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Review article

# Ubiquitin-proteasome system and hereditary cardiomyopathies

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ABSTRACT

Adequate protein turnover is essential for cardiac homeostasis. Different protein quality controls are involved in the maintenance of protein homeostasis, including molecular chaperones and co-chaperones, the autophagy-lysosomal pathway, and the ubiquitin-proteasome system (UPS). In the last decade, a series of evidence has underlined a major function of the UPS in cardiac physiology and disease. Particularly, recent studies have shown that dysfunctional proteasomal function leads to cardiac disorders. Hypertrophic and dilated cardiomyopathies are the two most prevalent inherited cardiomyopathies. Both are primarily transmitted as an autosomal-dominant trait and mainly caused by mutations in genes encoding components of the cardiac sarcomere, including a relevant striated muscle-specific E3 ubiquitin ligase. A growing body of evidence indicates impairment of the UPS in inherited cardiomyopathies as determined by measurement of the level of ubiquitinated proteins, the activities of the proteasome and/or the use of fluorescent UPS reporter substrates. The present review will propose mechanisms of UPS impairment in inherited cardiomyopathies, summarize the potential consequences of UPS impairment, including activation of the unfolded protein response, and underline some therapeutic options available to restore proteasome function and therefore cardiac homeostasis and function. This article is part of a Special Issue entitled "Protein Quality Control, the Ubiquitin Proteasome System, and Autophagy".



**Contents**



## 1. Introduction

In mammalian cells, most of the proteins are in a dynamic state of flux. The balance of protein synthesis and degradation in each cell is highly regulated and occurs in a specific manner to maintain cellular homeostasis. However, under the circumstances of cardiac remodeling during heart disease this balance can be altered leading to accumulation

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of potentially toxic proteins. To ensure that these misfolded or aberrant proteins are either repaired or removed, a set of molecular mechanisms works in collaboration or separately as a quality control of the cell. This quality control consists of molecular chaperones and co-chaperones, the autophagy-lysosomal pathway (ALP), and the ubiquitin-proteasome system (UPS). In the past years, the functional significance of the UPS in cardiovascular physiology and disease has become evident. Particularly, UPS alteration is rapidly gaining recognition as a major player in the pathogenesis of several cardiac disorders, including inherited cardiomyopathies.

Cardiomyopathies are myocardial diseases, characterized by abnormal cardiac structure and function, in the absence of other causes that could produce these abnormalities, such as coronary artery disease, hypertension, valvular disease, or congenital heart disease [1,2]. Phenotypically, they are classified into four main forms: hypertrophic, dilated, restrictive, and arrhythmogenic right ventricular cardiomyopathy [3]. The two most prevalent familial forms are hypertrophic (HCM) and dilated (DCM) cardiomyopathies, which are typically associated with mutations in genes encoding proteins of the sarcomere, cytoskeleton, sarcoplasmic reticulum, T-tubules and others [1].

This review will highlight the current knowledge of UPS function in the context of HCM and DCM, discuss potential mechanisms and consequences of UPS dysfunction and propose potential therapeutic interventions.

## 2. The ubiquitin-proteasome system

The UPS is indispensable for the highly selective degradation of most intracellular cytosolic, nuclear, and myofibrillar proteins. It controls many fundamental biological processes such as cell proliferation, adaptation to stress and cell death, and its major function is to prevent accumulation of damaged, misfolded and mutant proteins [4]. Degradation of proteins by the UPS is an ATP-dependent multistage process that requires first ubiquitination of the target protein prior to its degradation by the 26S proteasome [5,6].

Ubiquitination of the target protein is achieved via an enzymatic cascade involving the concerted action of E1 (ubiquitin-activating), E2 (ubiquitin-conjugating) and E3 (ubiquitin ligase) enzymes. While there are two E1, about 40 E2 and more than 600 E3 enzymes have been described in mammals [6,7]. The process of ubiquitination occurs with spatial, temporal and substrate specificity, which is dictated by the E3 ubiquitin ligases. The E3 ubiquitin ligases have been broadly classified into 2 main categories based on structural similarities: the RING (really interesting new gene) finger domain-containing proteins including the RING-related E3s such as the U-box proteins, and the HECT (homologous to E6-AP carboxy-terminus) domain-containing proteins [7,8]. In addition, hybrids of RING-finger and HECT E3 ubiquitin ligases exist. Recent reviews, including one in the current issue, gave an update on the known striated muscle-specific E3 ubiquitin ligases ([9,10], Willis et al., in press). The last identified cardiac-specific E3 ubiquitin ligase is the F-box protein Fbxl22, which promotes degradation of α-actinin and filamin C [11].

The eukaryotic 26S proteasome is a large, multicatalytic protein complex composed of two subcomplexes: the 20S core particle capped by either one or two 19S regulatory particles (for a detailed description, see [12]). The function of the 19S regulatory particle is to recognize, deubiquitinate, and unfold target proteins, and then to translocate them into the 20S core particle, which houses the proteolytic activities within its central chamber. Three distinct proteolytic activities exist, namely the chymotrypsin-like, trypsin-like and caspase-like activities, and each cleaves preferentially after particular amino acid residues. Many structurally diverse inhibitors of these activities have been discovered or developed and were recently summarized [13].

Over the last decade, methods for assessment of UPS function have been established. The evaluation of the UPS includes the determination of steady-state levels of ubiquitinated proteins, ubiquitinating and deubiquitinating enzymes, and proteasomal subunits by Western blot. In addition, measurements of the proteolytic activities using synthetic fluorogenic substrates are often performed, despite the disadvantage that these small substrates can easily enter the 20S core in an ubiquitination-independent manner and therefore do not reflect the highly-regulated entry of substances into the 20S core. To get insights into the dynamic behavior of the UPS in living cells, fluorescent-labeled UPS components, including fluorescent ubiquitin and fluorescent-tagged proteasomal subunits, have been generated [14]. These tools allowed the discovery of novel features of the UPS in diverse cellular processes, including its different locations such as in the inner surface of the nuclear envelope, the endoplasmic reticulum, or its homogenous distribution in cells. Related to these studies, transgenic reporter mouse models expressing fluorescent substrates of the UPS were then created to decipher the role of the UPS in the whole animal [15,16].

## 3. The ubiquitin-proteasome system in familial cardiomyopathies

Several disorders, including neurodegenerative and cardiovascular diseases exhibit organ failure due, at least in part, to toxic protein accumulation [5,9,17,18]. Most cases of heart failure with hypertrophic, dilated or ischemic cardiomyopathies exhibit accumulation of ubiquitinated proteins [19,20], abnormal protein aggregation such as preamyloid oligomer formation [21,22], and altered proteasomal activities [23–25]. A body of evidence indicates UPS alterations in inherited HCM and DCM and is discussed below.

### 3.1. The ubiquitin-proteasome system in hypertrophic cardiomyopathy

HCM is the most prevalent cardiac genetic disease (1:500), characterized by left ventricular hypertrophy (LVH), increased interstitial fibrosis, and diastolic dysfunction [26–28]. HCM is considered as a sarcomeropathy, transmitted in an autosomal-dominant fashion with an incomplete penetrance, and caused by more than 1000 individual mutations in (at least) 10 genes encoding proteins of the cardiac contractile unit [1,28–30]. Most of the disease genes exhibit missense mutations that are expected to produce stable full-length mutant proteins. However, in some HCM genes, mutations are mainly frameshift leading to a premature stop codon and C-terminal truncated polypeptides. This is the case for the most frequently mutated gene, *MYBPC3*, encoding cardiac myosin-binding protein C (cMyBP-C; [29]) and for a much less frequently mutated gene, *FHL1*, encoding four-and-a-half LIM domain protein 1 [31].

It has been shown that truncated cMyBP-Cs resulting from human *MYBPC3* mutations are unstable, not well incorporated into the sarcomere and finally degraded by the UPS after gene transfer in rat cardiac myocytes [32,33]. Continuous degradation of mutant cMyBP-C proteins led to UPS impairment as shown by the accumulation of the UPS substrate $Ub^{G76V}$-DsRed [33]. The muscle-specific E3 ubiquitin ligase involved was then found to be atrogin-1, whereas MuRF1 did contribute to lowering cMyBP-C level at the mRNA level after gene transfer in cardiac myocytes [34]. The expression of a missense E334K *MYBPC3* mutation also resulted in UPS impairment and accumulation of pro-apoptotic markers, ion channels and $Ca^{2+}$ handling proteins after gene transfer in cardiac myocytes [35,36]. The knock-in of the most frequent human *MYBPC3* mutation (c.772G>A; 13% of unrelated HCM patients, likely with a founder effect in Toscany; [37]) into the mouse genome revealed that its expression is regulated by both the nonsense-mediated mRNA decay (NMD) and UPS [38]. Moreover, in both homozygous *Mybpc3*-targeted knock-in (KI) and knock-out (KO) mice, which developed LVH with systolic and diastolic dysfunction [38–40], the activities of the proteasome were elevated during the first 3 months of age and positively correlated with the degree of LVH [41]. Interestingly, then, the global activity of the proteasome was impaired with aging only in the KI mice (but not in KO), as shown by the accumulation of the UPS substrate $Ub^{G76V}$-GFP protein in the heart [41]. Similarly, adrenergic stress induced the same extent

of LVH (but with a specific septum involvement) in heterozygous *Mybpc3*-targeted KI and KO mice as in wild-type mice, but induced a marked reduction in proteasome activity only in heterozygous KI mice [42]. Reduced proteasome activities were found in human myocardial tissue of HCM patients, particularly in those carrying *MYBPC3* gene mutations [24]. Whether UPS impairment contributes to the development of HCM in human is unclear but mouse studies strongly support the view that UPS impairment results from the combination of altered cardiac phenotype plus stress in mice exhibiting a *Mybpc3* mutation.

Besides *MYBPC3*, other disease genes need to be underlined, even if mutations were rare and found in isolated cases of HCM. For example, the expression of missense and truncating *FHL1* mutations as well as missense mutations in *ANKRD1*, encoding ankyrin repeat domain 1, were markedly regulated by the UPS after gene transfer in cardiac myocytes or in rat engineered heart tissue (EHT; [31,43]). ANKRD1 interacts with the sarcomere-specific MuRF1 and MuRF2 [44], suggesting that its degradation could be mediated by MuRF1. Interestingly, mutations in *TRIM63* encoding MuRF1 cause isolated cases of HCM and reduced the UPS-mediated degradation of mTOR-S6K hypertrophic signaling pathway in transgenic mutant mice [45].

### 3.2. The ubiquitin-proteasome system in dilated cardiomyopathy

DCM is characterized by increased ventricular dimensions, contractile dysfunction and myocardial fibrosis [2]. In 20–50% of cases DCM is familial and inherited primarily in an autosomal-dominant mode [1]. The genetic basis of DCM is far more heterogeneous than that of HCM. More than 50 single genes are associated with DCM, several of which also cause HCM [46]. The DCM genes encode components of the sarcomere, sarcolemma, nuclear envelope, cytoskeleton, mitochondria, and proteins involved in $Ca^{2+}$ handling [1,46]. Most of DCM cases result from sarcomere gene mutations, with the majority (25%) attributed to truncating mutations in *TTN*, encoding titin [47].

As for HCM, UPS impairment might also play a role in human or experimental models of familial DCM. While *MYBPC3* is the paradigm for UPS impairment in HCM, for DCM these are *CRYAB* encoding α-B-crystallin and *DES* encoding desmin. Mutations in *CRYAB* or *DES* resulted in accumulation of mutant proteins and severe DCM in desmin-related (cardio)myopathy (DRM; [48,49]). A mouse model of DRM, obtained by overexpression of the R120G mutant *CRYAB* ($CryAB^{R120G}$) recapitulated the human phenotype [50] and exhibited marked UPS impairment as revealed by GFPdgn-based UPS reporter mice before the development of hypertrophy and heart failure [51]. Similar observations were made in mutant *Des-D7* transgenic mice [52,53]. In both DRM mouse models, UPS impairment started before the cardiac phenotype and seems to concern the delivery of ubiquitinated proteins into the 20S proteasome.

Other genes associated with DCM are also subject to UPS-mediated regulation. This is the case for *LMNA* encoding lamins A/C, which are proteins of the nuclear envelope. *LMNA* mutations cause DCM with conduction and/or rhythm defects [54]. Heterozygous $Lmna^{\Delta K32/+}$ mice developed DCM and heart failure, and finally died between 35 and 70 weeks of age [55]. DCM was triggered by lamin haploinsufficiency, due to rapid degradation of ΔK32-lamin mutant by the UPS, followed by UPS impairment, leading to accumulation of toxic ΔK32-lamin [55]. A missense mutation in *NKX2.5*, associated with congenital heart disease and adult-onset DCM, resulted in UPS impairment after gene transfer in COS cells [56]. In a recent unbiased approach that aimed at identifying modifying pathways in mouse models of DCM carrying mutant muscle LIM protein, calsarcin-1 or δ-sarcoglycan, alterations of gene expression of UPS components emerged as the most significant predictor of impaired contractile function [57]. In human DCM and end-stage heart failure, marked accumulation of ubiquitinated proteins is a common feature, whereas contradictory findings were obtained for proteasomal activities [19,23–25]. Whether UPS impairment contributes to the development of DCM in human is not resolved yet, but mouse studies support this view.

### 3.3. Potential mechanisms leading to UPS impairment in cardiomyopathies

The mechanisms by which gene mutations lead to UPS impairment are not fully elucidated. In the absence of external stress, the expression of the mutation is regulated at several levels by quality control mechanisms in order to reduce as much as possible the amount of misfolded or aberrant poison polypeptides, which could induce damage in cardiac myocytes. Missense mutations are expected to produce stable full-length mutant mRNAs and proteins. Misfolded mutant proteins are recognized by chaperones (such as Hsp70, Hsp90) and co-chaperones (such as CHIP, Bag1, Bag3) that will make the decisions about refolding or degrading them by the UPS and/or the ALP [6]. Therefore, in some cases, the expression of missense mutations may be tightly regulated by the protein quality control systems, resulting in low level of full-length mutant proteins. In the specific case of frameshift or nonsense mutations, an additional quality control takes place at the mRNA level, which is the NMD (Fig. 1 [58]). Low levels (or absence) of mutant proteins and the assumed 50% of wild-type proteins, as expected for autosomal-dominant disease such as cardiomyopathies, result in protein haploinsufficiency, which leads to the cardiac phenotype. In most cases, expression of the wild-type allele partially compensates for protein deficiency. For example, >70% of wild-type cMyBP-C proteins were detected in septal myectomy of HCM patients with *MYBPC3* mutations, even for patients with missense mutations [59–61]. Similarly, heterozygous *Mybpc3*-targeted KO mice, which are considered as pure models of haploinsufficiency, exhibited 75% of cMyBP-C and then developed septal hypertrophy at 10–11 months of age [40], and heterozygous *Mybpc3*-targeted KI mice exhibited 79% of cMyBP-C and developed diastolic dysfunction at 10 weeks of age [38,39]. Finally, heterozygous $Lmna^{\Delta K32/+}$ mice exhibited haploinsufficiency before the development of DCM [55].

Several mechanisms could lead separately or in combination to UPS impairment. First, it has been shown that the continuous degradation of mutant proteins by the UPS saturates this system after gene transfer in cardiac myocytes or EHTs [31,33,35,55] and in the $CryAB^{R120G}$ DRM mouse model [51,53]. Second, the combination of external stress and overwhelmed UPS could precipitate the system into impairment (Fig. 1). This is the case in some HCM and DCM disease mouse models, in which the UPS continuously degraded mutant proteins in young adult mice carrying *Lmna* or *Mybpc3* mutation and became saturated or impaired only after adrenergic stress or aging [38,41,42,55]. In one of these studies, stress-induced decreased chymotrypsin-like activity was mainly due to reduced level of the β5-subunit of the proteasome in the cytosol, which could be due to translocation of the proteasome to another cellular compartment [41]. Reversible localization of proteasomal components was observed in other conditions. For example, yeast cells that stop cell cycling relocated the proteasome from the nucleus to the cytosol into storage granules [62]. In neurodegenerative disorders, the proteasome system was also relocalized into intracytoplasmic inclusions [63]. Third, misfolded proteins escaping the surveillance of chaperones and UPS tend to form aggregates, which are potentially toxic to the cell. Supporting this, the group of Robbins showed that intracellular amyloidosis was highly prevalent in cardiac myocytes derived from human HCM or DCM hearts [21]. Furthermore, protein aggregation itself impaired proteasome function in cardiac myocytes [64], forming a vicious cycle. Fourth, increased oxidative stress also results in protein aggregation. In the case of aging, this could be due to increased free radicals production by damaged aging mitochondria [65]. Oxidative stress induced protein oxidation and aggregation of oxidized proteins, which bind to the 20S proteasome and irreversibly inhibit its activity [66]. This could cause a vicious cycle and also lead to accumulation of oxidized proteins, which are normally degraded by the proteasome system. Fifth, an altered assembly of the proteasome or a switch in the

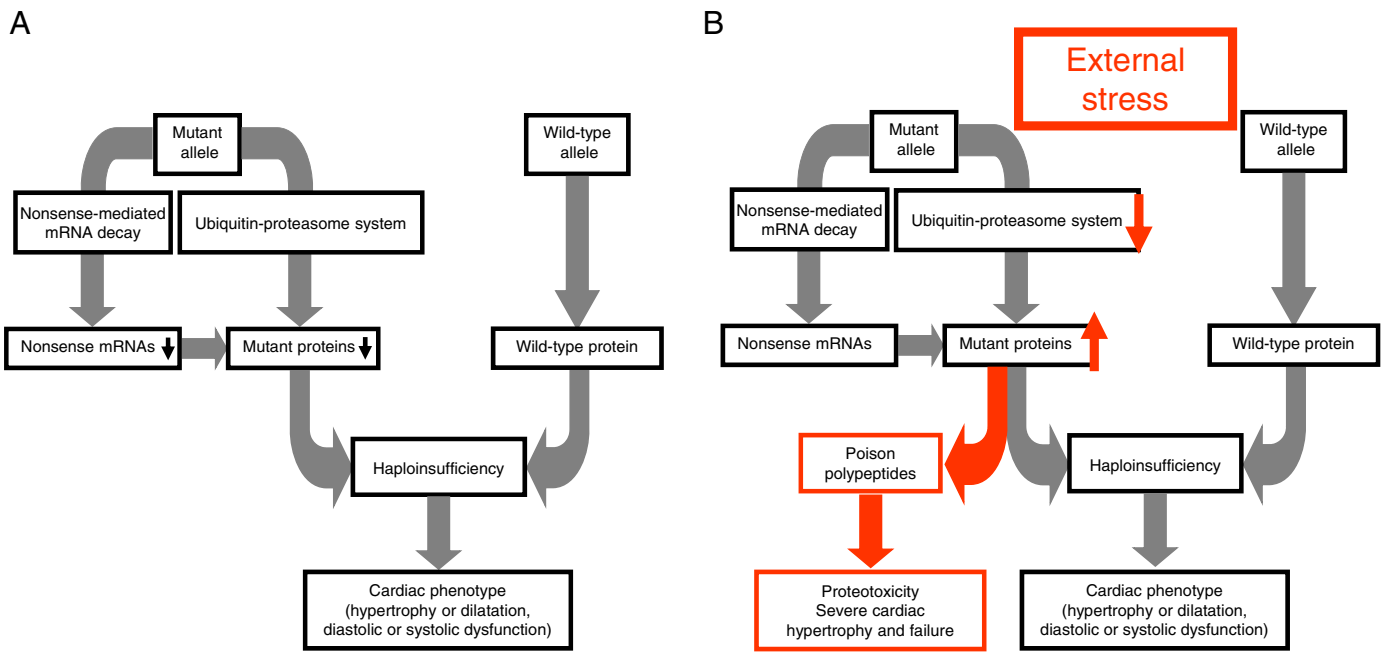


**Fig. 1.** Potential mechanisms leading to UPS impairment in genetic forms of cardiomyopathies. A) The nonsense-mediated mRNA decay at the mRNA level, and the ubiquitin-proteasome system (UPS) at the protein level regulate the expression of the mutant allele, leading to reduced amount of mutant proteins that could be toxic for cardiac myocytes. Together with the (normal) expression of the wild-type allele, this results in protein haploinsufficiency and the development of the cardiac phenotype (hypertrophy or dilatation, diastolic or systolic dysfunction). B) External stress, e.g. adrenergic stress or aging, could induce impairment of the UPS in cardiomyopathies. Although the exact mechanism is not known, one may suggest that adrenergic stress induces desensitization of the β-adrenoceptors leading to decreased activities of PKA and therefore reduced phosphorylation of proteasome components, known to be associated with decreased proteasome activity. Impaired UPS leads to accumulation of mutant proteins, which could act as poison polypeptides and further impair the phenotype (severe form of cardiac hypertrophy and heart failure). Figure was made from findings obtained in mouse models of hypertrophic and dilated cardiomyopathies [41,42,55].

distribution of proteasomal subpopulations [67] could lead to UPS impairment. A recent study demonstrated an impaired docking of the 19S to the 20S in human end-stage heart failure [68], which could affect the degradation capacity of the proteasome and may explain the diminished proteasomal activity found in human failing hearts [24]. Finally, regulation of the proteasome system involves post-translational modifications of proteasomal subunits, such as phosphorylation, acetylation or oxidation. For example, in vitro PKA phosphorylation of proteasomal subunits increased proteasomal assembly and activities in the heart [69,70]. Given the evidence of altered β-adrenergic signaling in the diseased heart and particularly in heart failure [71–73], it is possible that the reduced PKA-mediated phosphorylation of the proteasome

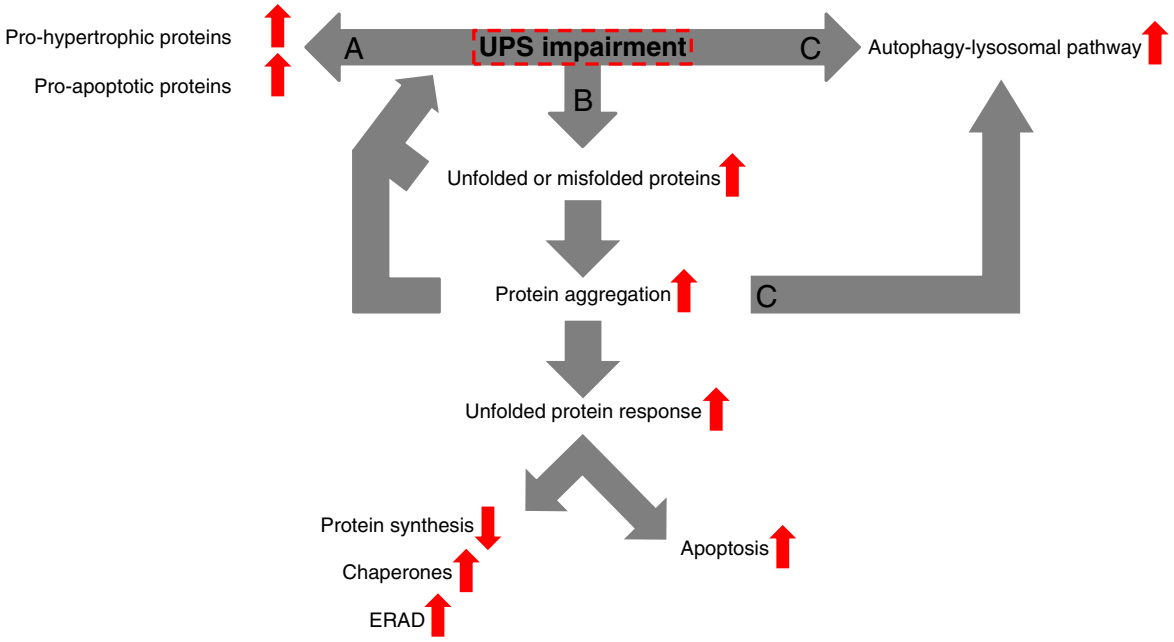


**Fig. 2.** Potential consequences of UPS impairment in cardiac myocytes. UPS impairment could lead to: A) Accumulation of proteins involved in hypertrophic signaling (e.g. calcineurin) or apoptotic pathway (e.g. p53), which are normally degraded by the UPS. B) Accumulation of unfolded or misfolded proteins in the endoplasmic reticulum (ER), which leads to protein aggregation. This ER stress activates the unfolded protein response, characterized by attenuation of protein synthesis, upregulation of chaperones genes (e.g. GRP78) and ERAD (ER-associated protein degradation). Prolonged stress will lead to apoptosis. Accumulation and/or aggregation of misfolded proteins could itself force UPS impairment. C) Activation of the autophagy-lysosomal pathway directly or indirectly (by protein aggregation) in order to contribute to protein degradation.

components contributes to UPS impairment in cardiomyopathies. Interestingly, reduced PKA-mediated phosphorylation of contractile proteins, such as cardiac troponin I and cMyBP-C was found in HCM [60,61,74] and more generally in human and experimental models of heart failure, such as chronic adrenergic stimulation [75,76].

### 3.4. Potential consequences of UPS impairment

UPS impairment could have several consequences in cardiac myocytes (Fig. 2). A number of key proteins involved in cardiac hypertrophy and apoptosis pathways are either targets or components of the UPS. For instance, several signaling proteins, such as β-catenin and calcineurin, which mediate cardiac growth (including pathological hypertrophy) are normally degraded by the UPS [77,78]. Similarly, p53 is a target of the E3 ubiquitin ligase MDM2 [79]. Therefore, proteasomal impairment could result in increased levels of pro-hypertrophic and pro-apoptotic factors. It has been shown that UPS impairment activated the calcineurin-NFAT pathway and promoted maladaptive remodeling in cardiac myocytes [80]. Furthermore, reduced proteasomal activities were associated with increased levels of pro-apoptotic p53 in human HCM and failing hearts [24]. Finally, the expression of the HCM p.Glu334Lys *MYBPC3* mutation in cardiac myocytes induced UPS impairment, accumulation of pro-apoptotic factors and alteration of $Ca^{2+}$ handling that could result in arrhythmias [35,36].

UPS impairment could also lead to accumulation of unfolded or misfolded proteins and aggregation of proteins (Fig. 2). This might result in ER stress, leading to an adaptive response, which is known as the unfolded protein response (UPR). The UPR promotes attenuation of protein synthesis, transcriptional activation of chaperone genes and activation of ER-associated degradation (ERAD) in order to reduce the load of misfolded proteins [81,82]. If the attempts to resolve the ER stress fail or the UPR is prolonged, UPR-mediated signaling pathways that lead to apoptosis are initiated. An inadequately working UPS (or in this case ERAD) probably stimulates the switch from adaptive to pro-apoptotic response. This hypothesis is supported by the demonstration that proteasome inhibition induced ER-initiated cardiac myocyte death via CHOP-dependent pathways [83]. Of note, accumulation and/or aggregation of misfolded proteins could itself force UPS impairment, forming thereby a detrimental feedback loop.

Whereas the UPS usually degrades the majority of proteins, the ALP is the other proteolytic system which is primarily responsible for degradation of (generally) long-lived or aggregated proteins and cellular organelles [6,84]. The ALP engulfs proteins or organelles into autophagosomes, which subsequently fuse with lysosomes to form auto(phago)lysosomes, in which lysosomal proteases degrade autophagosomal content [85]. Although autophagy is generally considered to be independent of the UPS, growing lines of evidence indicate that the UPS and ALP act as a consortium in the removal of misfolded proteins [84,86,87]. Another potential consequence of UPS inhibition is the ALP activation. Several proteins such as p62, NBR1 and HDAC6 seem to play a major role in the interplay between the UPS and ALP [6,84]. Proteasome inhibition activated autophagy in vitro and in vivo, likely as a compensatory mechanism to alleviate proteotoxic stress [87–89].

## 4. Potential therapeutic UPS interventions

Since the UPS plays a role in many fundamental biological processes, targeting this system for therapy is complex. In the last decade, inhibition of the proteasome has come into focus for the treatment of cardiac diseases. The irreversible proteasome inhibitor epoxomicin has been demonstrated to completely prevent the development of LVH in a mouse model of short-term pressure overload induced by transverse aortic constriction (TAC; [90]). Similarly, partial inhibition of the proteasome with low doses of the reversible proteasome inhibitor bortezomib significantly attenuated hypertrophic heart growth in hypertensive Dahl salt-sensitive rats [91]. Furthermore, administration of epoxomicin two weeks after TAC, i.e. at a stage of pronounced hypertrophy, resulted in regression of hypertrophy and stabilization of cardiac function in mice [92]. Comparably, treatment with the irreversible proteasome inhibitor PS-519 significantly diminished isoprenaline-induced hypertrophy in mice [93]. However, conflicting data exist that argue against a cardioprotective role of proteasome inhibition. Chronic administration of bortezomib induced LVH to a similar extent as induced by TAC and resulted in heart failure and premature death in mice [80]. Chronic treatment with the reversible inhibitor MLN-273, an analogue of bortezomib, led to LVH, diastolic dysfunction and a reduction in cardiac output in pigs [94]. Importantly, while bortezomib is generally well tolerated by patients with multiple myeloma, this therapy was associated with the occurrence of cardiac complications, including cardiac dysfunction or even heart failure in elderly patients or patients with preexisting cardiac problems [95–98]. In summary, while complete and sustained proteasome inhibition, particularly under circumstances in which the UPS is already dysfunctional, is expected to rather worsen than to rescue the phenotype, partial or short-term proteasome inhibition may mediate a protective effect in the heart. An alternative therapeutic approach would consist in specifically targeting E3 ubiquitin ligases to reduce UPS-mediated protein degradation. For example, small molecule inhibitors of MDM2 have been developed to induce cancer cell death by stabilizing p53 protein levels [99]. However, they would be not suitable in the therapy of cardiac diseases due to enhanced cardiac apoptosis. Similarly, it has been shown that the UPS-mediated degradation of the cyclin-dependent kinase inhibitor p27 mediated pathological cardiac hypertrophy [100]. Therefore, stabilization of p27 level by targeting its specific E3 ubiquitin ligase SCF-SKP2 or by preventing its degradation using a specific inhibitor [101] could be beneficial. So far, no molecules targeting specifically the cardiac E3 ubiquitin ligases have been developed.

On the other hand, and in light of reduced proteasomal activities or global reduction of proteasome function that was observed in human and experimental models of cardiomyopathies [24,33,35,36,41,42,51,55,102], a reactivation of the proteasome function is expected to be more appropriate and beneficial. Support for this hypothesis came from a recent study showing that proteasomal enhancement induced by overexpression of proteasome activator 28 alpha (PA28α) attenuated cardiac hypertrophy, delayed premature death, and protected against acute myocardial ischemia/reperfusion injury in a mouse model of DRM [103]. However, no drugs are currently available to mimic this effect. The first small compound capable of enhancing proteasome-mediated protein degradation via inhibiting the deubiquitinase USP14 was recently reported and used for neurodegenerative disorders associated with proteotoxicity [104]. However, it still remains to be evaluated in cardiac myocytes and in the heart in vivo. Another way to enhance proteasome function would be to target protein kinase G (PKG). PKG is activated by the PDE5 inhibitor sildenafil, which raises cGMP level. Sildenafil elicited reverse remodeling and improved LV diastolic function in failing patients and animal models [105–107]. Recently, the group of Wang showed that sildenafil activated the proteasome system [108]. Therefore, stimulation of PKG by sildenafil administration is potentially a novel therapeutic strategy to treat cardiomyopathies associated with UPS impairment.

## 5. Conclusion — future directions

The UPS regulates several functions involved in cardiac physiology. The recent identification of HCM gene mutations in a ubiquitin E3 ligase and dysregulation of several UPS components in HCM or DCM support the view that the UPS contributes to the pathogenesis of inherited cardiomyopathies. In light of the findings of UPS impairment, we believe that global proteasome inhibition is likely to be harmful, at least as a long-term treatment for inherited cardiomyopathies. On the other hand, global activation of the proteasome is likely to be a more promising approach to pursue. Finally, a comprehensive understanding of the mechanisms of UPS impairment in different models of inherited

cardiomyopathies, including cellular and animal models as well as human failing ventricular samples should result in the discovery of cardiac-specific targets within the complexity of the UPS for therapeutic benefit.

## Disclosures

None.

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