REVIEW

Cardiac myosin binding protein C phosphorylation in cardiac disease

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Abstract Perturbations in sarcomeric function may in part underlie systolic and diastolic dysfunction of the failing heart. Sarcomeric dysfunction has been ascribed to changes in phosphorylation status of sarcomeric proteins caused by an altered balance between intracellular kinases and phosphatases during the development of cardiac disease. In the present review we discuss changes in phosphorylation of the thick filament protein myosin binding protein C (cMyBP-C) reported in failing myocardium, with emphasis on phosphorylation changes observed in familial hypertrophic cardiomyopathy caused by mutations in *MYBPC3*. Moreover, we will discuss assays which allow to distinguish between functional consequences of mutant sarcomeric proteins and (mal)adaptive changes in sarcomeric protein phosphorylation.

During recent years it has become increasingly evident that cardiac cMyBP-C exerts an important role in the regulation of sarcomere function with consequences for in vivo cardiac performance. The functional role of cMyBP-C is tightly regulated by kinase-mediated phosphorylation. The most important kinase which is known to phosphorylate cMyBP-C in vivo is protein kinase A (PKA), which is

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activated upon stimulation of the *β*-adrenergic receptors during increased stress as occurs during exercise. At the sarcomere level, *β*-adrenergic receptor activation reduces the sensitivity of myofilaments to calcium, reduces passive stiffness and increases the kinetics of cardiac contraction due to PKA-mediated phosphorylation of the three sarcomeric target proteins, cardiac troponin I (cTnI), titin, and cMyBP-C. Although cTnI exerts a "dominant" role in the reduction of myofilament Ca^{2+}-sensitivity upon PKA-mediated phosphorylation, recent studies indicated a modulatory role for cMyBP-C in this process (Cazorla et al. 2006; Cuello et al. 2011; Kooij et al. 2010b). The most important regulatory role of cMyBP-C seems to be the effect on cross-bridge kinetics of sarcomere contraction (Stelzer et al. 2006a, 2006b). Involvement of cMyBP-C and its phosphorylation in stretch activation has been demonstrated in mice by Stelzer et al. (2006b, 2007). This stretch activation might play an important role in the development of systolic pressure (Steiger 1977; Stelzer et al. 2006a). It has been proposed that cMyBP-C acts as a structural constraint limiting cross-bridge formation and that phosphorylation of cMyBP-C accelerates cross-bridge kinetics which is required for enhanced rates of relaxation and force development in diastole and systole, respectively.

cMyBP-C phosphorylation in end-stage heart failure

Systolic heart failure (SHF or heart failure with reduced left ventricular ejection fraction) is the end-stage of various cardiac diseases (e.g., ischemic heart disease, valve defects) and is characterized by ineffective functioning of the heart which then cannot supply sufficient blood to meet the body's demands. The body tries to compensate for the reduced cardiac output by sympathetic stimulation, in an

attempt to maintain vital organ perfusion via an increase in heart rate and cardiac contractility. Because of the chronic nature of the disease, this leads to a prolonged increase in catecholamine levels in the serum. This in turn results in the down-regulation and desensitization of the beta-adrenergic receptor (Bristow et al. 1982) and reduced phosphorylation of downstream PKA target proteins in the end-stage failing heart (El-Armouche et al. 2004; Schwinger et al. 1999). In addition to reduced PKA signaling, increased activity and expression of protein phosphatase 1 (PP1) has been reported in a swine model of post-myocardial infarction (MI) cardiac remodeling (Duncker et al. 2009) as well as in patients with heart failure (Neumann et al. 1997). PP1 dephosphorylates many PKA target proteins and its activity is indirectly regulated by PKA via phosphorylation of the PP1 inhibitor protein. In addition to the changes in PP1 expression/activity, a decreased activity of this endogenous inhibitor of PP1 was observed in heart failure patients (El-Armouche et al. 2004).

In end-stage failing human myocardium, reduced phosphorylation of all PKA sarcomeric target proteins has been reported. Total phosphorylation of cTnI was decreased in end-stage failing myocardium (Bodor et al. 1997; van der Velden et al. 2003; Zaremba et al. 2007). This decrease could at least partially be attributed to reduced PKA-mediated phosphorylation, as phosphorylation of the PKA-specific sites Ser23/24 was lower in failing compared to non-failing donor heart tissue (Hamdani et al. 2010; Messer et al. 2007; van der Velden et al. 2006). In addition, phosphorylation of the giant protein titin is reduced in patients with systolic and diastolic heart failure (Borbely et al. 2009; Kruger et al. 2009).

As indicated above, the other main target of PKA in the sarcomere is cMyBP-C and in cardiac tissue from end-stage heart failure patients its phosphorylation is also decreased (El-Armouche et al. 2007; Jacques et al. 2008; Zaremba et al. 2007), irrespective if the underlying cause of heart failure (Copeland et al. 2010; Hamdani et al. 2010). Using 1D gel electrophoresis and staining with the phospho-specific stain ProQ Diamond we observed reduced phosphorylation of cMyBP-C in end-stage heart failure patients with ischemic (ISHD) or idiopathic (IDCM) cardiomyopathy (Fig. 1a). Protein phosphorylation can be studied by a number of methods, one of which is 2D gel electrophoresis. This technique is based on the iso-electric point of a protein, which is reciprocally correlated to the amount of phosphorylation. With this method, it was shown that the cMyBP-C protein spots separated on the 2D gel (Fig. 1b) were shifted to the basic side (higher pI) in failing compared to donor cardiac samples in support for decreased cMyBP-C phosphorylation in both ischemic and idiopathic heart failure (Fig. 1c). Another method to quantitatively study protein phosphorylation is by phosphate affinity gel

electrophoresis, in which the degree of phosphorylation is inversely related to the migration speed in the gel (Kinoshita et al. 2006). In human donor heart tissue, it was shown that most of the cMyBP-C exists in mono-, bi-, tri-, or tetra-phosphorylated forms with very little of the unphosphorylated form, while in hearts from end-stage heart failure patients the unphosphorylated form is predominant, with only some mono-phosphorylated cMyBP-C (Copeland et al. 2010). Protein phosphorylation can also be studied by using phosphorylation site-specific antibodies. cMyBP-C can be phosphorylated in vivo on at least three sites, all of which are located in the cardiac isoform specific M region, i.e., Ser273, Ser282, and Ser 302 (Barefield and Sadayappan 2010). At least one other site should exist in humans (Copeland et al. 2010) and multiple sites are predicted from studies in animal models (Yuan et al. 2006) or on the basis of in vitro studies (Jia et al. 2010). Using antibodies specific for these sites, it was shown that Ser282 phosphorylation was markedly reduced in end-stage failing heart tissue (El-Armouche et al. 2007), as was phosphorylation of Ser273 and Ser302 (Copeland et al. 2010).

cMyBP-C in familial hypertrophic cardiomyopathy (FHCM)

Another class of cardiac disease consists of the inherited cardiomyopathies (Watkins et al. 2011). In these forms of cardiomyopathy, cardiac dysfunction, and altered morphology are caused by genetic mutations rather than an external cause, such as coronary artery disease or hypertension. FHCM is the most prevalent inherited cardiac disease and has a prevalence of 1:500 (Maron 2004). FHCM is most frequently caused by mutations in genes coding for sarcomeric proteins (Richard et al. 2003). Interest in cMyBP-C grew when it became apparent that ~40% of the FHCM causing mutations were located in the MYBPC3 gene (Richard et al. 2003).

The sarcomeric phosphorylation pattern in HCM shows a great deal of overlap with that in heart failure. Similar to heart failure samples, phosphorylation of cTnI was found to be reduced in FHCM (Hoskins et al. 2010; van Dijk et al. 2008; van Dijk et al. 2009b). Cardiac MyBP-C phosphorylation was also lower in FHCM patients (Hoskins et al. 2010; Jacques et al. 2008; van Dijk et al. 2008; van Dijk et al. 2009b), and Copeland et al. (2010) showed a shift towards unphosphorylated and mono-phosphorylated cMyBP-C similar to end-stage failing myocardium. The lower cMyBP-C phosphorylation in FHCM hearts was found in patients in whom no mutation was found in the genes coding for cMyBP-C, β-myosin heavy chain and troponin T. Figure 2a shows protein data from patients (NYHA class II) who underwent myectomy surgery to
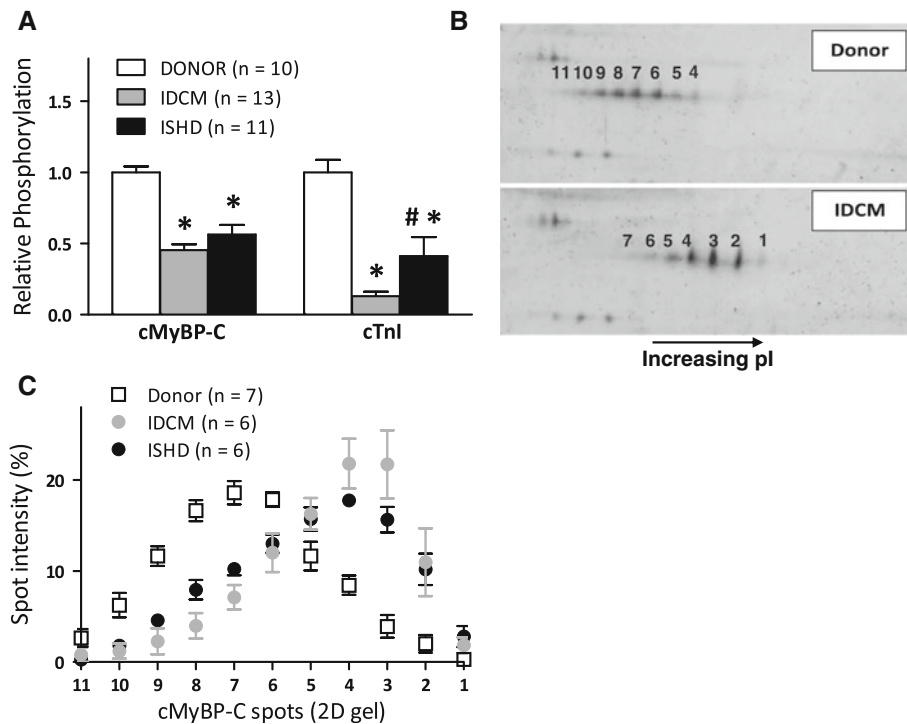
Fig. 1 Cardiac MyBP-C phosphorylation in end-stage heart failure. **a** Samples from donor, end-stage heart failure patients with IDCM or ISHD cardiomyopathy were analyzed for cMyBP-C and cTnI phosphorylation with ProQ Diamond stained gels and normalized to total SYPRO-stained cMyBP-C. Phosphorylation of cardiac cMyBP-C and cTnI is higher in donor compared to the ISHD and IDCM samples. Phosphorylation in the groups was normalized to donor, which was set to one. Figure adapted from Hamdani et al. (2010) with kind permission from Springer Science + Business Media. **b** Representative 2D-gels from donor and IDCM, showing a shift of cMyBP-C towards higher pI spots (*left*) in IDCM compared with donor, indicating less phosphorylation. Figure adapted from Copeland et al. (2010) with permission from Elsevier. **c** Quantification of the different spots from 2D gel analysis showing similar shifts in cMyBP-C phosphorylation in IDCM and ISHD compared to donor. *$P < 0.05$ versus donor in one-way ANOVA followed by post-test Bonferroni; #$P < 0.05$ versus IDCM in one-way ANOVA followed by post-test Bonferroni analysis

restore left ventricular outflow and from explanted heart samples of end-stage failing FHCM patients. In the Netherlands two founder mutations in *MYBPC3* account for 35% of all HCM mutations (Alders et al. 2003). Although these mutations are predicted to result in truncated proteins, no mutant protein was found, indicating haplo insufficiency rather than a toxic peptide as the cause of FHCM (van Dijk et al. 2009a). In this patient population cMyBP-C protein levels were 33% lower than donor levels, but surprisingly relative phosphorylation of cMyBP-C was not different between patients and donors, whereas cTnI phosphorylation was notably reduced (van Dijk et al. 2009a) (Fig. 2b). The observation that cMyBP-C phosphorylation was similar in patients with *MYBPC3* mutations compared to donors may be explained by an altered stoichiometry between kinase activities in the cardiac cells and cMyBP-C protein levels. The reduction in cMyBP-C protein level may match the reduction in kinase activities in FHCM with *MYBPC3* mutations.

In both end-stage failing and most FHCM hearts phosphorylation was markedly lower than in donor hearts.

It has been debated whether donor hearts truly represent the normal situation and can therefore be used as controls (Jweied et al. 2007; Marston and de Tombe 2008). Especially the fact that many donors receive positive inotropic support and may have brain damage leading to a catecholamine surge is of concern to studies of protein phosphorylation, as these conditions would lead to enhanced PKA activity. Furthermore, it has recently been shown that different tissue procurement strategies can affect the phosphorylation of sarcomeric proteins (Walker et al. 2011). Noteworthy, in the latter study cMyBP-C phosphorylation did not change with different procurement strategies.

The observation that cMyBP-C is less phosphorylated in a host of different cardiac disease states does not directly establish the functional consequences of lower phosphorylation of cMyBP-C. To study the functional consequences of cMyBP-C phosphorylation, either transgenic animal models can be used or studies can be performed by modulating phosphorylation in vivo or in vitro.
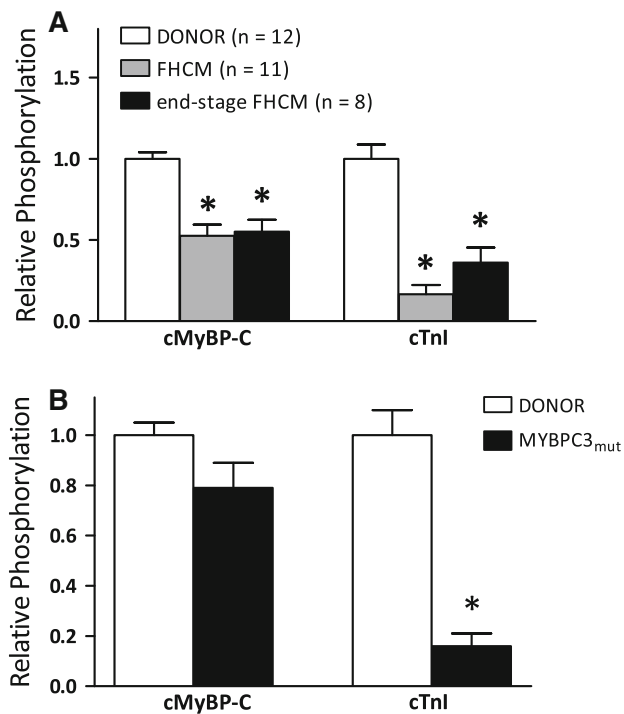
Fig. 2 Cardiac MyBP-C phosphorylation in FHCM. a Phosphorylation status of cMyBP-C and cTnI was assessed by ProQ Diamond stained gels and signal was normalized to the SYPRO Ruby stained cMyBP-C and α-actinin band respectively as described before (Zaremba et al. 2007). Phosphorylation status of cMyBP-C and cTnI was lower in cardiac tissue obtained from myectomy operation from FHCM patients (NYHA class II) and in cardiac tissue obtained from explanted hearts from end-stage FHCM patients (NYHA class IV) compared to donor samples. Phosphorylation in the groups was normalized to donor, which was set to one. b In a subgroup of FHCM patients with *MYBPC3* mutations ($MYBPC3_{mut}$), the phosphorylation status of cMyBP-C was similar between FHCM and donor samples, while cTnI phosphorylation was lower. Phosphorylation was normalized to donor, which was set to one. *$P < 0.05$ versus donor in one-way ANOVA followed by post-test Bonferroni analysis. Figure adapted from van Dijk et al. (2009a) with permission

Transgenic animal models

The effects of cMyBP-C phosphorylation on its physiological function have been demonstrated with transgenic animal models. It has been established that cMyBP-C is vital for normal cardiac performance as complete knock-out of cMyBP-C (Carrier et al. 2004; Harris et al. 2002) or homozygous expression of a mutated *MYBPC3* gene, resulting in less than 10% expression of truncated cMyBP-C (McConnell et al. 1999; Sadayappan et al. 2005), both led to a dilated and dysfunctional heart with cardiomyocyte disarray and fibrosis. Sadayappan and colleagues provided evidence that apart from cMyBP-C expression level also its phosphorylation is essential for cardiac performance. They showed that transgenic expression of wild-type cMyBP-C could rescue the phenotype of mice carrying homozygous

mutated *MYBPC3,* while expression of cMyBP-C protein in which the three well-known phosphorylation sites (Ser273, 282, and 302) were replaced by unphosphorylatable alanines (cMyBP-C^{AllP-}) did not correct dysfunction (Sadayappan et al. 2005). Furthermore, transgenic expression of cMyBP-C in which the known phosphorylation sites were replaced by the negatively charged aspartic acid (cMyBP-C^{AllP+}), to mimic constitutive tri-phosphorylation, was able to rescue the phenotype of the null mutant (Sadayappan et al. 2006). Cardiomyocytes isolated from cMyBP-C^{AllP-} mice showed a reduced stretch activation after PKA treatment compared to mice expressing wild-type cMyBP-C (Tong et al. 2008). In addition, the hearts of these animals showed an attenuated dobutamine-induced (Dob) contractile reserve compared with animals expressing the wild-type protein. To further elucidate the role of the individual phosphorylation sites in cMyBP-C, Sadayappan et al. (2011) used a transgenic mouse model in which the Ser282 site was either converted to an alanine or to an aspartic acid and bred into the cMyBP-C null mutant. This revealed that phosphorylation of the Ser302 depends on Ser282 phosphorylation, while the Dob-induced increase in cardiac contractility was dependent on all three sites being phosphorylated (Sadayappan et al. 2011). Taken together this illustrates the vital role of cMyBP-C phosphorylation for its physiological function and a proper cardiac performance.

Phosphorylation of cMyBP-C is not only important in contraction, but also seems to exert a protective effect against protein degradation as a canine model of low flow ischemia showed cMyBP-C dephosphorylation and degradation (Decker et al. 2005). Similar degradation was seen in a mouse model of ischemia–reperfusion, in which cMyBP-C was protected against degradation in mice with cMyBP-C^{AllP+} (Sadayappan et al. 2006). cMyBP-C^{AllP+} also protected against ischemia–reperfusion injury, as the ischemic area and apoptosis were reduced and fractional shortening was increased compared with wild-type cMyBP-C (Sadayappan et al. 2006, 2009).

Modulation of cMyBP-C phosphorylation

Kinases

To establish which kinases are responsible for cMyBP-C phosphorylation, both in vivo and in vitro studies have been used. The first clue that PKA could phosphorylate cMyBP-C was the observation that phosphorylation of cMyBP-C was increased after beta-adrenergic receptor stimulation in rat hearts (Jeacocke and England 1980) and frog atria (Hartzell and Titus 1982). In a follow-up study it was shown that PKA could phosphorylate isolated

cMyBP-C in vitro (Hartzell and Glass 1984). In agreement, PKA incubation of skinned cardiomyocytes from end-stage failing heart tissue showed a shift towards cMyBP-C forms with a lower iso-electric point (increased pH) on 2D gel electrophoresis, indicating increased phosphorylation (Fig. 3a, b). In vitro phosphorylation of isolated cMyBP-C by PKA in the presence of [^{32}P] ATP, followed by proteolysis and sequencing could pinpoint the three phosphorylation sites to the cardio-specific region between the C1 and C2 domains of the protein (Gautel et al. 1995; Mohamed et al. 1998). Functionally it was found that phosphorylation of cMyBP-C with PKA lead to changes in thick filament structure and an increased level of weakly bound cross-bridges at low Ca^{2+}-levels, which could lead to increased force generating cross-bridges during systole (Levine et al. 2001).

An area that has received a lot of attention in the last years, is the compartmentalization of PKA signaling, which enhances its specificity. This spatial regulation is mediated by so-called A-kinase anchoring proteins (AKAPs) that bind PKA and are able to localize to specific subcellular compartments (Fink et al. 2001; Ruehr et al. 2004). The thin filament protein troponin T has been identified as a sarcomeric AKAP, which provides a pool of PKA that can quickly phosphorylate myofilament proteins upon activation (Sumandea et al. 2011). Recently, myomegalin was shown to interact with cMyBP-C and act as an AKAP (Uys et al. 2011).

To test the role of PKA-mediated cMyBP-C phosphorylation in cardiac pathology, phosphorylation changes were studied in remodeled myocardium of swine 3 weeks after MI at baseline and upon in vivo administration of the beta-adrenergic receptor agonist Dob (Boontje et al. 2011; Duncker et al. 2009). At baseline there were no differences in cMyBP-C and cTnI phosphorylation in the post-MI animals compared with sham operated animals (Fig. 3c, d). Dob administration had a discordant effect on the PKA targets, as the increase in cTnI phosphorylation was markedly attenuated in the post-MI hearts compared to sham, while cMyBP-C was not different between post-MI

Fig. 3 PKA-mediated cMyBP-C phosphorylation in vitro and in vivo. a 2D-gel analysis of cMYBP-C from IDCM myocardium before and after PKA treatment, showing an shift towards lower pI species, indicating increased phosphorylation. b Quantification of the changes in phosphorylation after PKA treatment. c, d Effect of high and low dose Dob administration (2 and 10 µg/kg/min; Dob2 and Dob10) on the phosphorylation of the PKA-specific Ser23/24 cTnI site (n = 5) and Ser282 cMyBP-C site (n = 6) in sham and post-MI remodeled myocardium from pigs. cTnI phosphorylation increased significantly in sham animals but this increase was attenuated in MI animals. cMyBP-C was not different between MI and sham animals. Figure adapted from Boontje et al. (2011) with permission from Elsevier. *P < 0.05, effect of Dob in a 1-way ANOVA
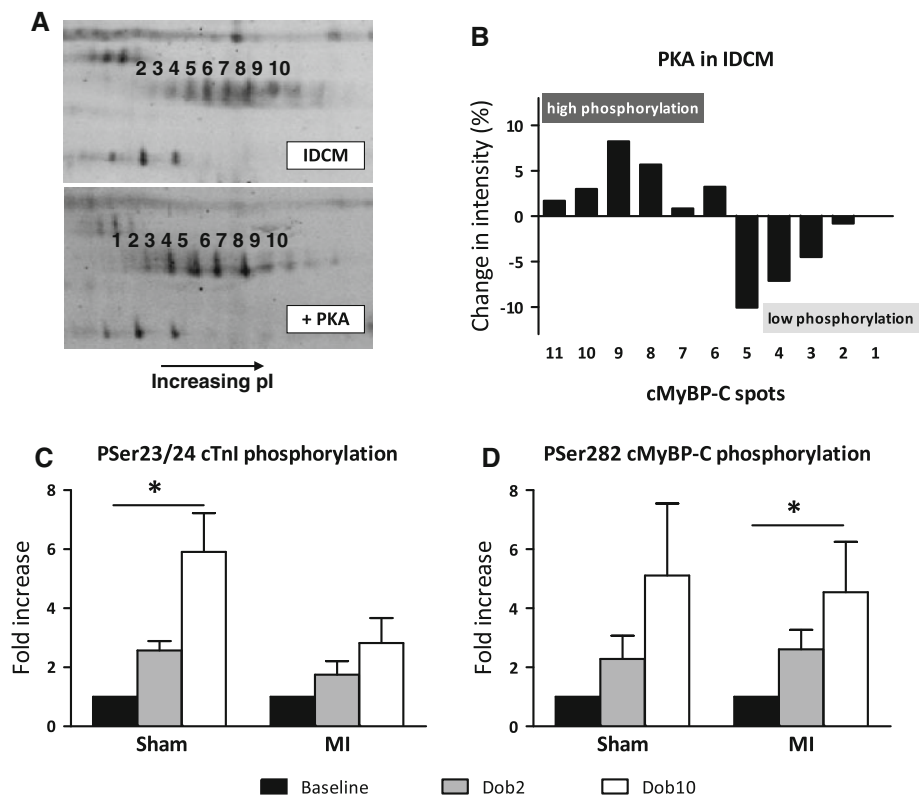
and sham hearts (Fig. 3c, d). This might be explained by an increased activation of Ca^{2+}-dependent calmodulin kinase II (CaMKII) in post-MI myocardium (Boontje et al. 2011).

Calmodulin kinase II was first found to phosphorylate cMyBP-C as a Ca^{2+}-calmodulin dependent kinase co-purified with cMyBP-C isolated from chicken hearts (Hartzell and Glass 1984), which was subsequently identified as CaMKII (Schlender and Bean 1991). Whereas PKA could add 3 mol of phosphates per mole of cMyBP-C, CaMKII could add only one (Gautel et al. 1995). Furthermore, it seems that the site now identified as Ser282 is the target site for CaMKII (Gautel et al. 1995) and that this site needs to be phosphorylated first to facilitate phosphorylation of Ser302 (Sadayappan et al. 2011). Functionally, CaMKII phosphorylation seems to be important in the frequency-dependent increase in force as this was depressed in intact muscle treated with a CaMKII inhibitor (Tong et al. 2004).

Protein kinase C can also phosphorylate cMyBP-C, as revealed by in vitro phosphorylation studies with recombinant PKC in vitro (Lim et al. 1985; Venema and Kuo 1993) or by PKC stimulation in intact cardiomyocytes (Venema and Kuo 1993). The phosphorylation sites of PKC on cMyBP-C overlap with those of PKA, as two of the three PKA-sites were also phosphorylated by PKC (Mohamed et al. 1998). PKC incubation combined with phosphorylation site-specific antibodies revealed that Ser273 and Ser302 are PKC target sites (Sadayappan et al. 2011). Protein kinase C is composed of a family of kinases, of which in the heart PKC-α is known to be upregulated in heart failure (Bowling et al. 1999). To study the effect of different PKC isoforms on cMyBP-C and cTnI phosphorylation, incubations with PKC-α and the novel–non Ca^{2+}-activated-PKC-ε in skinned cardiomyocytes from failing tissue were performed (Kooij et al. 2010a). Both isoforms could phosphorylate cMyBP-C and cTnI, albeit with different substrate affinities (Fig. 4). Phosphorylation of cMyBP-C by another isoform of PKC, namely PKCζ, was shown by expressing a constitutively active form in cardiomyocytes (Wu and Solaro 2007). PKC phosphorylation of cMyBP-C is proposed to cause a decrease in actomyosin ATPase, which could be cardioprotective (Pyle et al. 2003).

Recently, Protein kinase D (PKD) and p90 ribosomal S6 kinase (p90 RSK or RSK) are added to the gamut of kinases able to phosphorylate cMyBP-C. PKD phosphorylates cTnI at the same sites as PKA (Haworth et al. 2004), while it phosphorylates cMyBP-C only at Ser302 (Bardswell et al. 2010). RSK on the other hand phosphorylates cMyBP-C at Ser-282 (Cuello et al. 2011). Phosphorylation of cMyBP-C with RSK was accompanied by a reduction in Ca^{2+}-sensitivity of force development and acceleration of
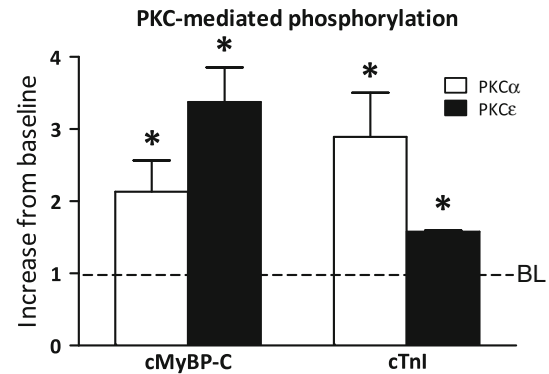

Fig. 4 Phosphorylation of cTnI and cMyBP-C by protein kinase C. Phosphorylation of cMyBP-C and cTnI after incubation of failing tissue samples with PKCα ($n = 5$) or PKCε ($n = 2$). Phosphorylation was assessed by ProQ Diamond stained gels and normalized to total SYPRO-stained cMyBP-C. Both PKC isoforms increased phosphorylation of cMyBP-C and cTnI, albeit with different specificities. *$P < 0.05$ versus baseline. *BL* baseline. Figure adapted from (Kooij et al. 2010a) with permission

cross-bridge kinetics, independent from cTnI phosphorylation (Cuello et al. 2011).

Phosphatases

In contrast to the extensive body of work published about kinase-mediated phosphorylation of cMyBP-C, only a limited number of studies have focused on the effects of phosphatases. As was mentioned above, cardiac PP1 expression and activity is increased in heart failure (Neumann et al. 1997). Purified cMyBP-C that was first phosphorylated by PKA, was subsequently dephosphorylated by 30–40% by incubation with the catalytic subunit of PP1 (Schlender et al. 1987). The same extent of dephosphorylation was seen after incubation of skinned donor heart tissue with PP1 (Yang et al. 2008; Zaremba et al. 2007) as well as in skinned mice cardiomyocytes (Yang et al. 2008). Incubations of donor tissue with a high concentration of PP1 resulted in almost complete dephosphorylation of cTnI, while cMyBP-C dephosphorylation was moderate (Fig. 5). PP1 incubations led to the decrease of the tri- and tetra-phosphorylated cMyBP-C and an increase in the unphosphorylated form (Copeland et al. 2010). Functionally, PP1 incubation in donor tissue resulted in a marked increase in the Ca^{2+}-sensitivity of force development, which could be reversed by subsequent PKA incubation (Neulen et al. 2007). Although in another study, PP1 incubation had no effect on Ca^{2+}-sensitivity in donor cells, but increased Ca^{2+}-sensitivity after pre-incubation with PKC (Belin et al. 2007). The effect of PP1 on Ca^{2+}-sensitivity is likely explained by the dephosphorylation of TnI, rather than cMyBP-C phosphorylation (Duncker et al. 2009).
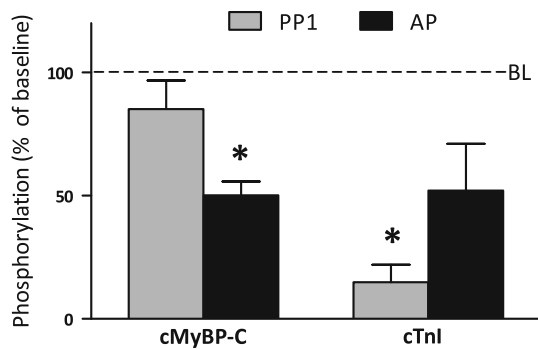
Fig. 5 Dephosphorylation of cTnI and cMyBP-C by protein PP1 and AP. Incubations of skinned donor tissue with PP1 and AP were performed as described before (Zaremba et al. 2007). Briefly, human donor tissue ($n = 3$) was obtained during cardiac surgery and frozen and stored in liquid N_2. Samples were homogenized in buffer containing 0.5% (v/v) Triton X-100. Samples were washed twice in buffer without Triton and 100 μl sample was subsequently incubated with 10 μl PP1 (catalytic subunit, Sigma) or 10 μl AP (calf intestinal; New England Biolabs). Phosphorylation was assessed by ProQ Diamond stained gels and normalized to total SYPRO-stained α-actinin. PP1 preferentially dephosphorylates cTnI, while AP also dephosphorylates cMyBP-C. *$P<0.05$ versus baseline. *BL* baseline

Alkaline phosphatase (AP) is a widely available phosphatase that is commonly used for in vitro dephosphorylation assays. AP mainly dephosphorylates troponin T in the sarcomere, while cMyBP-C and TnI are significantly but to a lesser degree dephosphorylated (Kooij et al. 2010a; Zaremba et al. 2007) (Fig. 5). Functionally, incubation of myofilaments from donor hearts with AP leads to a slight, but significant increase in Ca^{2+}-sensitivity of force development and passive force (Kooij et al. 2010a), but the role of cMyBP-C dephosphorylation herein is unclear.

Protein phosphatase 2a (PP2a) is able to dephosphorylate cMyBP-C (Schlender et al. 1987), although incubation of skinned cardiomyocytes with PP2a showed only a low degree of cMyBP-C dephosphorylation (Zaremba et al. 2007). A proteomic study on rat cardiomyocytes showed that the PP2a regulatory subunit B56α, is present in the myofilaments after skinning and that B56α level decreases after β-adrenergic receptor stimulation (Yin et al. 2010). PP2a's sarcomeric localization is regulated via its interaction with P^{21}-activated kinase-1 (PAK1) (Sheehan et al. 2007). Cultured cardiomyocytes with increased PAK1 activity and thus sarcomeric localization of PP2a, had lower phosphorylation of cMyBP-C and cTnI (Ke et al. 2004). Conflicting data is published about the effect of PP2a incubation on skinned cardiomyocytes. While Belin et al. (2007) found no changes on myofilament function in either non-failing or failing rat cardiac tissue incubated with PP2a, a recent paper by Wijnker et al. (2011) showed an increased Ca^{2+}-sensitivity of force development in skinned cardiomyocytes isolated from human donor hearts, but not from end-stage failing hearts. This effect

was attributed to dephosphorylation of cTnI, as PP2a did not dephosphorylate cMyBP-C (Wijnker et al. 2011). Further research is warranted to see if cMyBP-C is a target of PP2a in vivo.

Employing kinases and phosphatases to specifically phosphorylate or dephosphorylate cMyBP-C should help to distinguish between functional consequences of mutant sarcomere proteins and (mal)adaptive changes in sarcomeric protein phosphorylation. While a number of kinases have been identified that readily could phosphorylate cMyBP-C, no specific phosphatase has been found that can dephosphorylate cMyBP-C to a large extent. Whether dephosphorylation pathways play a role in modulating the (patho)physiological role of cMyBP-C warrants further study.

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