C

the initiation of myocardial hypertrophy (Kubis et al. 2003).

The hypothesis that sarcomeric HCM mutations lead to inefficient ATP utilization was proposed by the Watkins group and is an attractive model, because it is able to explain many different features found in HCM (Ashrafian et al. 2003). According to this theory a lower ATP/ADP + P ratio may contribute to energy depletion in critical compartments, which may affect calcium re-uptake and lead to an increase in calcium concentrations and thus activation of calcium dependent enzymes such as calcineurin. Another consequence is the activation of adenosine monophosphate kinase (AMPK), which is also able to induce hypertrophy and yet another consequence is the increase in mitochondrial demands which may lead to an increase in reactive oxygen species, mitochondrial abnormalities and apoptosis—all of which have been observed in various patients affected by HCM (Ishikawa et al. 2005; Kavantzas et al. 2000; Unno et al. 2009).

As pointed out above most *MYBPC3* mutations lead to haploinsufficiency but some missense mutations might escape targeted removal and might well be expressed in the myocardium. This might particularly be true for the reported aminoterminal mutations G5R, R35W and T59A in the human genome (Sarcomere Protein Gene Mutation Data Base) as well as for the A31P mutation observed in Maine Coon cats (Meurs et al. 2005), all of which are linked to HCM. Ratti and co-workers (Ratti et al. 2011) studied above mentioned missense mutations. While G5R had only mild effects, A31P could not be expressed as a soluble protein and T59A was not chosen as in many other species a threonine residue is substituted by an alanine and no extreme effects were expected, the R35W seemed to be important for binding to RLC.

MYBPC3 mutations by modulating calcium sensitivity may also have effects, either directly or indirectly, on mechanosensation and mechanotransduction. For example, changes in calcium transients caused either via an increase in calcium sensitivity or via inefficient ATP usage, may have effects on calcium dependent enzymes which may in turn affect mechanosensory effects. For example, calcineurin mediated NFAT and GATA4 activation, protein kinase C mediated effects on MYBPC3 itself or calmodulin kinase II effects on phospholamban and or ryanodine receptor phosphorylation may contribute to changes in gene expression and myocardial remodelling. Myocardial hypertrophy and remodelling itself may lead to changes in bio-mechanical properties of the heart, which feeds back to changes in intracellular signalling (Knöll et al. 2002, 2010). The sarcomeric Z-disc is a central nodal point for signalling (Knöll et al. 2011a) and changes in Z-disc structure or composition may well affect its function. In this context, the E3 ubiquitin ligase atrogin 1 has been found to colocalize at the
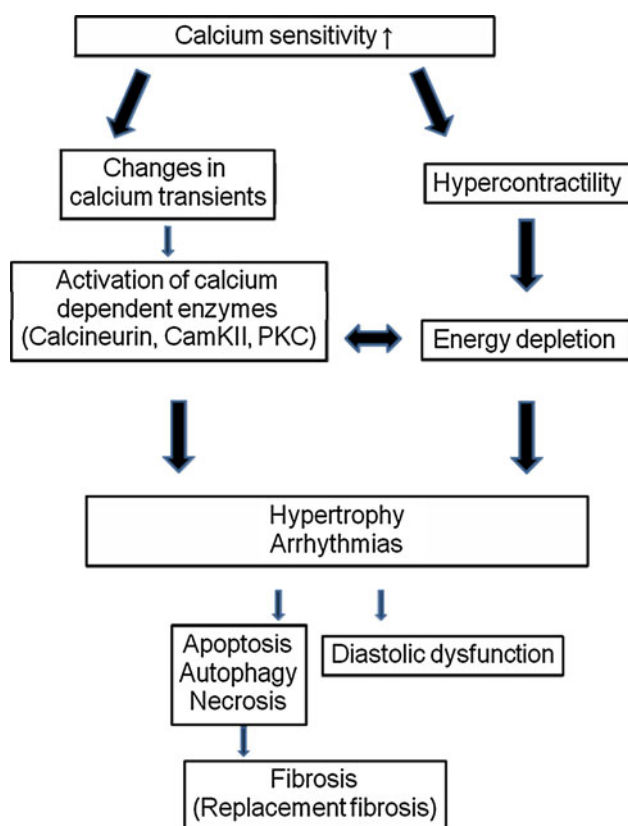
sarcomeric Z-disc with a truncated MYBPC3 which may have consequences for Z-disc mediated signalling (Mearini et al. 2010) (see also later). However it remains unclear whether above mentioned changes in intracellular signalling, if not alone able to cause effects, will sensitize the myocardium at predisposed locations to maladaptation. This may include the possibility of increased shear stress causing septal hypertrophy. It also remains to be seen whether Z-disc mediated apoptotic events play a role in MYBPC3 related HCM (Knöll et al. 2011c).

It remains also unclear, how *MYBPC3* mutations are linked to fibrosis as well to intracellular and cellular myofibrillar disarray. It might well be that fibrosis, which is probably an early event in HCM, causes myofibrillar disarray by displacing existing cardiac myocytes, but it remains unclear how intracellular disarray develops. In this context, myofibrillar disarray contributes certainly to inefficient energy use (i.e. cardiac myocytes are not aligned properly). A recent study on HCM myocardial biopsies, including those from patients affected by *MYBPC3* mutations, supports the view that fibrosis is an early event and is present even before the onset of hypertrophy (Ho et al. 2010). Inhibition of fibrosis, either via anti TGFβ antibodies or the angiotensin II type 1 receptor antagonist losartan prevented onset of hypertrophy, non myocyte proliferation and fibrosis in genotype positive but hypertrophy negative (pre-hypertrophic) animals (Teekakirikul et al. 2010). These data point as well to the importance of other cell types than cardiac myocytes which are inherent in the myocardium and which contribute significantly to the phenotype (Knöll et al. 2011b).

A novel link between an increase in myofibrillar calcium sensitivity and arrhythmias has been uncovered recently. An increase in calcium sensitivity led to a change in the shape of the ventricular action potentials in mice which results in shorter effective refractory periods, greater beat to beat variability of action potential durations and increased dispersion of ventricular conduction velocities at higher heart rates—all of which may predispose to the occurrence of arrhythmias. These effects were greatest in HCM causing troponin T mutant animals and most importantly, these effects were reproduced when the calcium sensitizer EMD57033 was used and they were reversible when blebbistatin, a calcium de-sensitizer, was employed. Another important finding of this study is that the degree of myocardial hypertrophy does not correlate with the arrhythmia risk (Baudenbacher et al. 2008). Other mechanisms may involve above mentioned energy depletion with consecutive effects on energy dependent ion exchanges (Ashrafian et al. 2003), loss of cardiac myocytes via apoptosis, necrosis or autophagy which may lead to electric isolation of single cardiac myocytes and fibrosis (Teekakirikul et al. 2010), all of which have been shown to contribute to arrhythmogenic events.

Another important approach has been used by Lucie Carrier's group, when they analyzed the degradation process of MYBPC3. This group found that the E3 ubiquitin ligase atrogin 1 interacts with both, mutant (a 32 kDa MYBPC3 truncated protein, based on a human mutation and which comprises only of domains C0 and parts of C1 termed M7t-cMyBP-C) and wildtype MYBPC3, but only the mutant MYBPC3 was targeted for degradation. This makes atrogin 1 also a novel MYBPC3 interacting protein, but whether the interaction of atrogin 1 and full length wildtype MYBPC3 is physiologically relevant remains to be seen. Interestingly atrogin 1 and M7t-cMyBP-C co-localize at the sarcomeric Z-disc, which points to the importance of this structure in protein degradation processes (Mearini et al. 2010) (reviewed in: (Knöll et al. 2011a)). Their data also indicated that muscle ring finger protein 1 (MuRF1) regulates MYBPC3 mRNA and protein via a posttranscriptional mechanism (Mearini et al. 2010). Also, calpain 1, a ubiquitously expressed calcium dependent protease and which localizes to the sarcomeric Z-disc and I-band via interactions with titin, interacts with MYBPC3 as well and might contribute to MYBPC3 turnover (Jiang et al. 2002; Kontrogianni-Konstantopoulos et al. 2009). In addition, the lysosomal inhibitor bafilomycin A1 caused an increase in truncated MYBPC3 proteins, which points to the possible involvement of autophagy in MYBPC3 related degradation processes.

As briefly mentioned above, domains C5–C8 have been hypothesized to interact with one another, forming a "trimeric collar" that constrains the thick filament. Interestingly, mutations R654H and N755K, both are HCM causing and located in C5, decreased the affinity to C8 about 2-fold and by at least 10-fold, respectively (Moolman-Smook et al. 2002). Disturbances of intra- MYBPC3 interactions by mutations are a completely novel approach to explain the complex phenotype observed in genetically altered animal models as well as in human patients.

Implications for therapy

MYBPC3 mutations like the majority of all other cardiomyopathy causing mutations are associated with incomplete, age and gender dependent penetrance. Powerful epigenetic and environmental factors are thought to have a major impact on the phenotype, which also implies the possibility of effective therapeutical interventions.

If increased calcium sensitivity is the primary event which leads to HCM in *MYBPC3* mutation carriers then calcium desensitizers might be able to influence positively disease progression, which has been shown in animal models (Baudenbacher et al. 2008) (see also above). However DCM is associated with decreased calcium sensitivity and precise

calibration of calcium sensitivity is certainly important and needs to be monitored properly, if applied in therapy. Another although still very experimental approach is to use short oligonucleotides to intervene in mRNA processing such that "exon skipping" events can be used therapeutically to prevent the synthesis of pathologic mRNAs or to use siRNAs/miRNAs to suppress pathologic mRNAs—an approach potentially important for *MYBPC3* mutations. Calcium antagonists such as diltiazem have been successfully used to suppress HCM in the R403Q alpha MHC transgenic mouse (Semsarian et al. 2002) as well as in a mouse model carrying the TnT-I79 N mutation (Westermann et al. 2006), which led to an ongoing human trial (NCT00319982). However it will be important to find out, whether *MYBPC3* mutation positive patients will respond appropriately to this type of treatment.

Also overexpression of SERCA2a in a Glu180Gly tropomyosin (Pena et al. 2010) as well as parvalbumin overexpression in a Glu180Gln tropomyosin animal model rescued the phenotype in both of these lines, which points to the possibility of manipulating the calcium metabolism in order to treat the disease (Coutu et al. 2004). However while phospholamban ablation in muscle LIM protein deficient (Minamisawa et al. 1999) and in Glu180Gly tropomysin transgenic animals (Alves et al. 2010; Gaffin et al. 2011) is favourable, ablation of the very same gene in *Mybpc3* transgenic animals is not (Song et al. 2003). It remains to be determined whether these differences are due to the well known differences between human and mouse physiology or whether these differences reflect differences in the underlying molecular mechanisms.

Another possibility might be to intervene at the level of energy metabolism via perhexiline which shifts substrate utilization away from free fatty acids to carbohydrates by inhibiting the enzyme carnitine palmitoyl transferase. Perhexiline improved cardiac energetics, normalized exercise diastolic dysfunction and increased exercise capacity in patients (Abozguia et al. 2010; Lele et al. 1995).

It remains also to be seen whether moderate physical activity in *MYBPC3* mutation carriers is favourable, as suggested by animal experimentation (Konhilas et al. 2006), and anti-apoptotic strategies may prove helpful as well. Indeed anti-apoptotic gene expression has been observed when animals were subjected to limited physical exercise (Konhilas et al. 2006). Also calcineurin inhibition may have favourable effects as suggested by animal experimentation (Sussman et al. 1998), but application of available calcineurin inhibitors such as cyclosporine A and tacrolimus, at doses needed to affect calcineurin activity in the heart, is associated with significant side effects such as hypertension, nephro-toxicity and diabetes, which precludes their application in human individuals.

Another way to inhibit hypertrophy is to use statins (3-hydroxy-3-methyglutarylcoenzyme A reductase inhibitors), which act via reducing membrane bound Ras and thus reducing pERK44/42 and ERK1/2 activation which reduced HCM related symptoms in a R403Q beta MHC transgenic rabbit model (Patel et al. 2001; Senthil et al. 2005). However human pilot studies based on these animal experiments remained disappointing (Bauersachs et al. 2007; Nagueh et al. 2010). Therefore it remains to be determined whether this approach is not applicable in general or whether a genotype–phenotype specific analysis will identify patient subgroups more susceptible to this type of treatment.

Summary

Although MYBPC3 has been the subject of intense research for almost over four decades, our understanding of its function in physiology and patho-physiology is still limited. However, *MYBPC3* mutations are a major cause of human cardiomyopathy and associated heart failure, with some disease causing mutations having frequencies of up to 4% in various populations. Most *MYBPC3* mutations lead to haploinsufficiency and to a primary increase in calcium sensitivity. This may explain major features observed in patients such as the hypercontractile phenotype, changes in calcium transients, myocardial hypertrophy, the defect in relaxation and effects on remodelling including the development of myofibrillar disarray as well as the link to live threatening arrhythmias (Fig. 4).

Future research will focus on the identification of pathways leading from a single *MYBPC3* mutation to the complex disease phenotype in patients. These pathways should be defined for every single "private" mutation. Although challenging and certainly difficult to achieve, this approach will undoubtedly result in the development and design of patient specific, novel therapies. Inducible pluripotent stem cells (iPS), gene therapy approaches, whole genome sequencing and determination of the explicit genotypes, as well as medicinal chemistry will have to be combined to increase our knowledge in this field.

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