

# Functional alterations of cardiac proteasomes under physiological and pathological conditions

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The cardiac proteasome is a complex, heterogeneous, and dynamic organelle. Its function is regulated by its molecular organization, post-translational modifications, and associated partner proteins. Pressure overload, ischaemic heart disease, or genetic mutations in contractile proteins can cause heart failure, during which misfolded protein levels are elevated. At the same time, numerous interconnected signal transduction pathways are activated that may modulate any of the three proteasomal regulatory mechanisms mentioned above, resulting in functional changes in cardiac proteasomes. Many lines of evidence support the important role of the ubiquitin-proteasome system (UPS) in the development of heart diseases. Many researchers have focused on the UPS, applying new drug discovery methods not only in the field of cancer research but also in cardiovascular fields such as cardiac hypertrophy and ischaemic heart diseases. More understanding of UPS in the pathophysiology of heart diseases will lead to new routes for therapy.

**Keywords** Proteasome regulation • Molecular organization • Post-translational modification • Associating partner protein • Heart failure

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## 1. Introduction

Cellular homeostasis and function require properly folded proteins. Aberrant three-dimensional structures in misfolded or damaged proteins can result from mutated protein sequences or environmental factors, such as thermal, osmotic, or oxidative stress.<sup>1,2</sup> Protein misfolding may expose hydrophobic amino acid residues that should be located inside the protein, which often results in aggregation,<sup>1,3</sup> impairment of protein and cellular function, and potentially cell death.<sup>4</sup> To address damaged proteins, eukaryotic cells have developed multilayered protein quality control mechanisms that are carried out primarily by chaperones, the ubiquitin-proteasome system (UPS), and autophagy. Among these processes, the UPS functions to eliminate ubiquitinated proteins as well as damaged, oxidized, and unfolded proteins before they aggregate.<sup>5</sup> This is particularly important in non-dividing cells, such as neurons and cardiomyocytes, in which such proteins are not diluted during cell proliferation.<sup>6</sup>

Strong associations between proteasomal dysfunction and cell death have been observed in many neurodegenerative diseases,

suggesting that aberrant protein quality control may have catastrophic consequences.<sup>7</sup> Recently, a growing body of evidence suggests that alteration of proteasome-mediated protein degradation also contributes to the initiation and/or progression of cardiac diseases.<sup>8–10</sup>

## 2. Regulation of the cardiac proteasome

In eukaryotic cells, the UPS maintains homeostasis via regulating a variety of cellular functions. Poly-ubiquitination of target protein through lysine 48 (Lys48) results in degradation by the 26S proteasome, while poly-ubiquitination through Lys63 or mono-ubiquitination of lysine residues can be used for other purposes such as DNA repair, re-localization, or endocytosis.<sup>11</sup> In particular, the UPS is a non-lysosomal intracellular protein degradation pathway; approximately 80–90% of intracellular proteins are degraded by proteasomes.<sup>12</sup>

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## 2.1 Molecular organization of the proteasome 20S core particle

The 20S proteasome is composed of four stacked rings, each of them containing seven subunits. The two internal rings ( $\beta$ -rings) contain the catalytic subunits ( $\beta 1$ ,  $\beta 2$ , and  $\beta 5$ ), whereas the outer rings ( $\alpha$ -rings) form a gated channel through which polypeptides enter the central chamber where they are exposed to multiple proteolytic sites. The passage of proteins and peptides through this channel is restricted by the N-terminal regions of the  $\alpha$ -subunits, which function as a gate.<sup>13</sup> The closed gate maintains the 20S proteasome in a latent state with low activity. Opening of the gate is tied to an ATP- and ubiquitin-independent interaction between the 20S proteasome and the exposed hydrophobic residues of oxidized proteins; in this manner, the 20S proteasome plays a major role in the degradation of oxidized proteins in the cytoplasm, nucleus, and endoplasmic reticulum (ER) of eukaryotic cells.<sup>5</sup>

### 2.1.1 Variable form of 20S core subunits

In higher eukaryotes, the 20S core is observed in a variety of forms. For example, interferon- $\gamma$  (IFN- $\gamma$ ) results in replacement of the constitutive catalytic subunits  $\beta 1$ ,  $\beta 2$ , and  $\beta 5$  with the immunosubunits  $\beta 1i$ ,  $\beta 2i$ , and  $\beta 5i$ , respectively.<sup>14</sup> Nitric oxide and heat shock are also reported to upregulate immunoproteasome levels.<sup>15,16</sup> The different proteasome complexes, such as the regular proteasome and immunoproteasome, exhibit different catalytic efficiencies.<sup>14,17</sup> These catalytic alternations in the immunoproteasome produce well-defined effects on the production of major histocompatibility complex (MHC) class I antigenic peptides.<sup>18</sup> Each cell type or tissue may contain specific proportions of regular 20S proteasomes and immunoproteasomes.<sup>19</sup> This ratio can also vary in response to environmental factors, such as cytokines<sup>20</sup> or ageing.<sup>21</sup> Such changes have been observed in cardiomyocytes. The cardiac proteasome contains a variable 20S proteasome complex consisting of different proportions of  $\beta$ -subunits.<sup>22,23</sup> Alternations in proteasome subunit composition affect overall proteasome proteolytic activity, which, in turn, may alter the specificity and selectivity of the proteasome for various substrates under certain conditions. The physiologic consequences of such changes, however, have not yet been elucidated.

### 2.1.2 Regulatory particles

The  $\alpha$ -rings create attachment sites that activate such regulatory particles (RPs) as the 19S RP (PA700), 11S RP (PA28 $\alpha$ ,  $\beta$ , and  $\gamma$ ), and PA200 as well as inhibitory RPs, including PI31.<sup>14</sup> Thus, the proteasome assumes multiple forms: the free 20S proteasome, 26S proteasome (20S proteasome with one PA700), 30S proteasome (20S proteasome with two PA700), immunoproteasome (20S proteasome associated with PA28), and hybrid proteasome (20S proteasome with one PA700 and one PA28 or PA200),<sup>14</sup> presenting another layer of proteasome complexity and heterogeneity.

The 26S proteasome is responsible for the recognition and ATP-dependent degradation of polyubiquitinated proteins. Mammalian 19S RPs are subdivided into those that assemble as the base and lid subcomplexes, which are connected by Rpn10.<sup>14,24</sup> The base of the 19S RP is composed of six AAA ATPase subunits (Rpt1-6) and three non-ATPase subunits (Rpn1, Rpn2, and Rpn13). The six

ATPase subunits form a hexameric ring, which binds to the  $\alpha$ -ring of the 20S core particle, leading to opening of the 20S gated pore and increased proteolytic activity.<sup>14,24</sup> The lid of the 19S RP is composed of Rpn3, Rpn5-9, Rpn11, Rpn12, and Rpn15.<sup>24</sup> Importantly, the 19S RP recognizes substrates (polyubiquitinated proteins) directly via Rpt5 and Rpn10<sup>25,26</sup> and indirectly via Rpn1 and Rpn2,<sup>14,27</sup> deubiquitinates substrates using Rpt11,<sup>14</sup> unfolds substrates using the ATPase activity,<sup>28</sup> and allows substrates to enter into the 20S core proteolytic particle through the activity of the base subcomplex, in particular Rpt2.<sup>13</sup> Gomes *et al.*<sup>23</sup> reported that 19S subunits from murine hearts contained two alternatively spliced isoforms of Rpn10 (Rpn10a and Rpn10b), although the functional implications of these variants are unknown.

PA28 $\alpha$  and  $\beta$  are expressed in many organs, with particularly high levels observed in immune tissues. PA28 $\gamma$  is abundantly expressed in brain compared with moderate expression levels in other organs. PA28 $\alpha$  and  $\beta$  are mainly cytoplasmic, whereas PA28 $\gamma$  is confined to the nucleus.<sup>29</sup> The PA28 $\alpha$  and  $\beta$  subunits form a heteroheptamer, whereas PA28 $\gamma$  forms a homoheptamer. One study found that PA28 $\alpha$  was associated with the 20S proteasome, but was limited in 26S proteasomes purified from hearts.<sup>23</sup> The binding of 11S complexes to the 20S core particle induces opening of the 20S gate and increased proteolytic activity.<sup>29</sup> PA28 $\alpha$  and  $\beta$  cleave peptide backbones after basic, acidic, and hydrophobic residues, whereas PA28 $\gamma$  activates proteasome hydrolysis of peptides with basic residues with modest cleavage observed after acidic and hydrophobic residues.<sup>30</sup> Like immunosubunits, the expression of PA28 $\alpha$  and  $\beta$  subunits are induced by INF- $\gamma$ ,<sup>29</sup> suggesting that the 11S complex contributes to antigen processing. PA28 $\alpha$  and  $\beta$  levels are also upregulated by continuous motor nerve stimulation,<sup>31</sup> serum withdrawal, amino acid starvation,<sup>32</sup> and ageing.<sup>33</sup> In contrast, PA28 $\gamma$  is unaffected by INF- $\gamma$  and can show markedly reduced levels during infection.<sup>34</sup> PA28 $\gamma$  is reportedly associated with cell-cycle progression and apoptosis.<sup>35</sup> Interestingly, recent studies suggest a role for the immunoproteasome in the removal of damaged or senescent proteins.<sup>21,36</sup>

PA200, an asymmetric, hollow, dome-like structure, binds to the 20S core particle as a monomer. PA200 binding also induces opening of the entrance and exit gates in the 20S proteasome, resulting in enhanced cleavage after acidic residues relative to basic or hydrophobic amino acid.<sup>37,38</sup> PA200 expression is confined to the nucleus. In heart, PA200 was found in total heart lysate, but not in purified cytosolic 26S or 20S proteasome samples.<sup>23</sup>

## 2.2 Post-translational modification of proteasome subunits

Post-translational modifications (PTMs) are key regulatory mechanisms for proteasomes. Phosphorylated subunits of 20S proteasomes reportedly include  $\alpha 2$ ,  $\alpha 3$ ,  $\alpha 4$ ,  $\alpha 5$ ,  $\alpha 6$ ,  $\alpha 7$ , and  $\beta 6$  in both yeast and mammalian tissues.<sup>39,40</sup> Phosphorylation of  $\alpha 7$  by protein kinase CK2 stabilizes the 26S proteasome, whereas phosphorylation of Rpt6 in the 19S RP regulates the assembly and activity of the 26S proteasome through a direct association with the 20S proteasome  $\alpha 3$  subunit.<sup>41-43</sup> INF- $\gamma$  decreases the level of phosphorylation of

both  $\alpha 3$  and  $\alpha 7$ , suggesting that downregulation of 26S proteasome levels after INF- $\gamma$  treatment results from dephosphorylation-dependent destabilization of 26S proteasome.<sup>44</sup>

26S proteasomes purified from the livers of ethanol-fed rats are hyperphosphorylated and show decreased chymotrypsin-like activity.<sup>45</sup> On the other hand, phosphorylation of purified 20S proteasomes from murine hearts using active protein kinase A (PKA) was shown to enhance 20S proteolytic activities.<sup>40</sup> In this study, novel phosphorylation profiles of various proteasome subunits, including  $\alpha 1$ ,  $\beta 2$ ,  $\beta 3$ , and  $\beta 5$ , were identified.<sup>40</sup> Based on isoelectric points, a measure largely reflective of the phosphorylation state, heart contains a different 20S proteasome subpopulation compared with the liver. These different subpopulations have unique molecular compositions and proteolytic activity profiles.<sup>22</sup> Phosphorylation of 11S also contributes to the regulation of proteasome activity.<sup>39</sup>

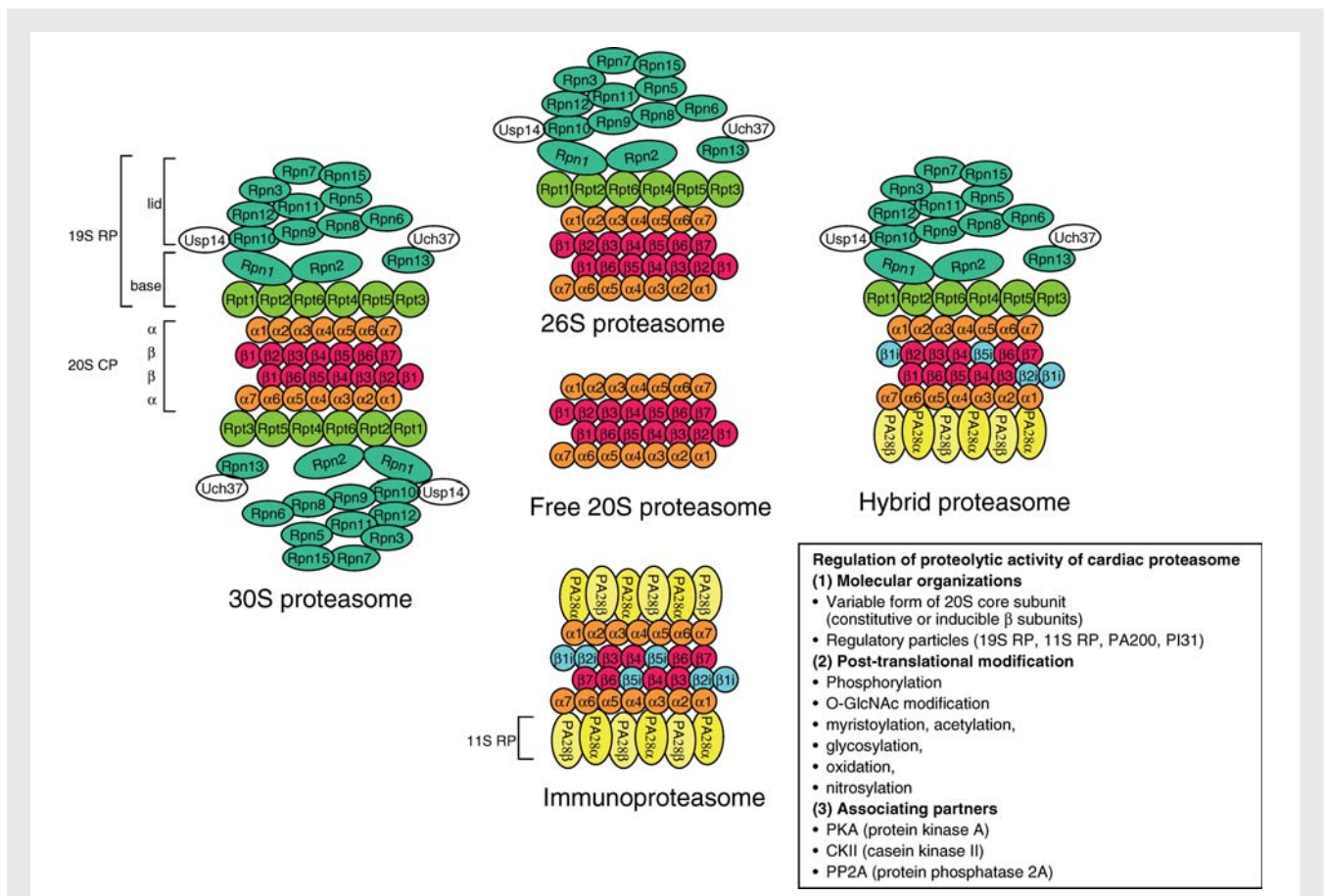
Interestingly, mammalian proteasomes are modified and regulated by metabolic factors. For instance, proteasome activity in muscle cells is stimulated during starvation.<sup>46</sup> O-GlcNAc modification of Rpt2 by O-GlcNAc transferase reversibly inhibits proteasome function.<sup>47</sup> This proteasome modification may allow the organism to respond to its metabolic needs by controlling the availability of amino acids and regulatory proteins.

Other PTMs, such as N-terminal myristoylation (Rpt2) and N-terminal acetylation of several proteasome subunits ( $\alpha 2$ ,  $\alpha 7$ ,  $\alpha 5$ ,  $\beta 3$ ,  $\beta 4$ , Rpt3, Rpt6, Rpn1, Rpn5, and Rpn6), have also been observed in the murine heart.<sup>23</sup> Recently, Zong *et al.*<sup>48</sup> characterized additional PTMs of cardiac 20S proteasomes, including glycosylation, phosphorylation, oxidation, and nitrosylation, using two-dimensional electrophoresis-based approaches supported by antibodies, capture reagents, and mass spectroscopy. The functional significance of most of these PTMs remains unknown, however.

### 2.3 Associating partners

Another important element that regulates proteasome function is the involvement of associating partners. PKA and casein kinase II (CKII) were previously reported as proteasome-associated kinases.<sup>49,50</sup> In murine heart, PKA and protein phosphatase 2A were found to associate with the 20S proteasome and increase and decrease the proteolytic activity, respectively.<sup>40,51</sup> Recently, it was demonstrated that many of cardiac 20S proteasome subunits are phosphorylated by active PKA.<sup>51</sup>

Thus, the cardiac proteasome is a heterogeneous and dynamic organelle that is regulated by at least three mechanisms: molecular organization, PTM, and associating partners<sup>52</sup> (Figure 1).



**Figure 1** Regulation of proteolytic activity of cardiac proteasome. Abbreviations used are: Rpn, regulatory particle non-ATPase; Rpt, regulatory particle triple-A; CP, core particle; RP, regulatory particle; PA, proteasome activator; PI, proteasome inhibitor; Uch, ubiquitin carboxy-terminal hydrolase; Usp, ubiquitin specific peptidase. Reprinted from Tanaka *et al.*<sup>14</sup> with permission from Proceedings of the Japan Academy.

### 3. The regulation of proteasome in failing hearts

Congestive heart failure (CHF) is a complex clinical syndrome that can result from virtually any form of heart disease.

#### 3.1 Pressure overload

Pressure overload due to hypertension is a common cause of cardiac hypertrophy. Cardiac hypertrophy is an early milestone during the clinical course of heart failure. The development of cardiac hypertrophy requires increased protein synthesis that exceeds protein degradation in individual cardiomyocytes.<sup>53</sup> An increase in protein synthesis could potentially result in increased levels of misfolded proteins, which are targeted for proteasomal degradation before they aggregate.<sup>3,54</sup> Production of misfolded proteins beyond the capacity of the proteasomes or proteasomal dysregulation, however, may result in aggregate deposition in cardiomyocytes. Recent studies have detected an accumulation of ubiquitinated proteins and abnormal protein aggregation in the cardiomyocytes of patients with decompensated cardiac hypertrophy.<sup>55–58</sup> It also has been shown that the transcript levels of some of the  $\alpha$ - and  $\beta$ -subunits of the 20S proteasome are downregulated in failing human hearts compared with non-failing hearts.<sup>59</sup> These findings suggest that impaired removal of abnormal proteins by proteasomes contributes to the pathophysiology of CHF. In fact, we demonstrated that depressed proteasome activities may contribute to the transition from compensated cardiac hypertrophy to CHF in a pressure-overloaded mouse model.<sup>56</sup>

On the other hand, cardiac proteasome activity increased together with an upregulation of both 20S and 19S proteasome subunit levels during the development of cardiac hypertrophy caused by either pressure overload or cardiac-specific expression of Hsp22 in mice.<sup>60–62</sup> The authors reproduced proteasome activation *in vitro* using cardiomyocytes treated with either acute adenovirus-mediated overexpression of Hsp22 or prohypertrophic stimuli.<sup>62</sup> Also of interest, the proteasomes were redistributed to the nuclear compartment in the hypertrophic hearts of cardiac-specific Hsp22 transgenic mice.<sup>62</sup> Based on these findings, studies into the effects of proteasome inhibitors on cardiac hypertrophy have been conducted. Depre et al.<sup>61</sup> showed that proteasome inhibition in mice prevents pressure overload-induced cardiac hypertrophy without affecting ventricular contractile function. The authors also demonstrated that proteasome inhibition has reverse cardiac remodelling effects in overloaded hearts and stabilizes contractile function by repressing collagen accumulation via reduced NF- $\kappa$ B activity.<sup>60</sup> The effects of proteasome inhibition on both the prevention and regression of cardiac hypertrophy also were demonstrated in a pharmacologically induced cardiac hypertrophic model.<sup>63</sup> Meiners et al.<sup>64</sup> showed that proteasome inhibition effectively suppressed cardiomyocyte hypertrophy in cultured cardiomyocytes, as evidenced by reduced cell size and suppressed expression of several hypertrophic marker genes.

Potential benefits of proteasome inhibition in cardiac remodeling induced by hypertension have also been reported in rat hypertension models.<sup>64,65</sup> The proteasome inhibition suppressed either cardiomyocyte hypertrophy<sup>64</sup> or cardiac fibrosis<sup>65</sup> by down-

regulating key signalling pathways of hypertrophic growth or by interfering collagen and matrix metalloproteinase expression, respectively. Interestingly, the enhanced ubiquitin-proteasome activity was demonstrated in atherosclerotic lesions of patients with morning blood pressure surge, which were associated with inflammatory-dependent unstable plaque phenotype.<sup>66</sup> These findings indicated that proteasome plays a key role in the pathophysiology of hypertension.

Important questions remain, however. First, why is proteasome activation required for the development of cardiac hypertrophy? One possible reason is that regulatory proteins that prevent cardiac hypertrophy are targeted to the proteasome during pressure overload due to activation of a specific ubiquitin ligase. Alternatively, activation of the proteasome during stress may be necessary to maintain a pool of amino acids for the synthesis of nascent proteins.<sup>62,67</sup> Second, is long-term treatment with a proteasome inhibitor beneficial to these patients?<sup>68</sup> It is known that significant inhibition of the proteasome induces apoptosis in various cell types, including cardiomyocytes, because of ER stress or a direct toxic effect of the accumulated misfolded proteins.<sup>69,70</sup> Importantly, an unexpected cardiotoxicity of bortezomib, a selective and irreversible inhibitor of 20S proteasome, has been identified in clinical settings.<sup>71–74</sup> Finally, is the reversal of hypertrophy in the presence of prohypertrophic signalling of benefit for the function and survival of heart muscle?<sup>68,74</sup> All of these questions must be clearly replied to evaluate the potential of proteasome inhibitors for treatment of cardiac hypertrophy.

Angiotensin II (ANGII) plays a critical role in the development of cardiac hypertrophy. In skeletal muscle myotubes, ANGII increased proteasome chymotrypsin-like activity as well as an increase in protein expression of proteasome  $\alpha$ -subunits, Rpt1 and Rpt4 subunits.<sup>75</sup> ANGII also increased mRNA levels of the ubiquitin ligases MURF-1 (muscle ring finger-1) and atrogenin-1 *in vivo*,<sup>76</sup> suggesting a critical role of ANGII in muscle wasting in patients with cardiac cachexia. However, there is little information about the effects of ANGII on cardiac proteasome, it is worthy of consideration to investigate it.

#### 3.2 Familial cardiomyopathies

More than 200 different mutations in 14 genes encoding sarcomeric proteins have been identified in patients with hypertrophic cardiomyopathy (HCM), with MYBPC3 among the most frequently mutated genes.<sup>77</sup> MYBPC3 encodes cardiac myosin-binding protein C (cMyBP-C); identified mutations frequently result in a frameshift, leading to the production of a truncated protein product. In addition to a 'poison polypeptide' mode of action, haploinsufficiency is thought to contribute to the disease, because the expected truncated proteins were not detected on western blots of cardiac tissue from patients carrying MYBPC3 frameshift mutations.<sup>78</sup> Sarikas et al.<sup>79</sup> clearly showed that truncated cMyBP-C proteins are preferentially degraded by the UPS. A more important finding from this study, however, is that the truncated cMyBP-C proteins were not only substrates of the UPS, but also impaired the degradation of other UPS substrates, probably due to competitive inhibition; this suggests that the truncated cMyBP-C mutants create a constant additional workload for the UPS in the heart, resulting in decreased proteasome-mediated



degradation of abnormal proteins caused by ageing or oxidative stress.<sup>79</sup> Like truncated cMyBP-C variants, the E334K missense mutation in MYBPC3 also causes UPS-dependent destabilization of the protein and proteasome impairment, which may contribute to cardiac dysfunction in HCM.<sup>80</sup> Bahrudin *et al.*<sup>80</sup> identified five novel MYBPC3 mutations in Japanese patients with HCM. Among them, only E334K made cMyBP-C unstable via the UPS. The expression of the E334K variant impaired 20S proteasome activity and induced apoptosis in cardiomyocytes. In addition, patients carrying the E334K mutation showed smaller ejection fractions and larger left ventricular end-diastolic diameters compared with healthy subjects and patients carrying other MYBPC3 mutations.

Another example that demonstrates the possible role of UPS in the pathophysiology of familial cardiomyopathy is familial desmin-related myopathy (DRM), which is caused by a R120G mutation in the gene encoding  $\alpha$ B-crystallin (CryAB).<sup>81</sup> CryAB, an abundant heat shock protein, acts as a chaperone protein to assist the assembly of and modulate interactions between desmin filaments.<sup>59,82</sup> Transgenic mice expressing CryABR120G develop cardiomyopathy that resembles DRM because CryABR120G acts as a dominant negative mutant, leading to disruption of myofibril alignment and formation of desmin-positive aggregates.<sup>83</sup> Interestingly, analysis of cardiac-specific CryABR120G transgenic mice crossed with GFPdn reporter mice that express a UPS reporter substrate revealed marked proteasome dysfunction in the heart before the development of CHF.<sup>84</sup> Furthermore, CryABR120G-induced proteasome dysfunction in cultured cardiomyocytes was attenuated by inhibition of aberrant protein aggregation by Congo red.<sup>84</sup> These findings indicate that proteasome dysfunction contributes to cardiac remodelling and failure in this model, highlighting an additional pathogenic mechanism of CryABR120G-related DRM.<sup>84</sup> The mechanism underlying impaired proteasome function is unclear, however; insufficient delivery of substrate proteins into 20S proteasomes, depletion of key components of the 19S subcomplex, and direct inhibition of proteasomes by aggregated proteins may play a role.<sup>84,85</sup>

### 3.3 Ischaemia

Ischaemic heart disease is one of the most common causes of CHF; myocardial ischaemia contributes to the pathophysiology and progression of CHF. Ubiquitinated protein levels increase in the hearts of patients with ischaemic heart disease,<sup>57</sup> suggesting that myocardial ischaemia disturbs the UPS in heart. Bulteau *et al.*<sup>86</sup> first demonstrated declines in the caspase-like, trypsin-like, and chymotrypsin-like activities of 20S proteasomes along with increased levels of oxidized and ubiquitinated proteins in rat hearts after coronary occlusion/reperfusion. Reduced 26S proteasome activity has been also observed in hearts after ischaemia/reperfusion.<sup>87</sup> A correlation between recovery of post-ischaemic function and levels of oxidized and/or ubiquitinated proteins has been observed.<sup>88</sup> The mechanism of ischaemia/reperfusion-induced inactivation of proteasome activity remains unknown, although modification of proteasome subunits by 4-hydroxy-nonenal (4-HNE) is likely involved in this process. Incubation of purified 20S proteasomes from heart with 4-HNE resulted in specific subunit modification and proteasome inactivation.<sup>89</sup> The 26S proteasome subunit S6 ATPase was also identified as a major intracellular target protein that is deactivated by oxidative

stress.<sup>90</sup> 4-HNE cross-links proteins or oxidized proteins and can inhibit proteasome activity.<sup>68,91,92</sup> Interestingly, oxidative stress caused selective rather than global inhibition of proteasome activity.<sup>93</sup> These data suggest oxidative stress plays a major role in heart proteasome dysfunction during ischaemia/reperfusion. Depletion of ATP may also be an important contributing factor to proteasome dysfunction during ischaemia because degradation of polyubiquitinated protein by the 26S proteasome requires ATP,<sup>94,95</sup> whereas the removal of oxidized proteins during myocardial ischaemia is mediated by the 20S proteasome.<sup>88</sup>

On the other hand, oxidative stress can also offer signal preconditioning protection, not simply injurious, and induce stress responses that lead to survival.<sup>96</sup> Costa *et al.*<sup>97</sup> demonstrated a protective effect of reactive oxygen species via proteasome inhibition and a resulting upregulation of HSP70 levels. The cytoprotective increases in heat shock protein levels and anti-inflammatory effects observed following proteasome inhibition<sup>98</sup> led to investigations of proteasome inhibitors in experimental cerebral ischaemia.<sup>99,100</sup> There are conflicting results, however, about whether proteasome inhibition is cardioprotective<sup>101–104</sup> or cardiotoxic<sup>87</sup> in ischaemic hearts. Beneficial effects include significant reductions in cardiac infarct size, preserved ventricular function, and suppression of malignant ventricular arrhythmia. These effects are mediated by inhibition of NF $\kappa$ B activation,<sup>101,103</sup> the induction of heat shock protein expression,<sup>102,105</sup> inhibition of G-protein signalling,<sup>104</sup> and subsequent attenuation of inflammatory responses. On the other hand, Powell *et al.*<sup>87</sup> demonstrated that pre-ischaemic treatment of isolated rat heart with a proteasome inhibitor results in dose-dependent decreases in post-ischaemic cardiac function and enhanced accumulation of ubiquitinated and oxidized proteins. Thus, the effects of proteasome inhibition in the heart, which inhibitors should be used (reversible or irreversible) at which doses (degree of inhibition), the appropriate time-course of inhibition (when and how long), and whether the effects are extracardiac or cardiac all require further examination.

Ischaemic preconditioning and pharmacologic preconditioning with nicorandil increase post-ischaemic proteasome activities.<sup>87,106</sup> Cai *et al.*<sup>107</sup> investigated the role of immunoproteasomes in ischaemic preconditioning. The cardioprotective effects of ischaemic preconditioning were lost in  $\beta$ 1i knockout mice due to impaired degradation of phosphatase, a tensin homologue deleted from chromosome 10 (PTEN), and activation of the downstream protein kinase Akt. Impaired PTEN degradation was associated with defective immunoproteasomes and decreased proteolytic activities.<sup>107</sup>

Diabetes mellitus is a major cause of myocardial ischaemia. Marfella *et al.*<sup>108</sup> demonstrated that 20S proteasome activity (chymotrypsin-like) in the myocardia of diabetic ischaemic patients was enhanced compared with non-diabetic ischaemic patients. Ubiquitin and proteasome 20S immunoreactivity was observed in both cardiomyocytes and macrophages from the diabetic ischaemic area, whereas this immunoreactivity was primarily found in the inflammatory cells from the non-diabetic ischaemic area.<sup>108</sup> Experimental hyperglycaemia induced in rats using streptozotocin results in diabetic cardiomyopathy. In these hyperglycaemic hearts, 20S  $\beta$ 5 proteasome activity increased, 26S  $\beta$ 5 proteasome activity decreased, and the levels of ubiquitinated proteins and oxidized proteins increased.<sup>109</sup> Interestingly, PA28 $\alpha$  and PA28 $\beta$  levels were upregulated in the

hyperglycaemic hearts, suggesting a response to oxidative protein damage was triggered by hyperglycaemia.<sup>21,36,109</sup>

### 3.4 Myocarditis

Viral myocarditis leads to dilated cardiomyopathy (DCM), which may result in sudden death or end-stage CHF. Coxsackievirus B3 (CVB3), an enterovirus from the Picornavirus family, is a frequent cause of human viral myocarditis and the associated sequela, DCM.<sup>110</sup> Various viruses use the UPS to facilitate their replication by manipulating host cell signalling and regulating host proteins; for instance, the UPS plays a critical role in CVB3 replication in cardiomyocytes.<sup>111</sup> Cardiomyocyte HL1 cells infected by CVB3 show no significant changes in proteasome activity, although proteasome inhibition effectively attenuates CVB3 replication by preventing both viral RNA transcription and protein synthesis in cultured murine cardiomyocytes.<sup>111</sup> The regulation and roles of the UPS were also examined *in vivo* using myocarditis-susceptible A/J mice.<sup>112</sup> Ubiquitinated proteins abnormally accumulated in CVB3-infected hearts 9 days after infection, which may be caused by increased expression of the ubiquitin-activating enzyme E1A/E1B, the ubiquitin-conjugating enzyme UBCH7, and the deubiquitinating enzyme UCLH1. Furthermore, although no alteration of proteasome activity was observed in CVB3-infected hearts, a proteasome inhibitor attenuated CVB3-induced myocardial injury.<sup>112</sup> The underlying mechanisms by which proteasome inhibition attenuates viral replication and CVB3-induced myocardial injury are not fully determined, however.

On the other hand, microarray analysis demonstrated increased gene expression of low molecular mass protein 2 (LMP2, known as  $\beta$ 1i) in CVB3-infected mouse hearts.<sup>113</sup> Szalay et al.<sup>114</sup> demonstrated that the hearts of myocarditis-susceptible A.BY/SnJ mice express increased levels of immunoproteasomes and show decreased chymotrypsin-like and caspase-like 20S proteasome activities during the acute phase of CVB3 infection compared with those from resistant C57BL/6 mice. This was accompanied by elevated expression of genes involved in the antigen-presenting machinery, such as MHC class I molecules,  $\beta$ 2-microglobulin, TAP, and tapasin, suggesting that enhanced immunoproteasome formation affects the generation of antigenic peptides and alters T-cell-mediated immune responses to CVB3 in susceptible mice.<sup>114</sup>

### 3.5 Clinical application of a proteasome inhibitor

The proteasome inhibitor bortezomib (PS341) is approved by the FDA for the treatment of relapsed multiple myeloma and mantle cell lymphoma, and has been used as an anti-cancer agent against hematologic and solid malignancies.<sup>115</sup> However, this agent has been associated with unexpected cardiac complications ranging from arrhythmia to CHF.<sup>71–73</sup> The voltage-gated potassium channel Kv1.5 mediates an ultrarapid delayed rectifier current (Ik<sub>r</sub>), which plays an important role in maintenance of the membrane potential. The pore-forming, inwardly rectifying K<sup>+</sup> channel core Kir6.2 is primarily responsible for K<sup>+</sup> permeance. Both Kv1.5 and Kir6.2 are degraded by proteasomes and modulate the stability of these channel proteins,<sup>116,117</sup> which may contribute to arrhythmia following bortezomib therapy. Recently, Fu et al.<sup>69</sup> investigated

the cardiotoxic effect of a proteasome inhibitor on cardiomyocytes. They found that proteasome inhibition caused ER stress without a compensatory increase in ER chaperone expression, resulting in apoptosis via the CHOP-dependent pathway. Further, adenovirus-mediated overexpression of GRP78, an ER chaperone, attenuated CHOP expression and rescued cardiomyocytes from the apoptosis induced by proteasome inhibition.

If the cardiotoxic effects of bortezomib are a result of decreased cardiac proteasome activity, interventions that enhance or restore this activity may decrease cardiac side effects during bortezomib treatment. Recently, Asai et al.<sup>106</sup> showed that ischaemic preconditioning and intracoronary administration of PKA activators enhance cardiac 26S proteasome assembly and activity *in vivo*. Although further studies are necessary to elucidate this mechanism, manipulation of proteasome function may provide treatment avenue for a wide spectrum of heart diseases in which depressed cardiac proteasome activity contributes to pathogenesis or disease progression.<sup>56,86–88,118</sup>

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### References

1. Wetzel R. Mutations and off-pathway aggregation of proteins. *Trends Biotechnol* 1994;**12**:193–198.
2. Kelly SM, Vanslyke JK, Musil LS. Regulation of ubiquitin-proteasome system mediated degradation by cytosolic stress. *Mol Biol Cell* 2007;**18**:4279–4291.
3. Johnston JA, Ward CL, Kopito RR. Aggresomes: a cellular response to misfolded proteins. *J Cell Biol* 1998;**143**:1883–1898.
4. Bucciantini M, Calloni G, Chiti F, Formigli L, Nosi D, Dobson CM et al. Prefibrillar amyloid protein aggregates share common features of cytotoxicity. *J Biol Chem* 2004;**279**:31374–31382.
5. Grune T, Merker K, Sandig G, Davies KJ. Selective degradation of oxidatively modified protein substrates by the proteasome. *Biochem Biophys Res Commun* 2003;**305**:709–718.
6. Kopito RR. Aggresomes, inclusion bodies and protein aggregation. *Trends Cell Biol* 2000;**10**:524–530.
7. Ross CA, Poirier MA. Opinion: what is the role of protein aggregation in neurodegeneration? *Nat Rev Mol Cell Biol* 2005;**6**:891–898.
8. Powell SR. The ubiquitin-proteasome system in cardiac physiology and pathology. *Am J Physiol Heart Circ Physiol* 2006;**291**:H1–H19.
9. Wang X, Su H, Ranek MJ. Protein quality control and degradation in cardiomyocytes. *J Mol Cell Cardiol* 2008;**45**:11–27.
10. Patterson C, Ike C, Willis PWt, Stouffer GA, Willis MS. The bitter end: the ubiquitin-proteasome system and cardiac dysfunction. *Circulation* 2007;**115**:1456–1463.
11. Welchman RL, Gordon C, Mayer RJ. Ubiquitin and ubiquitin-like proteins as multifunctional signals. *Nat Rev Mol Cell Biol* 2005;**6**:599–609.
12. Lee DH, Goldberg AL. Proteasome inhibitors: valuable new tools for cell biologists. *Trends Cell Biol* 1998;**8**:397–403.
13. Groll M, Bajorek M, Kohler A, Moroder L, Rubin DM, Huber R et al. A gated channel into the proteasome core particle. *Nat Struct Biol* 2000;**7**:1062–1067.
14. Tanaka K. The proteasome: overview of structure and functions. *Proc Jpn Acad Ser B Phys Biol Sci* 2009;**85**:12–36.
15. Kotamraju S, Matalon S, Matsunaga T, Shang T, Hickman-Davis JM, Kalyanaram B. Upregulation of immunoproteasomes by nitric oxide: potential antioxidative mechanism in endothelial cells. *Free Radic Biol Med* 2006;**40**:1034–1044.
16. Callahan MK, Wohlfert EA, Menoret A, Srivastava PK. Heat shock up-regulates Imp2 and Imp7 and enhances presentation of immunoproteasome-dependent epitopes. *J Immunol* 2006;**177**:8393–8399.

17. Dahlmann B, Ruppert T, Kuehn L, Merforth S, Kloetzel PM. Different proteasome subtypes in a single tissue exhibit different enzymatic properties. *J Mol Biol* 2000;**303**:643–653.
18. Van den Eynde BJ, Morel S. Differential processing of class-I-restricted epitopes by the standard proteasome and the immunoproteasome. *Curr Opin Immunol* 2001;**13**:147–153.
19. Noda C, Tanahashi N, Shimbara N, Hendil KB, Tanaka K. Tissue distribution of constitutive proteasomes, immunoproteasomes, and PA28 in rats. *Biochem Biophys Res Commun* 2000;**277**:348–354.
20. Rivett AJ, Bose S, Brooks P, Broadfoot KI. Regulation of proteasome complexes by gamma-interferon and phosphorylation. *Biochimie* 2001;**83**:363–366.
21. Husom AD, Peters EA, Kolling EA, Fugere NA, Thompson LV, Ferrington DA. Altered proteasome function and subunit composition in aged muscle. *Arch Biochem Biophys* 2004;**421**:67–76.
22. Drews O, Wildgruber R, Zong C, Sukop U, Nissum M, Weber G et al. Mammalian proteasome subpopulations with distinct molecular compositions and proteolytic activities. *Mol Cell Proteomics* 2007;**6**:2021–2031.
23. Gomes AV, Zong C, Edmondson RD, Li X, Stefani E, Zhang J et al. Mapping the murine cardiac 26S proteasome complexes. *Circ Res* 2006;**99**:362–371.
24. DeMartino GN. Purification of PA700, the 19S regulatory complex of the 26S proteasome. *Methods Enzymol* 2005;**398**:295–306.
25. Elsasser S, Finley D. Delivery of ubiquitinated substrates to protein-unfolding machines. *Nat Cell Biol* 2005;**7**:742–749.
26. Lam YA, Lawson TG, Velayutham M, Zweier JL, Pickart CM. A proteasomal ATPase subunit recognizes the polyubiquitin degradation signal. *Nature* 2002;**416**:763–767.
27. Elsasser S, Gali RR, Schwickart M, Larsen CN, Leggett DS, Muller B et al. Proteasome subunit Rpn1 binds ubiquitin-like protein domains. *Nat Cell Biol* 2002;**4**:725–730.
28. Glickman MH, Ciechanover A. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. *Physiol Rev* 2002;**82**:373–428.
29. Rechsteiner M, Realini C, Ustrell V. The proteasome activator 11 S REG (PA28) and class I antigen presentation. *Biochem J* 2000;**345**:1–15.
30. Realini C, Jensen CC, Zhang Z, Johnston SC, Knowlton JR, Hill CP et al. Characterization of recombinant REGalpha, REGbeta, and REGgamma proteasome activators. *J Biol Chem* 1997;**272**:25483–25492.
31. Ordway GA, Neuffer PD, Chin ER, DeMartino GN. Chronic contractile activity upregulates the proteasome system in rabbit skeletal muscle. *J Appl Physiol* 2000;**88**:1134–1141.
32. Fuertes G, Martin De Llano JJ, Villarroya A, Rivett AJ, Knecht E. Changes in the proteolytic activities of proteasomes and lysosomes in human fibroblasts produced by serum withdrawal, amino-acid deprivation and confluent conditions. *Biochem J* 2003;**375**:75–86.
33. Gromov P, Skovgaard GL, Palsdottir H, Gromova I, Ostergaard M, Celis JE. Protein profiling of the human epidermis from the elderly reveals up-regulation of a signature of interferon-gamma-induced polypeptides that includes manganese-superoxide dismutase and the p85beta subunit of phosphatidylinositol 3-kinase. *Mol Cell Proteomics* 2003;**2**:70–84.
34. Khan S, van den Broek M, Schwarz K, de Giuli R, Diener PA, Groettrup M. Immunoproteasomes largely replace constitutive proteasomes during an antiviral and antibacterial immune response in the liver. *J Immunol* 2001;**167**:6859–6868.
35. Rechsteiner M, Hill CP. Mobilizing the proteolytic machine: cell biological roles of proteasome activators and inhibitors. *Trends Cell Biol* 2005;**15**:27–33.
36. Ferrington DA, Husom AD, Thompson LV. Altered proteasome structure, function, and oxidation in aged muscle. *FASEB J* 2005;**19**:644–646.
37. Ustrell V, Hoffman L, Pratt G, Rechsteiner M. PA200, a nuclear proteasome activator involved in DNA repair. *EMBO J* 2002;**21**:3516–3525.
38. Schmidt M, Haas W, Crosas B, Santamaria PG, Gygi SP, Walz T et al. The HEAT repeat protein Bim10 regulates the yeast proteasome by capping the core particle. *Nat Struct Mol Biol* 2005;**12**:294–303.
39. Bose S, Mason GG, Rivett AJ. Phosphorylation of proteasomes in mammalian cells. *Mol Biol Rep* 1999;**26**:11–14.
40. Zong C, Gomes AV, Drews O, Li X, Young GW, Berhane B et al. Regulation of murine cardiac 20S proteasomes: role of associating partners. *Circ Res* 2006;**99**:372–380.
41. Satoh K, Sasajima H, Nyoumura KI, Yokosawa H, Sawada H. Assembly of the 26S proteasome is regulated by phosphorylation of the p45/Rpt6 ATPase subunit. *Biochemistry* 2001;**40**:314–319.
42. Satoh K, Nishikawa T, Yokosawa H, Sawada H. Phosphorylation of proteasome substrate by a protein kinase associated with the 26 S proteasome is linked to the ATP-dependent proteolysis of the 26 S proteasome. *Biochem Biophys Res Commun* 1995;**213**:7–14.
43. Zhang F, Hu Y, Huang P, Toleman CA, Paterson AJ, Kudlow JE. Proteasome function is regulated by cyclic AMP-dependent protein kinase through phosphorylation of Rpt6. *J Biol Chem* 2007;**282**:22460–22471.
44. Bose S, Stratford FL, Broadfoot KI, Mason GG, Rivett AJ. Phosphorylation of 20S proteasome alpha subunit C8 (alpha7) stabilizes the 26S proteasome and plays a role in the regulation of proteasome complexes by gamma-interferon. *Biochem J* 2004;**378**:177–184.
45. Bardag-Gorce F, Venkatesh R, Li J, French BA, French SW. Hyperphosphorylation of rat liver proteasome subunits: the effects of ethanol and okadaic acid are compared. *Life Sci* 2004;**75**:585–597.
46. Jagoe RT, Goldberg AL. What do we really know about the ubiquitin-proteasome pathway in muscle atrophy? *Curr Opin Clin Nutr Metab Care* 2001;**4**:183–190.
47. Zhang F, Su K, Yang X, Bowe DB, Paterson AJ, Kudlow JE. O-GlcNAc modification is an endogenous inhibitor of the proteasome. *Cell* 2003;**115**:715–725.
48. Zong C, Young GW, Wang Y, Lu H, Deng N, Drews O et al. Two-dimensional electrophoresis-based characterization of post-translational modifications of mammalian 20S proteasome complexes. *Proteomics* 2008;**8**:5025–5037.
49. Pereira ME, Wilk S. Phosphorylation of the multicatalytic proteinase complex from bovine pituitaries by a copurifying cAMP-dependent protein kinase. *Arch Biochem Biophys* 1990;**283**:68–74.
50. Ludemann R, Lerea KM, Etlinger JD. Copurification of casein kinase II with 20 S proteasomes and phosphorylation of a 30-kDa proteasome subunit. *J Biol Chem* 1993;**268**:17413–17417.
51. Lu H, Zong C, Wang Y, Young GW, Deng N, Souda P et al. Revealing the dynamics of the 20 S proteasome phosphoproteome: a combined CID and electron transfer dissociation approach. *Mol Cell Proteomics* 2008;**7**:2073–2089.
52. Young GW, Wang Y, Ping P. Understanding proteasome assembly and regulation: importance to cardiovascular medicine. *Trends Cardiovasc Med* 2008;**18**:93–98.
53. Sugden PH, Clerk A. Cellular mechanisms of cardiac hypertrophy. *J Mol Med* 1998;**76**:725–746.
54. Friehs I. Proteasome inhibition in hypertrophied myocardium. *Am J Physiol Heart Circ Physiol* 2008;**295**:H1373–H1374.
55. Hein S, Arnon E, Kostin S, Schonburg M, Elsasser A, Polyakova V et al. Progression from compensated hypertrophy to failure in the pressure-overloaded human heart: structural deterioration and compensatory mechanisms. *Circulation* 2003;**107**:984–991.
56. Tsukamoto O, Minamino T, Okada K, Shintani Y, Takashima S, Kato H et al. Depression of proteasome activities during the progression of cardiac dysfunction in pressure-overloaded heart of mice. *Biochem Biophys Res Commun* 2006;**340**:1125–1133.
57. Weekes J, Morrison K, Mullen A, Wait R, Barton P, Dunn MJ. Hyperubiquitination of proteins in dilated cardiomyopathy. *Proteomics* 2003;**3**:208–216.
58. Birks EJ, Latif N, Enesa K, Folkvang T, Luong le A, Sarathchandra P et al. Elevated p53 expression is associated with dysregulation of the ubiquitin-proteasome system in dilated cardiomyopathy. *Cardiovasc Res* 2008;**79**:472–480.
59. Zolk O, Schenke C, Sarikas A. The ubiquitin-proteasome system: focus on the heart. *Cardiovasc Res* 2006;**70**:410–421.
60. Hedhli N, Lizano P, Hong C, Fritzy LF, Dhar SK, Liu H et al. Proteasome inhibition decreases cardiac remodeling after initiation of pressure overload. *Am J Physiol Heart Circ Physiol* 2008;**295**:H1385–H1393.
61. Depre C, Wang Q, Yan L, Hedhli N, Peter P, Chen L et al. Activation of the cardiac proteasome during pressure overload promotes ventricular hypertrophy. *Circulation* 2006;**114**:1821–1828.
62. Hedhli N, Wang L, Wang Q, Rashed E, Tian Y, Sui X et al. Proteasome activation during cardiac hypertrophy by the chaperone H11 Kinase/Hsp22. *Cardiovasc Res* 2008;**77**:497–505.
63. Stansfield WE, Tang RH, Moss NC, Baldwin AS, Willis MS, Selzman CH. Proteasome inhibition promotes regression of left ventricular hypertrophy. *Am J Physiol Heart Circ Physiol* 2008;**294**:H645–H650.
64. Meiners S, Dreger H, Fechner M, Bieler S, Rother W, Gunther C et al. Suppression of cardiomyocyte hypertrophy by inhibition of the ubiquitin-proteasome system. *Hypertension* 2008;**51**:302–308.
65. Meiners S, Hoher B, Weller A, Laule M, Stangl V, Guenther C et al. Downregulation of matrix metalloproteinases and collagens and suppression of cardiac fibrosis by inhibition of the proteasome. *Hypertension* 2004;**44**:471–477.
66. Marfella R, Siniscalchi M, Portoghese M, Di Filippo C, Ferraraccio F, Schiattarella C et al. Morning blood pressure surge as a destabilizing factor of atherosclerotic plaque: role of ubiquitin-proteasome activity. *Hypertension* 2007;**49**:784–791.
67. Vabulas RM, Hartl FU. Protein synthesis upon acute nutrient restriction relies on proteasome function. *Science* 2005;**310**:1960–1963.
68. Friguet B, Szveda LI. Inhibition of the multicatalytic proteinase (proteasome) by 4-hydroxy-2-nonenal cross-linked protein. *FEBS Lett* 1997;**405**:21–25.



69. Fu HY, Minamino T, Tsukamoto O, Sawada T, Asai M, Kato H et al. Overexpression of endoplasmic reticulum-resident chaperone attenuates cardiomyocyte death induced by proteasome inhibition. *Cardiovasc Res* 2008;**79**:600–610.
70. Okada K, Minamino T, Tsukamoto Y, Liao Y, Tsukamoto O, Takashima S et al. Prolonged endoplasmic reticulum stress in hypertrophic and failing heart after aortic constriction: possible contribution of endoplasmic reticulum stress to cardiac myocyte apoptosis. *Circulation* 2004;**110**:705–712.
71. Voortman J, Giaccone G. Severe reversible cardiac failure after bortezomib treatment combined with chemotherapy in a non-small cell lung cancer patient: a case report. *BMC Cancer* 2006;**6**:129.
72. Enrico O, Gabriele B, Nadia C, Sara G, Daniele V, Giulia C et al. Unexpected cardiotoxicity in hematological bortezomib treated patients. *Br J Haematol* 2007;**138**:396–397.
73. Hachaneftoglou A, Tarkun P, Gonullu E. Acute severe cardiac failure in a myeloma patient due to proteasome inhibitor bortezomib. *Int J Hematol* 2008;**88**:219–222.
74. Razeghi P, Taegtmeier H. Cardiac remodeling: UPS lost in transit. *Circ Res* 2005;**97**:964–966.
75. Sanders PM, Russell ST, Tisdale MJ. Angiotensin II directly induces muscle protein catabolism through the ubiquitin-proteasome proteolytic pathway and may play a role in cancer cachexia. *Br J Cancer* 2005;**93**:425–434.
76. Song YH, Li Y, Du J, Mitch WE, Rosenthal N, Delafontaine P. Muscle-specific expression of IGF-1 blocks angiotensin II-induced skeletal muscle wasting. *J Clin Invest* 2005;**115**:451–458.
77. Richard P, Charron P, Carrier L, Ledeuil C, Cheav T, Pichereau C et al. Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. *Circulation* 2003;**107**:2227–2232.
78. Vosberg HP. The ubiquitin-proteasome system may be involved in the pathogenesis of hypertrophic cardiomyopathy. *Cardiovasc Res* 2005;**66**:1–3.
79. Sarikas A, Carrier L, Schenke C, Doll D, Flavigny J, Lindenberg KS et al. Impairment of the ubiquitin-proteasome system by truncated cardiac myosin binding protein C mutants. *Cardiovasc Res* 2005;**66**:33–44.
80. Bahrudin U, Morisaki H, Morisaki T, Ninomiya H, Higaki K, Nanba E et al. Ubiquitin-proteasome system impairment caused by a missense cardiac myosin-binding protein C mutation and associated with cardiac dysfunction in hypertrophic cardiomyopathy. *J Mol Biol* 2008;**384**:896–907.
81. Vicart P, Caron A, Guicheney P, Li Z, Prevost MC, Faure A et al. A missense mutation in the alphaB-crystallin chaperone gene causes a desmin-related myopathy. *Nat Genet* 1998;**20**:92–95.
82. Lutsch G, Vetter R, Offhauss U, Wieske M, Grone HJ, Klemenz R et al. Abundance and location of the small heat shock proteins HSP25 and alphaB-crystallin in rat and human heart. *Circulation* 1997;**96**:3466–3476.
83. Wang X, Osinska H, Klevitsky R, Gerdes AM, Nieman M, Lorenz J et al. Expression of R120G-alphaB-crystallin causes aberrant desmin and alphaB-crystallin aggregation and cardiomyopathy in mice. *Circ Res* 2001;**89**:84–91.
84. Chen Q, Liu JB, Horak KM, Zheng H, Kumarapeli AR, Li J et al. Intrascapular amyloidosis impairs proteolytic function of proteasomes in cardiomyocytes by compromising substrate uptake. *Circ Res* 2005;**97**:1018–1026.
85. Bence NF, Sampat RM, Kopito RR. Impairment of the ubiquitin-proteasome system by protein aggregation. *Science* 2001;**292**:1552–1555.
86. Bulteau AL, Lundberg KC, Humphries KM, Sadek HA, Szweida PA, Friguet B et al. Oxidative modification and inactivation of the proteasome during coronary occlusion/reperfusion. *J Biol Chem* 2001;**276**:30057–30063.
87. Powell SR, Wang P, Katzeff H, Shringarpure R, Teoh C, Khaliulin I et al. Oxidized and ubiquitinated proteins may predict recovery of posts ischemic cardiac function: essential role of the proteasome. *Antioxid Redox Signal* 2005;**7**:538–546.
88. Divald A, Powell SR. Proteasome mediates removal of proteins oxidized during myocardial ischemia. *Free Radic Biol Med* 2006;**40**:156–164.
89. Farout L, Mary J, Vinh J, Szweida LI, Friguet B. Inactivation of the proteasome by 4-hydroxy-2-nonenal is site specific and dependant on 20S proteasome subtypes. *Arch Biochem Biophys* 2006;**453**:135–142.
90. Ishii T, Sakurai T, Usami H, Uchida K. Oxidative modification of proteasome: identification of an oxidation-sensitive subunit in 26 S proteasome. *Biochemistry* 2005;**44**:13893–13901.
91. Sitte N, Huber M, Grune T, Ladhoff A, Doecke WD, Von Zglinicki T et al. Proteasome inhibition by lipofuscin/ceroid during postmitotic aging of fibroblasts. *FASEB J* 2000;**14**:1490–1498.
92. Powell SR, Wang P, Divald A, Teichberg S, Haridas V, McCloskey TW et al. Aggregates of oxidized proteins (lipofuscin) induce apoptosis through proteasome inhibition and dysregulation of proapoptotic proteins. *Free Radic Biol Med* 2005;**38**:1093–1101.
93. Gurusamy N, Goswami S, Malik G, Das DK. Oxidative injury induces selective rather than global inhibition of proteasomal activity. *J Mol Cell Cardiol* 2008;**44**:419–428.
94. McNulty PH, Jagasia D, Cline GW, Ng CK, Whiting JM, Garg P et al. Persistent changes in myocardial glucose metabolism in vivo during reperfusion of a limited-duration coronary occlusion. *Circulation* 2000;**101**:917–922.
95. Hershko A, Ciechanover A. The ubiquitin system. *Annu Rev Biochem* 1998;**67**:425–479.
96. Becker LB. New concepts in reactive oxygen species and cardiovascular reperfusion physiology. *Cardiovasc Res* 2004;**61**:461–470.
97. Costa VM, Silva R, Ferreira R, Amado F, Carvalho F, de Lourdes Bastos M et al. Adrenaline in pro-oxidant conditions elicits intracellular survival pathways in isolated rat cardiomyocytes. *Toxicology* 2009;**257**:70–79.
98. Zollner TM, Podda M, Pien C, Elliott PJ, Kaufmann R, Boehncke WH. Proteasome inhibition reduces superantigen-mediated T cell activation and the severity of psoriasis in a SCID-hu model. *J Clin Invest* 2002;**109**:671–679.
99. Henninger N, Sicard KM, Bouley J, Fisher M, Stagliano NE. The proteasome inhibitor VELCADE reduces infarction in rat models of focal cerebral ischemia. *Neurosci Lett* 2006;**398**:300–305.
100. Zhang L, Zhang ZG, Liu X, Hozeska A, Stagliano N, Riordan W et al. Treatment of embolic stroke in rats with bortezomib and recombinant human tissue plasminogen activator. *Thromb Haemost* 2006;**95**:166–173.
101. Pye J, Ardeshirpour F, McCain A, Bellinger DA, Merricks E, Adams J et al. Proteasome inhibition ablates activation of NF-kappa B in myocardial reperfusion and reduces reperfusion injury. *Am J Physiol Heart Circ Physiol* 2003;**284**:H919–H926.
102. Stangl K, Gunther C, Frank T, Lorenz M, Meiners S, Ropke T et al. Inhibition of the ubiquitin-proteasome pathway induces differential heat-shock protein response in cardiomyocytes and renders early cardiac protection. *Biochem Biophys Res Commun* 2002;**291**:542–549.
103. Stansfield WE, Moss NC, Willis MS, Tang R, Selzman CH. Proteasome inhibition attenuates infarct size and preserves cardiac function in a murine model of myocardial ischemia-reperfusion injury. *Ann Thorac Surg* 2007;**84**:120–125.
104. Huang S, Patterson E, Yu X, Garrett MW, De Aoi I, Kem DC. Proteasome inhibition 1 h following ischemia protects GRK2 and prevents malignant ventricular tachyarrhythmias and SCD in a model of myocardial infarction. *Am J Physiol Heart Circ Physiol* 2008;**294**:H1298–H1303.
105. Luss H, Schmitz W, Neumann J. A proteasome inhibitor confers cardioprotection. *Cardiovasc Res* 2002;**54**:140–151.
106. Asai M, Tsukamoto O, Minamino T, Asanuma H, Fujita M, Asano Y et al. PKA rapidly enhances proteasome assembly and activity in vivo canine hearts. *J Mol Cell Cardiol* 2009;**46**:452–462.
107. Cai ZP, Shen Z, Van Kaer L, Becker LC. Ischemic preconditioning-induced cardioprotection is lost in mice with immunoproteasome subunit low molecular mass polypeptide-2 deficiency. *FASEB J* 2008;**22**:4248–4257.
108. Marfella R, Filippo CD, Portoghese M, Siniscalchi M, Martis S, Ferraraccio F et al. The ubiquitin-proteasome system contributes to the inflammatory injury in ischemic diabetic myocardium: the role of glycemic control. *Cardiovasc Pathol* 2009; in press.
109. Powell SR, Samuel SM, Wang P, Divald A, Thirunavukkarasu M, Koneru S et al. Upregulation of myocardial 11S-activated proteasome in experimental hyperglycemia. *J Mol Cell Cardiol* 2008;**44**:618–621.
110. McManus BM, Yanagawa B, Rezaei N, Luo H, Taylor L, Zhang M et al. Genetic determinants of coxsackievirus B3 pathogenesis. *Ann N Y Acad Sci* 2002;**975**:169–179.
111. Luo H, Zhang J, Cheung C, Suarez A, McManus BM, Yang D. Proteasome inhibition reduces coxsackievirus B3 replication in murine cardiomyocytes. *Am J Pathol* 2003;**163**:381–385.
112. Gao G, Zhang J, Si X, Wong J, Cheung C, McManus B et al. Proteasome inhibition attenuates coxsackievirus-induced myocardial damage in mice. *Am J Physiol Heart Circ Physiol* 2008;**295**:H401–H408.
113. Taylor LA, Carthy CM, Yang D, Saad K, Wong D, Schreiner G et al. Host gene regulation during coxsackievirus B3 infection in mice: assessment by microarrays. *Circ Res* 2000;**87**:328–334.
114. Szalay G, Meiners S, Voigt A, Lauber J, Spieth C, Speer N et al. Ongoing coxsackievirus myocarditis is associated with increased formation and activity of myocardial immunoproteasomes. *Am J Pathol* 2006;**168**:1542–1552.
115. Ludwig H, Khayat D, Giaccone G, Facon T. Proteasome inhibition and its clinical prospects in the treatment of hematologic and solid malignancies. *Cancer* 2005;**104**:1794–1807.
116. Kato M, Ogura K, Miake J, Sasaki N, Taniguchi S, Igawa O et al. Evidence for proteasomal degradation of Kv1.5 channel protein. *Biochem Biophys Res Commun* 2005;**337**:343–348.
117. Tanaka H, Miake J, Notsu T, Sonyama K, Sasaki N, Iitsuka K et al. Proteasomal degradation of Kir6.2 channel protein and its inhibition by a Na<sup>+</sup> channel blocker aprindine. *Biochem Biophys Res Commun* 2005;**331**:1001–1006.
118. Akazawa H, Komuro I. 'Change can happen' by PKA: proteasomes in vivo hearts. *J Mol Cell Cardiol* 2009;**46**:445–447.