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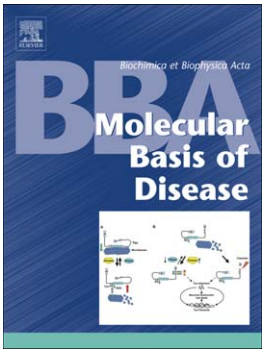

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Ubiquitin-proteasome system in cardiac dysfunction

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Abstract

Since proteins play crucial roles in all biological processes, the finely tuned equilibrium between their synthesis and degradation regulates cellular homeostasis. Controlling the quality of proteome informational content is essential for cell survival and function. After initial synthesis, membrane and secretory proteins are modified, folded, and assembled in the endoplasmic reticulum, whereas other proteins are synthesized and processed in the cytosol. Cells have different protein quality control systems, the molecular chaperones, which help protein folding and stabilization, and the ubiquitin-proteasome system (UPS) and lysosomes, which degrade proteins. It has generally been assumed that UPS and lysosomes are regulated independently and serve distinct functions. The UPS degrades both cytosolic, nuclear proteins, and myofibrillar proteins, whereas the lysosomes degrade most membrane and extracellular proteins by endocytosis as well as cytosolic proteins and organelles via autophagy. Over the last two decades, the UPS has been increasingly recognized as a major system in several biological processes including cell proliferation, adaptation to stress and cell death. More recently, activation or impairment of the UPS has been reported in cardiac disease and recent evidence indicates that autophagy is a key mechanism to maintain cardiac structure and function. This review mainly focuses on the UPS and its various components in healthy and diseased heart, but also summarizes recent data suggesting parallel activation of the UPS and autophagy in cardiac disease.

Keywords: ubiquitin-proteasome system; cardiomyopathy; autophagy; cardiac disease

1. The cardiac ubiquitin-proteasome system

The ubiquitin-proteasome system (UPS) is an ATP-dependent proteolytic system that requires the polyubiquitination of a protein intended for degradation by the 26S proteasome [1]. While it is known that the specificity of the system lies in the E3 ubiquitin ligases, recent evidence indicates that the cardiac 20S proteasome has functional partners involved in the β-adrenergic stimulation, underlying the importance of the UPS and its cardiac-specific components in the heart.

1.1. Protein ubiquitination

Protein ubiquitination consists of the covalent linkage of ubiquitin molecules to one or more lysine residues of a protein. In general, degradation by the UPS requires the "canonical" ubiquitination, characterized by the covalent attachment of one or more chains of polyubiquitin linked through their lysine 48 (K48) to the targeted protein (Figure 1). However, ubiquitination can form ubiquitin chains through linkage of other lysine residues (e.g. lysine 63, K63) and represents "non-canonical" ubiquitination (Figure 1). In this case, the K63 chains are more extended and elongated than compared to the more compact K48 chains (Figure 1). Polyubiquitination through K63 is involved in other mechanisms such as protein localization and cellular signaling. In addition, monoubiquitination exists and is involved in the regulation of protein function or localization (Figure 1). The process of ubiquitination involves the concerted action of ubiquitin enzymes composed of E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzymes) and E3 (ubiquitin ligases). Sometimes, efficient multiubiquitination requires an additional conjugation factor, named E4 [2]. While there is a single E1, about fifty E2 and more than hundred E3 enzymes have been described. The E3 ubiquitin ligases confer substrate specificity to the whole system. They are often grouped in two different categories depending on the presence of either an HECT (homologous to E6-AP carboxy-terminus) or a RING (really interesting new gene) finger as catalytic domain [3, 4]. It is worth to note that ubiquitination has important roles independent of the proteasome such as regulation of the activity of transcription factors, regulation of gene transcription and autophagy, and that the same target can be mono, multi and polyubiquitinated [5, 6]. Up to now, several hundred distinct E3 ubiquitin ligases have been identified, but only few are muscle-specific and are presented here (Figure 2 and Table 1).

1.1.1 Atrogin-1/MaFBx

Atrogin-1 (Atrophy gene 1), also known as MAFbx (Muscle Atrophy F-box), was first identified in 2001 as a muscle-specific E3 ubiquitin ligase highly expressed in muscle atrophy [7, 8].

Atrogin-1 contains a F-box motif [9, 10], which is a protein-protein interaction domain found in proteins that are component of the SCF (skp1, cullin, F-box protein) ubiquitin ligase complex, a subgroup of the RING finger E3 ubiquitin ligase (Figure 2F) [11]. Thus, atrogin-1 per se has no ligase activity but acts as a bridge between the substrate and the enzymatic complex. Other important domains were found along the protein: i) a nuclear localization signal (NLS), which suggests that atrogin-1 could also be involved in the regulation of muscle-specific transcription factors or other nuclear proteins involved in muscle growth [7], and ii) a PDZ domain [12] at the C-terminus, which probably mediates the interaction with the different substrates. However, leucine-rich regions and WD40 repeats, which are known to be the domains for interaction with protein substrate in other F-box proteins, are not present in atrogin-1.

Northern blot analysis showed that atrogin-1 expression is restricted to the cardiac and skeletal muscles in mice, rats and humans [7, 8]. After adenoviral gene transfer in cardiomyocytes atrogin-1 was found in the Z-line of the sarcomere, where it colocalizes with α-actinin and calcineurin [13]. Moreover, atrogin-1 decreases the level of calcineurin and participates in a $SCF^{atrogin-1}$ complex to ubiquitinate calcineurin in vitro [13]. The expression of atrogin-1 is under the control of the FoxO (Forkhead box O) family of transcription factors [14, 15], and therefore linked to the network of PI3K/Akt signaling pathway involved in the physiological hypertrophic response [16]. Recently, it was shown that FoxO1 and FoxO3 are substrates for atrogin-1-mediated "non-canonical" ubiquitination through K63 linkage chains [17]. The presence of K63 chains is known to modulate protein function [18], and indeed K63-mediated polyubiquitination of FoxO1 and FoxO3 enhances their transcriptional activity rather than their degradation [17]. Another substrate for atrogin-1-mediated degradation is MyoD, a transcription factor involved in muscle development and differentiation [19]. No cardiac transcription factors are known to be targeted by atrogin-1 for degradation yet.

1.1.2. Muscle RING finger proteins

Muscle RING finger proteins (MuRF1, MuRF2 and MuRF3) are encoded by distinct genes but share high sequence homology. They are composed of few conserved structural domains: i) the RING finger domain, a zinc finger motif at the N-terminal sequence composed of cysteine and histidine residues, which plays a critical role for the ubiquitin ligase activity [20], ii) the B-box domain, a second zinc finger motif for which no specific function has been yet attributed, and iii) the coiled-coil domain mainly involved in protein-protein interaction, which therefore allows homo or heterodimerization (Figure 2C). Proteins characterized by these three domains are also called RBCC or TRIM proteins [21, 22]. Moreover, there is an additional highly conserved motif called MFC-motif (MURF-family conserved domain) between the RING finger and the B-box, which is exclusively found in MuRF proteins.

As for atrogin-1, the tissue distribution of MuRF transcripts is restricted to striated muscles, and MuRF1 expression is upregulated during skeletal muscle atrophy [8, 23, 24]. MuRF1 is mainly located in the M-band of the sarcomere, where it interacts with titin, but also at the Z-line and in the nucleus [24, 25]. MuRF2 is mainly diffused in the cytoplasm, where it colocalizes with microtubules and intermediate filaments. It can associate with the sarcomeric M-line, where it also interacts with titin, and can shuttle between the cytoplasm and the nucleus [26, 27]. MuRF3 is associated with microtubules and also located at the Z-line of the sarcomere [23]. Yeast-two hybrid screens of a cardiac cDNA library with MuRF1 or MuRF2 baits identified sarcomeric proteins, transcription and translation factors, ribosomal components and proteins involved in mitochondrial energy metabolism as binding partners [28-30]. Furthermore, the E3 ligase activity of MuRF1 was shown for cardiac troponin I (TnI), protein kinase C epsilon (PKCε), and muscle creatine kinase (M-CK) [29, 31, 32]. The identification of M-CK as a target for MuRF1-mediated degradation places MuRF1 under a new light since it could represent the connector between protein turnover and muscle metabolism [32]. Recently, four and a half LIM protein 2 (FHL2) and γ-filamin have been shown to be targeted by MuRF3 for proteasome-mediated degradation [33]. Interestingly, whereas MuRF1 has been shown to be a E3 ligase of both fast and slow/β-myosin heavy chain (β-MHC) in C2C12 myotubes treated with dexamethasone [34], both MuRF1 and MuRF3 are needed together for ubiquitination and degradation of MHC in vivo [35], To date, no substrate for MuRF2-mediated degradation has been described.

1.1.3. Other E3 ubiquitin ligases

Although atrogin-1 and MuRF proteins are the best characterized muscle-specific E3 ubiquitin ligases, there are others which are worth to be mentioned. Among them, CHIP (carboxyl-terminus of Hsp70-interacting protein) is highly expressed in the heart and has dual function as a cochaperone and E3 ubiquitin ligase. CHIP plays a very important role in the regulation of protein quality control [36, 37]. On the one hand, CHIP interacts with the chaperones Hsp70 and Hsp90, and increases refolding rates of stress-damaged proteins. On the other hand, it promotes proteasome-mediated degradation of irreversibly damaged proteins (Figure 2A). Moreover, CHIP not only enhances Hsp70 induction during acute stress, but also mediates Hsp70 turnover during the stress recovery process [38, 39]. In addition, CHIP also degrades p53 [40]. The catalytic domain for the ligase activity is a U-box, a RING finger-like domain without Zn-coordinating residues [41].

Several transcription factors are regulated by ubiquitination and by UPS-mediated degradation. Therefore, cell biology can be markedly affected by differential gene expression as a consequence of ubiquitination and degradation. One example is the altered gene expression mediated by the hypoxia-inducible transcription factor 1a (HIF-1α) and HIF-2α in response to hypoxia. The HIFs are basic helix-loop-helix transcription factors that regulate the expression of a wide variety of genes involved in a multitude of biological functions, including angiogenesis, apoptosis and cellular

metabolism [42]. The major level of HIF-1α regulation is posttranslational and involved the Hippel-Lindau protein (VHL), which is a major subunit in an E3 ubiquitin ligase complex and the UPS [42]. Under normoxic conditions, HIF-1α undergo prolyl hydroxylation that promotes VHL binding and subsequent ubiquitination of HIF [43]. Another example is the transcription factor p53, which is targeted by Mdm2 (murine double minute 2), another interesting RING finger E3 ligase, for proteasome-mediated degradation (Figure 2B). Mdm2-mediated ubiquitination of p53 results in inactivation of its transcriptional activity, nuclear export and degradation [44]. Upon β2-adrenergic stimulation, Mdm2 ubiquitinates β-arrestin that induces rapid internalization of the β2-adrenergic receptors [45]. In addition, Mdm2 interacts with and down-regulates the sarcomeric protein Tcap (telethonin) through the proteasomal pathway, but in an ubiquitin-independent manner, yet not completely described [46].

Ozz is another muscle-specific E3 ligase for the membrane-bound β-catenin (Figure 2D) [47]. It is a member of the suppressor of cytokine signaling (SOCS) family, and, similarly to F-box proteins, acts as a bridge between the substrate and the cullin, elonginB/C and Rbx1 SCF-like complex [48]. Ozz is also involved in maintaining the organization and alignment of sarcomeres and its inhibition or knock-down leads to accumulation of β-catenin causing myofibrillar abnormalities [47].

Recent studies pointed out that the Nedd4 (neuronal precursor cell expressed developmentally downregulated)-like family of HECT-type E3 ligases are very important in the regulation of cardiac voltage-gated sodium ($Na_v1.5$) and potassium (KCNQ1) channels (Figure 2E). Nedd4-like proteins contain two to four WW domains (protein-protein interaction), which interact with the conserved PY-motif (xPPxY) of target proteins [49]. PY-motifs are found at the C-terminus of the channels and mediate the interaction with and ubiquitination by the Nedd4-like ubiquitin ligase. This induces internalization and consequent decrease in surface channel density [50, 51].

The lastly identified muscle-specific E3 ligase is Trim32. As the MuRF proteins, Trim32 belongs to the TRIM protein family and is expressed primarily in skeletal muscle. It has been shown that Trim32 controls muscle protein turnover. A recent study demonstrated that Trim32 has E3 ubiquitin ligase activity and binds to the S1 and S2 region of myosin and ubiquitinates actin in vitro [52]. The involvement of Trim32 as an E3 ligase in the heart has not been described yet.

1.2. The cardiac proteasome

The eukaryotic 26S proteasome (named on the basis of its sedimentation coefficient) is a multicatalytic protein complex that is composed of two multisubunit subcomplexes, the 670 kDa 20S proteasome and the 900 kDa 19S regulatory complex (Figure 3) [53]. The 19S regulatory complex binds to either or both ends of the 20S proteasome resulting in a 26S proteasome with a molecular mass of 1500 kDa or 2400 kDa, respectively. The function of the 19S regulatory complex is to

recognize, bind, deubiquitinate and unfold polyubiquitinated proteins and to regulate the opening of the 20S core to channel the unfolded proteins into the 20S proteasome. The unfolded proteins are then degraded by peptidases in the cavity of the 20S proteasome.

1.2.1. The 20S proteasome

The 20S proteasome represents the proteolytic core of the 26S proteasome. It is a barrel-shaped protein complex containing 28 subunits consisting of two copies of fourteen different proteins. According to the nomenclature of Baumeister et al. [54] the subunits are named α1-α7 and β1-β7 (Figure 3). To reach the proteolytic core of the β-rings, proteins have to pass through a narrow, gated pore, which is formed by the seven subunits α1-α7 of the α-rings [55]. The N-terminal peptides of the α-subunits constitute a physical barrier and prevent the access of the unfolded proteins into the proteolytic core of the β-rings, when the 26S proteasome is in a nonactive state [55]. After activation of the 26S proteasome by binding of the 19S regulatory complex to the α-ring of the 20S proteasome, the α-subunits rearrange and the occlusion by their peptides is abolished resulting in the free entrance of the proteins into the proteolytic core.

The proteolytic activity of the 20S proteasome resides on the inner surface of the β-rings (Figure 3). The unfolded proteins are degraded by three major peptidase activities: the chymotrypsin-like, the trypsin-like and the caspase-like (or post-glutamyl peptide hydrolase) activities, which reside in the β5-, β2- and β1-subunit of each β-ring, respectively. The peptidase of the β1-subunit preferentially cleaves after acidic amino acids, the peptidase of the β2-subunit after basic amino acids and the peptidase of the β5-subunit after hydrophobic amino acids. All three peptidases exhibit the same catalytic mechanism, in which the N-terminal threonine residue is the active nucleophile [54, 56]. It is possible to measure the proteolytic activities in vitro using fluorogenic substrates, which are small peptides containing 3-4 amino acids and a fluorometric reporter at their C-terminal end. These fluorogenic substrates are cleaved by the proteolytic activities and the fluorometric reporter, mainly a 7-amino-4-methyl-coumarin or a β–naphtylamide, is released and its fluorescence can be determined. It is also possible to inhibit reversibly or irreversibly the peptidase activities by several inhibitors, peptide aldehydes (e.g. MG132), peptide boronates (e.g. MG262), β-lactone derivatives (e.g. lactacystin), peptide vinyl sulfones (e.g. NLVS) or peptide epoxyketones (e.g. epoxomicin) [57].

Under certain physiological states such as enhanced immune function, the β1-, β2- and β5-subunits can be replaced upon interferon-γ signaling by inducible immunoforms named β1i-, β2i- and β5i-subunits [54]. The replacement results in the formation of “immunoproteasomes”, which favor the generation of MHC class I antigenic peptides [58]. A recent study showed that these inducible immunoforms can exist in parallel with their constitutive counterparts in the normal adult mouse myocardium [59]. The role of the other four ß-subunits remains at the moment elusive. They are thought to be inactive [60], but one study proposed a novel N-terminal nucleophile hydrolase activity

for the β7-subunit [61]. The recent identification of protein phosphatase 2A and protein kinase A as functional partners of the cardiac 20S proteasome [62] suggests dynamic regulation of the proteasome function in the normal and diseased heart.

1.2.2. The 19S regulatory complex of the proteasome

The 19S regulatory complex, which is also known as PA700 (for proteasome activator of 700 kDa), is composed of at least 19 distinct subunits, which are distributed in a lid and in a base subcomplex. According to the nomenclature of Finley et al. [63] the subunits are named Rpn1-Rpn15 (regulatory particle non-ATPase) and Rpt1-Rpt6 (regulatory particle triple-A).

The lid subcomplex is composed of the subunits Rpn3, Rpn5-Rpn9, Rpn11, Rpn12 and Rpn15, whose functions remain mostly unclear. Rpn11 has a metalloprotease-like deubiquitinating activity and catalyzes the deubiquitination of polyubiquitinated proteins [64, 65]. Rpn4 was originally identified as a putative subunit of the lid subcomplex, but recent studies showed that it acts as a transcription factor regulating the expression of proteasome subunits via interaction with PACE (proteasome-associated control element) sequences, which were found in at least 32 genes encoding proteasome subunits [66, 67]. The subunit Rpn10 connects the lid and the base subcomplexes, and recognizes and binds polyubiquitin chains of proteins via its ubiquitin interaction motif (UIM) element [68-70]. The two non-ATPase subunits Rpn1 and Rpn2 bind a series of polyubiquitinated chain receptors and are therefore thought to mediate indirect targeting of polyubiquitinated proteins to the 19S regulatory complex [71, 72].

The subunits Rpt1-Rpt6 possess an AAA-family ATPase activity and represent together with the subunits Rpn1, Rpn2 and Rpn13 the base subcomplex. The six ATPase subunits form a hexameric ring, which is placed on the top of the α-ring of the 20S proteasome [73]. The interaction of the base and the α-ring activates the 26S proteasome leading to an opening of the regulated gates in the α-ring [55]. Especially Rpt2 is known to be involved in controlling both substrate access and product release from the 20S proteasome by opening the gated pore of the 20S proteasome. Another function of the base is to unfold the proteins and to channel them into the 20S core. The ATPases have a chaperone-like activity, which enables them to bind relatively non-specifically to protein surfaces and to change their tertiary structure by utilizing ATP resulting in the unfolding of the proteins [74, 75]. In addition to its ATPase activity, Rpt5 binds directly polyubiquitin chains [76]. Recent data showed that Rpn13 and Rpn14 are involved in the efficient recognition of ubiquitinated Gcn4p, which is a typical eukaryotic transcriptional activator [77].

1.2.3. Assembly of the 26S proteasome

A recent study proposed a multistep model for the assembly of the human 20S proteasome [78]. In this model, the subunits α5 and α7 associate with a heterodimer consisting of two chaperone proteins, the proteasome assembly chaperone 1 and 2 (PAC1, PAC2). The remaining α-subunits can then polymerize and the heptameric α-ring is formed. The β-subunits initially possess an N-terminal prosequence, which prevents their degradation and which is removed after their incorporation into the complex [79, 80]. These β-propeptides associate with the chaperone protein hUmp1 (human analog of ubiquitin-mediated proteolysis), and this complex associates in turn with the α-ring/PAC1:PAC2 complex forming an inactive half-proteasome. Two inactive half-proteasomes dimerize and hUmp1 catalyzes the cleavage of the prosequences of the β-subunits resulting in a 20S proteasome with active proteolytic sites [81, 82]. The prosequences, hUmp1 and the PAC1:PAC2 heterodimer are then released and degraded by the 20S proteasome.

Only rare information exists about the assembly of the 19S regulatory complex. A study suggests that the six ATPase subunits (Rpt1-Rpt6) first form the hexameric ring and then, after coupling with Rpn1 and Rpn2, associate with the 20S proteasome [83]. Like already mentioned above, the subunits β1, β2 and β5 can be replaced by their inducible immunoforms resulting in an altered composition of the 20S proteasome. In addition, various endogenous post-translational modifications, like N-terminal myristoylation, N-terminal acetylation and phosphorylation of the proteasome subunits can occur, which were shown to alter the function of the 26S proteasome [59, 62, 84, 85]. A recent report provides evidence of distinct proteasome subpopulations with different activities in murine hearts [86]. The alteration in proteasome subunit composition would afford specificity and selectivity for various protein substrates and could therefore play a major role in the regulation of protein degradation.

1.3. Investigation of the ubiquitin-proteasome system

The UPS function can be evaluated at the step of both ubiquitination and degradation by determining the steady-state levels of ubiquitinated proteins by Western blot and the chymotrypsin-like, trypsin-like, and caspase-like activities of the 26S proteasomes using specific synthetic fluorogenic substrates, respectively. These small fluorogenic substrates can easily enter the 20S proteolytic core in an ubiquitination-independent manner. However, they can also be cleaved by non-proteasomal peptidases such as calpain and do not reflect the highly-regulated entry of substances into the 20S proteasome. In addition, although both 20S and 26S proteasomes exist in cells, only the 26S proteasome has been proven to play a significant role in protein degradation. The 26S is an ATP-dependent degradation system, whereas the 20S functions independently of ATP [87]. Therefore, activities measured in the absence of ATP do not accurately reflect the proteasome function. To better evaluate the UPS proteolytic function in vitro or in the whole animal, a series of fluorescent protein

reporters were developed [88, 89]. Fluorescent protein reporters were modified in a way that they were targeted for ubiquitination and degradation by the UPS. These reporters use different ubiquitination signals, such as, modified ubiquitin (Ub) covalently linked to green/red fluorescent protein (eg, Ub^{G76V}-GFP or Ub^{G76V}-DsRed) [88, 90], a cleavable Ub fusion peptide that allows creation of an N-end rule substrate (eg, Ub-Arg-GFP), and the CL1 degron (GFP^{u}) [91-93]. GFP reporter transgenic mouse models of UPS proteolytic function have been created [94, 95]. These mice are important tools to decipher the role of the UPS in the whole animal in various pathologies including cardiac disease.

2. Role of the ubiquitin-proteasome system in cardiac disease

A large body of evidence indicates UPS alterations in human or experimental cardiac disease. Whereas accumulation of ubiquitinated proteins is a common feature of cardiac disease and suggests UPS impairment [96-104], the activities of the proteasome are not consistently depressed in affected hearts [95, 97, 99, 101-109]. Similarly, the expression of the UPS components such as E2 conjugating-enzymes, E3 ubiquitin ligases, or subunits of the proteasome are either increased [100-104, 106, 110, 111] or decreased [102, 103, 106, 112] in cardiac disease. On the other hand, recent studies suggest that the muscle-specific E3 ligases play a key role in the regulation of both cardiomyocyte and cardiac hypertrophy [13, 17, 30, 31, 33, 35, 113, 114]. A summary of the literature is given in Table 2 and Figure 4.

2.2. Cardiac hypertrophy and failure

Cardiac hypertrophy occurs as an adaptive response to increased workload to maintain cardiac function in response to physiological or pathological stress. While physiological hypertrophy can result from exercise, pathological hypertrophy responds to events such as volume or pressure overload, ischemia, or genetic abnormalities. Prolonged pathologic cardiac hypertrophy causes heart failure, and its mechanisms are largely unknown. Cardiac remodeling during hypertrophy and failure involves global increase in gene expression, including re-expression of fetal genes such as β-MHC and α-skeletal actin, or up-regulation of the expression of atrial/brain natriuretic factors [115-117]. One key element of cardiac hypertrophy is an adaptation in protein turnover. It refers to both protein synthesis and degradation, and interestingly, while synthesis has always been shown to be stimulated, protein degradation was either accelerated or unchanged in hypertrophic hearts, but inhibited by induction of cardiac work or high aortic pressure in Langendorff preparations [118, 119]. More recently, accumulation of ubiquitinated proteins has been reported in human heart failure [96-98, 104] suggesting impaired UPS. In an experimental mouse model of heart failure induced by transverse

aortic constriction (TAC) both increased steady-state levels of ubiquitinated proteins and depression of proteasome activities [97] as well as prolongation of endoplasmic reticulum (ER) stress characterized by accumulation of ER chaperones [120] were described. Recent data also showed that the transcription factor p53 accumulates during the transition from cardiac hypertrophy to heart failure [121]. These lines of evidence are consistent with the hypothesis that removal of abnormal proteins by the proteasome is insufficient in heart failure. However, depressed UPS was not detected consistently and recent data rather showed an activation of the UPS, including increased levels of UPS components and proteasome activities in murine, canine and feline models of TAC-induced cardiac hypertrophy [105, 122].

A recent series of data indicate that several muscle-specific E3 ligases protect against development of cardiomyocyte hypertrophy induced by drugs and physiological or pathological cardiac hypertrophy in the whole animal. Adenoviral overexpression of atrogin-1, MuRF1 or Mdm2, inhibits phenylephrine or endothelin-1-induced hypertrophy in cardiomyocytes (Figure 4A), whereas knock-down of atrogin-1 enhanced it [13, 31, 113]. Similarly, the overexpression of atrogin-1 in the heart of transgenic mice blunts both physiological hypertrophy induced by IGF-1 and growth hormone (GH) and pathological hypertrophy induced by TAC (Figure 4B) [13]. Conversely, whereas atrogin-1 knock-out (KO) mice do not exhibit any cardiac phenotype under basal conditions, they develop marked physiological cardiac hypertrophy in response to voluntary running exercise (Figure 4B) [17]. These data support the view that atrogin-1 regulates both physiological and pathological hypertrophy. On the one hand, atrogin-1 disrupts the Akt pathway by activating (by ubiquitinating) FoxO1/FoxO3 through the addition of “non-canonical” K63-linked chains [17]. On the other hand, atrogin-1 blocks hypertrophy by ubiquitinating and degrading calcineurin via the “canonical” K48-linked chains and therefore prevents nuclear translocation of the transcription factor NFATc4 [13]. Similar to atrogin-1 KO mice, single KO mice of either MuRF1, MuRF2 or MuRF3 gene do not show signs of cardiac hypertrophy under basal conditions [30, 33, 114]. However, while MuRF1 KO mice develop exaggerated hypertrophy after TAC [114], MuRF3 KO mice develop cardiac rupture after myocardial infarction (Figure 4B) [17]. Other evidences indicate that MurRF1 is a key regulator of the PKC-dependent hypertrophic response [31], while MuRF3 plays a key role in maintaining ventricular integrity after myocardial infarction by stimulating the turnover of FHL2 and γ-filamin [33]. Interestingly, the double MuRF1/MuRF2 KO mice develop a severe phenotype quickly after birth, and most of them die from chronic heart insufficiency and acute cardiac decompensation with heart failure [30]. Similarly, the double MuRF1/MuRF3 KO mice develop an extreme and lifelong muscle hypertrophy [35]. This demonstrates the cooperativity between MuRF1 and MuRF2 and between MuRF1 and MuRF3 at the genetic level. In contrast to MuRF1-KO and atrogin-1-KO, which do not develop a cardiac phenotype in the basal conditions, the cardiomyocyte-specific VHL-KO develop severe progressive structure degeneration and heart failure, malignant transformation and premature death by chronic activation of the HIF-1α hypoxia response pathway [123]. These data strongly

support that VHL is required for normal cardiac structure and function and that HIF-1α is central to this role of VHL.

2.2. Myocardial ischemia

Recent observations suggest that UPS impairment contributes to the pathophysiology of myocardial ischemia. The first evidence indicates loss of the 20S trypsin-like activity after 30 min of in vivo left anterior descending (LAD) occlusion [107]. Loss of activity was associated with oxidative modifications (4-hydroxy-nonenalyation, 4-HNE) of several proteasome subunits and accumulation of ubiquitinated proteins. These data were then confirmed in isolated perfused heart and indicate that not only the 20S activities were decreased but also the 26S activities, which was consistent with increased steady-state levels of ubiquitinated proteins [99]. The mechanism by which UPS dysfunction is induced during ischemia is not clear. The degree of proteasome inhibition depends on the duration of ischemia, and recent data showed that pretreatment of isolated hearts with the proteasome inhibitor lactacystin results in higher accumulation of oxidized proteins and decreased degradation of oxidized actin in the postischemic heart [124]. It was recently demonstrated that the 19S ATPase subunit Rpt6 is very sensitive to oxidative inactivation [125]. Moreover, modification of the 20S subunits by 4-HNE results in specific changes in 2D electrophoresis pattern associated with reduced 20S proteasomal activity [108].

A recent series of data indicate that muscle-specific E3 ligases play a key role in myocardial ischemia. Both CHIP and Mdm2 have been shown to modulate apoptosis and severity of disease in ischemia/reperfusion injury [36, 113]. CHIP KO mice are much more sensitive to ischemia/reperfusion injury than wild-type mice [36], suggesting that CHIP is cardioprotective. The mechanism of cardioprotection may be related to the ability of CHIP to bind damaged proteins in association with the chaperones Hsp70 and Hsp90, and therefore to coordinate their refolding or to mediate their degradation via its E3 ligase activity. The transcription factor p53 is an important effector of ischemia/reperfusion. Interestingly, the E3 ligase Mdm2 binds to p53 and inhibits its transcriptional activity but also acts as a E3 ubiquitin ligase by promoting ubiquitination and proteasomal degradation of p53 [44, 126].

2.3. Cardiac atrophy

Atrophy is characterized by a change in the balance of protein turnover toward proteolysis. Previous studies in skeletal muscle atrophy induced by different means have shown increased mRNA levels for ubiquitin, E2 ubiquitin-conjugating enzymes, E3 ubiquitin ligases such as atrogin-1 and MuRF1, and components of the proteasome, indicating UPS activation [127, 128]. Much less is known

about the UPS in cardiac atrophy, likely because this state is physiologically less relevant. However, unloading the heart with a left ventricular assist system is used in clinical practice for patients waiting for heart transplantation. Mechanical unloading of the heart can be experimentally induced in animals by heterotopic heart transplantation. This results in atrophic remodeling with reactivation of a "fetal gene program", such as observed in cardiac hypertrophy [129]. The recent demonstration of increased steady-state levels of ubiquitinated proteins, increased mRNA levels of the ubiquitin-conjugating enzyme UbcH2 and of downstream targets of mTOR supports the view of simultaneous activation of protein synthesis and UPS-mediated degradation in this model [100, 106, 112]. However, and in contrast to skeletal muscle atrophy, mRNA levels of atrogin-1 and MuRF1 decreased in cardiac atrophy [106, 112].

2.4. Other cardiac diseases

Doxorubicin, a potent anti-cancer agent of the anthracycline family induced cardiomyopathy. Recent evidence indicates that doxorubicin increases both the atrogin-1 mRNA levels in cardiac myocytes [111] and the UPS function in GFPdgn reporter mice [95], suggesting that UPS activation may contribute to acute cardiotoxicity of doxorubicin therapy.

Hyperglycemia is an independent risk factor for diabetic heart failure. However the mechanisms by which hyperglycemia induced cardiac damage remain poorly understood. Recent studies showed that the UPS becomes dysfunctional [101] and that the transcription factor GATA4 is down-regulated via the activation of its E3 ligase CHIP during experimental hyperglycemia [130]. These studies suggest that UPS alterations may also be involved in human diabetic heart failure.

2.5. Familial cardiomyopathies

Recent studies suggest that UPS alterations might play a role in familial cardiomyopathies, namely in familial hypertrophic cardiomyopathy (FHC) and desmin-related cardiomyopathy (DRM).

2.5.1 Familial hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is recognized on the basis of the most striking features of the condition – asymmetric septal hypertrophy, left ventricular outflow tract obstruction and premature sudden death. The disease prevalence has been estimated at approximately 1:500 in young adults [131]. HCM is the major cause of sudden death in young adults and particularly young athletes and is associated with a significantly increased risk of heart failure and malignant arrhythmia. HCM is associated with myocardial and myofibrillar disarray. Ultrastructural analysis of hypertrophied

ventricular tissue from patients with HCM showed a degeneration of cardiomyocytes [132]. Particularly, diseased myocytes exhibited a variety of ultrastructural alterations, such as myofibrillar lysis or marked development of the sarcoplasmic reticulum and mitochondria.

HCM is an autosomal-dominant familial disease (FHC) in most of the cases [133]. The typical forms of the disease involve more than 400 different mutations in at least 13 genes encoding sarcomeric proteins (for reviews, see [134, 135]). Out of them, *MYBPC3* is one of the most frequently mutated genes [136]. It encodes cardiac myosin-binding protein C (cMyBP-C) [137, 138], which is a major component of the thick filament and only expressed in the heart in mammals [139, 140]. cMyBP-C has both structural and regulatory roles in cardiac muscle [138]. Recent data using cMyBP-C KO cardiomyocytes or tissue demonstrated that cMyBP-C participates in the decreased Ca^{2+} sensitivity of the myofilament under β-adrenergic stimulation [141], is necessary for complete relaxation in diastole [142], and modulates the tunning of the molecular motor in the heart [143]. Most of the *MYBPC3* mutations result in a frameshift and should produce truncated cMyBP-C [144]. However, truncated cMyBP-C proteins were not detected in myocardial tissue of two unrelated FHC patients with frameshift mutations [145, 146], suggesting haploinsufficiency as a disease mechanism. Interestingly, heterozygous cMyBP-C KO mice carrying only one functional allele exhibit the key feature of human FHC that is asymmetric septal hypertrophy [147], which is preceded by gene expression changes including activation of the JNK and p38 parts of the MAPK pathway and induction of apoptosis [148]. Overexpression of human truncated cMyBP-Cs in cardiomyocytes or transgenic mice showed markedly less expression than the wild-type protein [90, 149, 150]. The absence of biomolecular interaction between truncated cMyBP-C and human β-MHC [151] suggests that lack of sarcomeric incorporation may be one of the mechanisms by which degradation of truncated cMyBP-C is promoted. This is supported by data showing that sarcomeric disassembly precedes degradation of cardiac MHC [152]. Truncated cMyBP-Cs have been shown to be rapidly and quantitatively degraded by the UPS and to a lower extent by the lysosome after adenoviral gene transfer in cardiomyocytes using specific inhibitors of both pathways [90]. The formation of ubiquitin-positive aggregates of truncated cMyBP-C and accumulation of the fluorescent UPS reporter Ub^{G76V}-DsRed in the presence of mutant, but not the wild-type cMyBP-C strongly suggest impaired UPS by the truncated cMyBP-C [90]. The mechanism by which the UPS is impaired is not elucidated yet. However, it is tempting to hypothesize that cMyBP-C mutants compete with other degradation-prone proteins for the UPS. This could result in accumulation of factors such as hypertrophic or apoptotic factors that can directly or indirectly lead to hypertrophy. Further analyses are needed to investigate such impairment in vivo.

2.5.2. Familial dilated cardiomyopathy

The role of the UPS may also be seen in familial dilated cardiomyopathies (F-DCM). DCM is characterized by left ventricular dilation and systolic dysfunction. The incidence has been evaluated at 3.5-8.5/100 000 individuals/year. It is the major cause of heart failure and heart transplantation [153]. Several inheritance forms have been identified, but the most common form is transmitted as an autosomal-dominant trait. In the pure form of F-DCM, 11 genes have been identified encoding different components of cardiomyocytes, including sarcomeric proteins, lamin A/C, and cytoskeletal proteins such as αB-crystallin (CryAB) and desmin (for detailed review, see [135]). Desmin aggregates have also been frequently found in human heart failure [154]. Experimentally, involvement of the UPS in F-DCM has been mainly evidenced in desmin-related myopathy (DRM), which was initially characterized by marked accumulation of desmin in skeletal and cardiac muscle and transmitted in an autosomal-dominant fashion [155]. The same group identified ten years later the corresponding R120G mutation in the gene encoding CryAB [156]. CryAB is the most abundant heat shock protein in the heart. Transgenic mice expressing a $CryAB^{R120G}$ mutant develop cardiomyopathy at 3 months and die at 6-7 months from heart failure [157]. These mice show perinuclear aggregates containing both desmin and preamyloid oligomer [158], suggesting that CryAB-DRM is a subclass of the aggresomal and amyloid-related neurodegenerative disorders such as Alzheimer's and Parkinson's diseases [159]. The cross between $CryAB^{R120G}$ mutant transgenic mice with the GFPdgn reporter mice revealed marked UPS impairment and accumulation of aggregates [102]. Interestingly, recent data demonstrated that voluntary exercise slows the progression to heart failure and reduced preamyloid aggregates formation in $CryAB^{R120G}$ mice [160]. Similar UPS impairment was found in another DRM mouse model associated with a desmin mutation [103, 161]. The molecular mechanisms by which aggregates impair the UPS remain elusive. It has been proposed that aggregated proteins themselves directly inhibit the 26S proteasome by "chocking" the proteases. Alternatively protein aggregates may indirectly interfere with UPS function by inactivating or depleting UPS components. Data obtained by Wang and collaborators indeed suggest a diminished entry of ubiquitinated protein into the 20S proteasome, likely due to depletion of key components of the 19S proteasomes [102, 103, 161].

3. Effect of proteasome inhibitors on cardiac function

Short term proteasome inhibition during ischemia/reperfusion injury and cardiac hypertrophy results in cardio-protection and cardiac hypertrophy regression in vivo. However, recent studies have demonstrated that proteasome inhibition therapy can result in significant cardiac side effects. In humans given Velcade® for the treatment of multiple myeloma, unexpected increases in cardiac

complications have been described. The therapeutic value of proteasome inhibition in cardiac disease in humans has not been tested to date.

3.1. Proteasome inhibition in cardiac hypertrophy

The development of cardiac hypertrophy depends on the function of the proteasome. Initial studies identified that NF-κB activity is essential in the development of hypertrophy, a process that is dependent on the proteasome-dependent degradation of the multimeric inhibitor of κB (IκB). When the hypertrophy driven activation of NF-κB is inhibited experimentally, agonist-induced cardiomyocyte hypertrophy is prevented [162]. Knowing that NF-κB is critical in the hypertrophic growth of cardiomyocytes, additional studies were performed using NF-κB inhibitors. Similarly, in vivo inhibition of NF-κB attenuates cardiac hypertrophy development [163, 164]. During the course of NF-κB activation, IκB is phosphorylated, and subsequently degraded, releasing NF-κB to the nucleus. The phosphorylated IκBα subunit is recognized specifically by the E3 ubiquitin ligase Skp-Cullin-F box β-transducin repeat-containing protein (SCF-βTrCP), which ubiquitinates it for degradation by the proteasome.

Knowing that NF-κB activation is dependent on proteasome activity, it was next determined if a broader inhibition of the proteasome could inhibit cardiac hypertrophy, by inhibiting NF-κB among other mechanisms. Indeed, partial inhibition of the proteasome suppresses the hypertrophic growth of neonatal rat cardiomyocytes in culture [165]. Inhibition of hypertensive Dahl-salt sensitive rats with the proteasome inhibitor Velcade® significantly similarly reduces cardiac hypertrophy, providing support for the therapeutic possibility of using low-dose proteasome inhibition as a therapy to interrupt the development of cardiac hypertrophy [165]. Recent studies have extended these findings, using experimental proteasome inhibitors to prevent not only the induction of hypertrophy, but also to induce the regression of established cardiac hypertrophy [166]. The ability of proteasome inhibition to prevent left ventricular hypertrophy development and promotion of its regression suggests that it may be useful clinically, but remains currently untested.

3.2. Proteasome inhibition in ischemia/reperfusion injury in the heart

When the 26S proteasome is experimentally inhibited before cardiac ischemia/reperfusion injury, a significant reduction in cardiac infarct size and concomitant left ventricular function occurs [167-172]. This transient inhibition of the proteasome may be cardioprotective by several mechanisms, including the induction of heat shock proteins and the inhibition of NF-kB activity [169-171, 173]. Proteasome inhibition after the induction of experimental ischemic events inhibits ventricular triplets,

which are highly predictive of sudden cardiac death during the first 24 hours after bortezomib treatment [174]. Malignant tachyarrthymias and sudden cardiac death are hypothesized to be suppressed through the inhibition of G-protein signaling [174, 175]. These mechanisms may be part of a process that occurs endogenously during ischemic injury. A recent study demonstrated that phosphorylated IκB accumulates after ischemic injury, indicating deficit proteasome activity [109]. However, other proteasome functions were intact, suggesting that a more specific proteasome inhibition occurs during ischemic injury, in contrast to the more global pharmacologic inhibition that occurs experimentally [109].

3.3. Chronic proteasome inhibition in cardiac hypertrophy

In contrast to the studies described above investigating the short term beneficial effects of proteasome inhibition, chronic inhibition of the cardiac proteasome may be detrimental. Bortezomib is approved by the FDA for the therapy of multiple myeloma, and its most common side effects include neurological symptoms and thrombocytopenia [176]. An unexpected increase in cardiac complications has been identified ranging from arrhythmias to heart failure [177]. In this study of 69 patients, 10% experience cardiotoxicity and were generally >60 years old and received a cumulative dose of at least 20.8 mg/m^2 [177]. Asymptomatic arrhythmia following bortezomib therapy has also been reported [178]. It is still unknown the mechanism by which bortezomib is cardiotoxic. However, there is currently a clear dichotomy between the benefits of short term bortezomib use in cardiac disease, and its cardiotoxicity seen after longer term use.

4. Role of autophagy in cardiac disease

A recent series of studies have outlined the parallel regulation of autophagy and the UPS, which may act in concert to regulate the necessary turnover of proteins. The process of autophagy involves the degradation of cytosolic components within lysosomes [179-183]. It occurs continuously to repair cellular components to maintain homeostasis, during both health and disease as a way to regulate protein quality control. Importantly, by degrading endogenous proteins, autophagy also supplies nutrients for survival during times of stress [184, 185]. Cardiac autophagy was first described in cultured cardiomyocytes [186]. Specifically, electron microscopic analysis identified autophagic vesicles containing damaged organelles in cells within 24 hours of culture [186]. Increased autophagy has subsequently been described in a wide range of cardiac disease states, including cardiomyopathy, heart failure, ischemic injury, but not in normal heart [110, 123, 187-191]. While autophagy is an

ongoing process in baseline conditions, it may be more apparent in disease because it is significantly up-regulated in response to hemodynamic stress [192].

Cardiomyocytes with deficient autophagy have increased levels of ubiquitinated proteins in autophagosomes, indicating that autophagy may work in parallel with the UPS to turnover cellular proteins. The process of autophagy involves numerous proteins, including ubiquitin-like proteins (e.g. Atg5 and Atg8), which participate in the formation of autophagosome vesicles (for a more complete review, see [193]). In mice lacking cardiac Atg5, autophagy is inhibited, resulting in cardiac hypertrophy, dilation, and contractile dysfunction [192]. Hearts from Atg5 deficient mice have increased levels of ubiquitinated proteins, disorganized sarcomeres, and mitochondrial aggregation [192]. These findings suggest a key role of UPS in the maintenance of essential cardiac structures that preserve contractility and energy production in the process of autophagy. While the presence of ubiquitinated cytoplasmic inclusions is a feature of cardiomyocytes during autophagy, the relationship between the proteasome and the degradation of these proteins is unclear in the process of autophagy. A final parallel between the UPS and the process of autophagy is their regulation. Recent studies demonstrated that the transcription factor FoxO3 is required for the induction of autophagy in skeletal muscle, regulating several autophagy-related genes such as LC3 (microtubule-associated protein 1 light chain 3) and Bnip3 (Bcl-2/adenovirus E1B 19 kDa-interacting protein) [194]. Similarly, it has been shown that the transcription factor HIF-1α, which is down-regulated by the UPS, also activates Bnip3 and the process of autophagy in the heart [123].

Cardiac FoxO3 inhibits cardiac hypertrophy by activating the transcription of E3 ubiquitin ligases such as atrogin-1 or MuRF1 [195], which themselves prevent cardiac hypertrophy [13, 17, 114]. Interestingly, a recent study demonstrated that FoxO3 coordinately regulates degradation by autophagy and the UPS (Figure 5) [196]. Further studies are needed to evaluate whether similar parallel regulation of autophagy and UPS exist in cardiomyocytes and in the whole animal.

5. Conclusion and future directions

The involvement of the UPS in the regulation of normal and pathological cardiac structure and function is now clearly established. However, whereas accumulation of ubiquitinated proteins is a common feature of cardiac dysfunction and suggests UPS impairment, the proteasome activities measured with fluorogenic substrates are not consistently depressed in affected hearts. The recent development of a series of UPS reporter adenovirus or mice have opened the way to better investigate the global function of the system in vivo. In addition, our understanding of the molecular mechanisms involved in UPS-mediated degradation in healthy and diseased heart is just at the beginning. A series

of recent evidence underlined the key role of the muscle-specific E3 ubiquitin ligases in blunting the development of physiological and pathological cardiac hypertrophy. Knowing that the E3 ligases give the specificity to the system, we expect that other cardiac-specific E3 ligases will be identified in the future providing additional molecular mechanisms and signaling pathways. Although short term and/or low doses or proteasome inhibition prevents left ventricular hypertrophy in animal models, chronic use in human treated with bortezomid is not without cardiotoxicity. Finally, the identification of the FoxO3 transcription factor coordinating the lysosomal and nonlysosomal pathways for degradation opens new perspectives of research and potential novel therapies for cardiac diseases.

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Figure legends

Figure 1. Models of ubiquitin chain formation. Ubiquitin contains seven Lys (K) residues, which can all be used for the formation of ubiquitin chains. **(A)** Chains linked via K48 ("canonical" ubiquitination) are recognized for UPS-mediated degradation of the target proteins. **(B)** The more extended chains linked via K63 ("non-canonical" ubiquitination) are implicated in DNA repair and activation of protein kinases. **(C)** Addition of a single ubiquitin at any K residue can result in a change in localization, transcriptional regulation and endocytosis.

Figure 2. Mechanism of action of muscle-specific E3 ligases on their substrates. (A to C) CHIP, Mdm2 and MuRF1 are monomeric E3 ubiquitin ligases that mediate the transfer of ubiquitin molecules from the E2 conjugating-enzyme to the target proteins (in green). The interaction with the E2 takes place at either the U-box domain (CHIP) or the RING finger domain (Mdm2 and MuRF1). **(D)** Ozz participates in the multi-subunit VCB complex for ubiquitination of phosphorylated (P) β-catenin. Ozz interacts through the SOCS domain with the adaptor protein elongin B/C which, together with cullin1 and Roc1, and therefore offers a platform for interaction with the E2 conjugating-enzyme. **(E)** The HECT-E3 ligase Nedd4-like forms an intermediate thioester-bond with ubiquitin and interacts through the WW domains with the conserved PY domain of the target proteins (voltage-gated channels). **(F)** Atrogin-1 contains a F-box motif but no ligase activity per se. It acts as a bridge between the enzymatic complex of the SCF (skp1, cullin, F-box protein) ubiquitin ligase complex, a subgroup of the RING finger E3 ubiquitin ligase. Peptides correspond to the digested substrates.

Figure 3. Schematic representation of the 26S proteasome. Molecular weight and function of the subunits are indicated. Abbreviations used are: Rpn, regulatory particle non-ATPase; Rpt, regulatory particle triple-A; UBL, ubiquitin-like domains.

Figure 4. Muscle-specific E3 ligases blunt development of experimental cardiomyocyte and cardiac hypertrophy. (A) Overexpression of atrogin-1, MuRF1 and Mdm2 prevent development of cardioymocyte hypertrophy induced by phenylephrine (PE) or endothelin-1 (ET-1). **(B)** Overexpression of MuRF1 blunts cardiac hypertrophy induced by transverse aortic constriction (TAC) in mice. Overexpression of atrogin-1 blocks both TAC-induced pathological hypertrophy and physiological hypertrophy induced by 2 week-treatment of IGF-1 and growth hormone (GH) in mice. Mice deficient in atrogin-1 gene develop exaggerated cardiac hypertrophy in response to voluntary running exercise. Mice deficient in MuRF3 gene exhibit a massive cardiac hypertrophy after myocardial infarction (MI). Adapted from [13, 17, 30, 31, 33, 35, 113, 114].

Figure 5. FoxO3 transcriptionally regulates both the autophagy-lysosomal pathway and key E3 ubiquitin ligases (i.e. atrogin-1) involved in the UPS-mediated degradation and therefore regulation of cardiac hypertrophy. (A) Upon external stimuli such as ischemia, autophagy is induced in cardiomyocytes. **(B)** Sequestering of cytosolic organelles and proteins by a double membrane vesicle occurs by a coordinated process involving numerous proteins such as Bnip3 and LC3. **(C)** The autophagosome fuses with a lysosome. **(D)** Following fusion with the lysosome, autophagosomes are degraded by lysosomal proteases. FoxO3 transcriptionally regulates proteins essential in autophagosome formation (Bnip3 and LC3). **(E)** FoxO3 regulates the expression of E3 ubiquitin ligase (e.g. atrogin-1), which is pivotal in degrading substrates (e.g. calcineurin, CnA) by the UPS. Peptides correspond to the digested substrates. Adapted from [13, 14, 17, 196].

Table 1. Muscle-specific E3 ubiquitin ligases

E3 ubiquitin ligase	Type	Substrate	References
Atrogin-1 (MAFbx)	RING finger, SCF complex	CnA, MyoD, FoxO1, FoxO3	[13, 17, 19]
MuRF1 (Trim63)	RING finger, single subunit	cTnI, PKCε, M-CK, β-MHC	[29, 31, 32, 35]
MuRF3 (Trim54)	RING finger, single subunit	FHL2, γ-filamin, β-MHC	[33, 35]
CHIP	U-box	misfolded proteins, Hsp70, GATA4	[39, 130]
Mdm2	RING finger, single subunit	p53, β-arrestin2, Tcap	[44-46, 197]
Ozz	RING finger, VCB complex	β-catenin	[47]
Nedd4-like	HECT domain	$Na_v1.5$, KCNQ1	[50, 51]
Trim32	RING finger	Actin	[52]

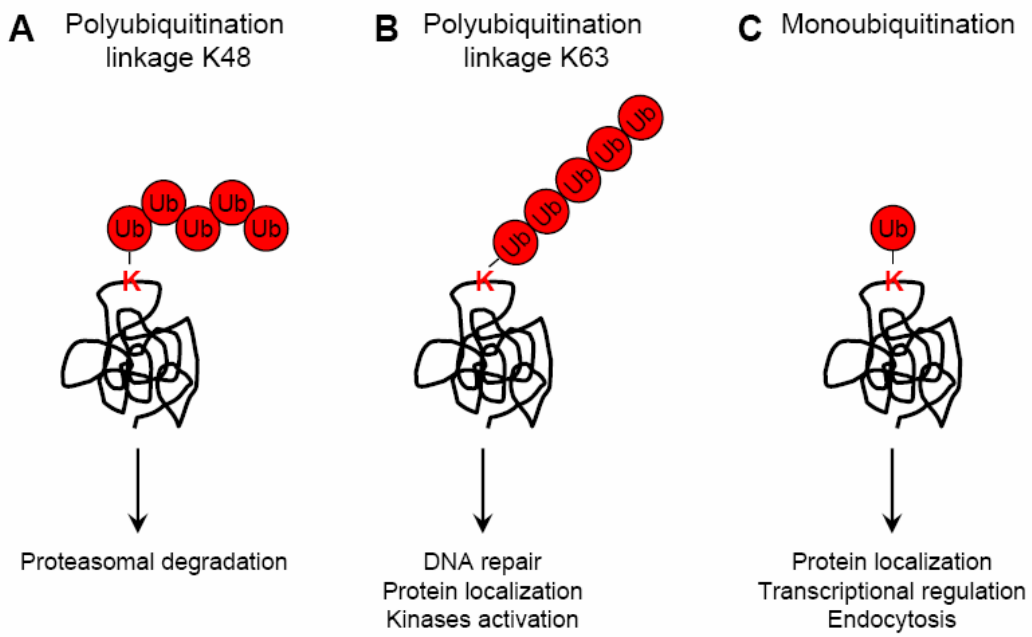
Abbreviations used are: β-MHC, β-myosin heavy chain; CHIP, carboxyl-terminus of Hsp70-interacting protein; CnA, calcineurin; cTnI, cardiac troponin I; FHL2, four and a half LIM protein-2; FoxO, Forkhead box O; GATA4, GATA binding protein 4; Hsp70, heat shock protein 70; KCNQ1, voltage-gated potassium channel; MAFbx, Muscle Atrophy F-box; M-CK, muscle creatine kinase; Mdm2, murine double minute 2; MyoD, myogenic differentiation factor; MuRF, muscle RING finger protein; Nedd4, neuronal precursor cell expressed developmentally downregulated; $Na_v1.5$, voltage-gated sodium channel; p53, protein 53; PKC, protein kinase C; Tcap, titin-cap, telethonin; Trim, TRIpartite interaction motif.

Table 2. Alterations of the UPS in cardiac disease

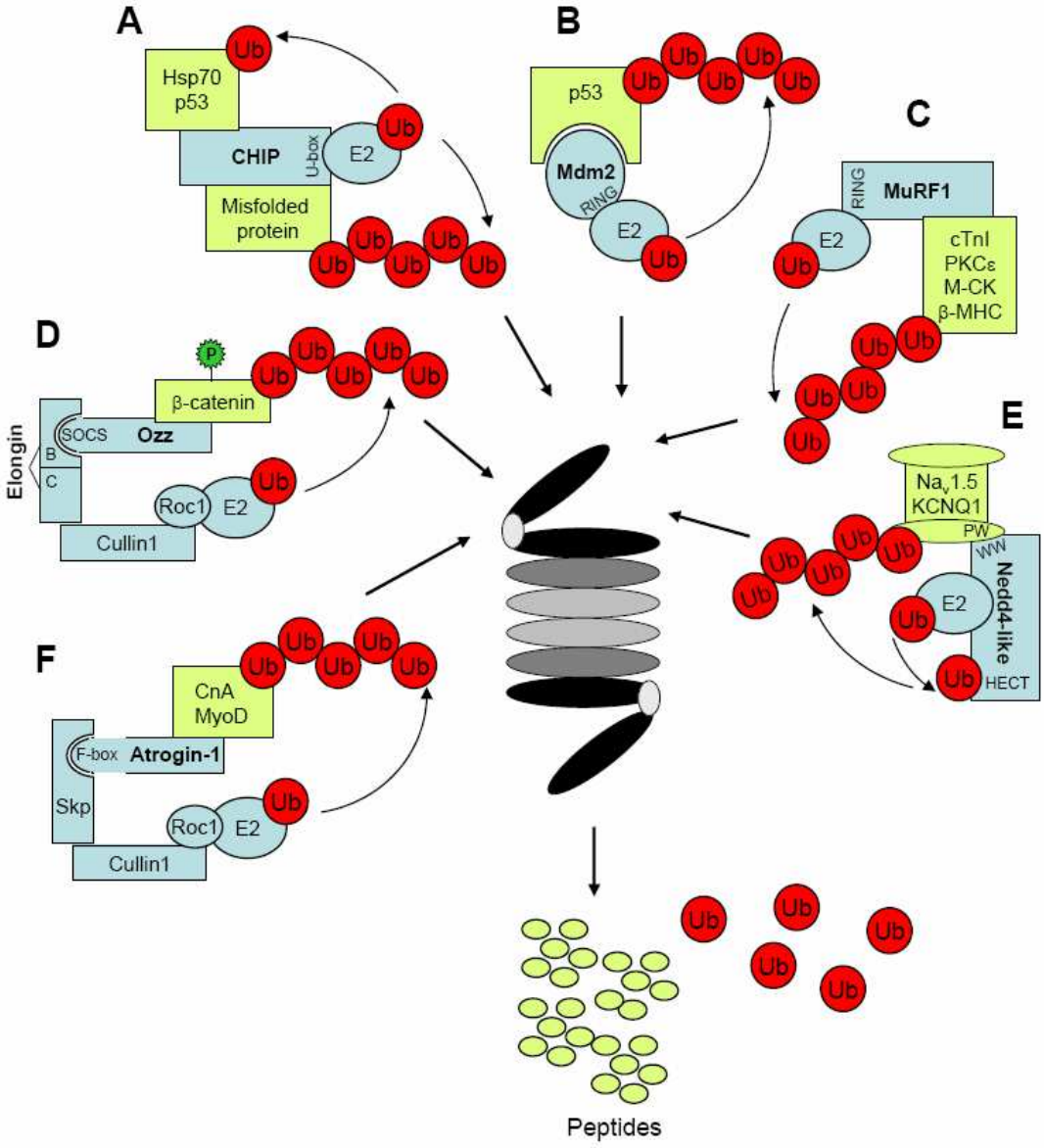
Disease	UPS alterations	References
Human heart failure	↑ ubiquitinated proteins	[96-98, 104]
	↑ E2 (Ubc2)	[110]
	↑ E3 (Mdm2)	[104]
	↑ deubiquitinases	[110]
Experimental cardiac hypertrophy and failure induced by TAC	↑ ubiquitinated proteins	[97, 122]
	↑ E2 (UbcH2)	[106]
	↑ E3 (atrogin-1, MuRF1)	[106]
	↑ E3 (Cb1, cIAPs, Mdm2)	[122]
	↑ proteasome subunits	[105, 106]
	↓ or ↑ proteasome activities	[97, 105]
Experimental myocardial ischemia	↑ ubiquitinated proteins	[99]
	↓ proteasome activities	[99, 107-109]
	Modifications of 20S subunits	[99, 107, 125]
Experimental cardiac atrophy induced by heterotopic heart transplantation	↑ ubiquitinated proteins	[100]
	↑ E2 (UbcH2)	[100]
	↓ E3 (atrogin-1, MuRF1)	[106, 112]
Doxorubicin-induced cardiomyopathy	↑ E3 (atrogin-1)	[111]
	↑ proteasome activities	[95]
Experimental hyperglycemia	↑ ubiquitinated proteins	[101]
	↓ 26S proteasome activities	[101]
	↑ 11S subunit of the proteasome	[101]
FHC cell model (truncated cMyBP-C)	↓ truncated cMyBP-C level in cardiomyocytes; impaired UPS	[90]
DRM mouse models ($CryAB^{R120G}$ or mutant desmin)	↑ ubiquitinated proteins	[102, 103]
	↑ proteasome activities	[102, 103]
	↑ 20S subunits	[102, 103]

	↓ 19S subunits	[102, 103]

Abbreviations used are: cIAPs, cellular inhibitors of apoptosis, CryAB, αB-crystallin; cMyBP-C, cardiac myosin-binding protein C; DRM, desmin-related myopathy; E2, ubiquitin-conjugating enzyme; E3, ubiquitin ligase, FHC, familial hypertrophic cardiomyopathy; TAC, transverse aortic constriction.



Mearini et al., Fig.1



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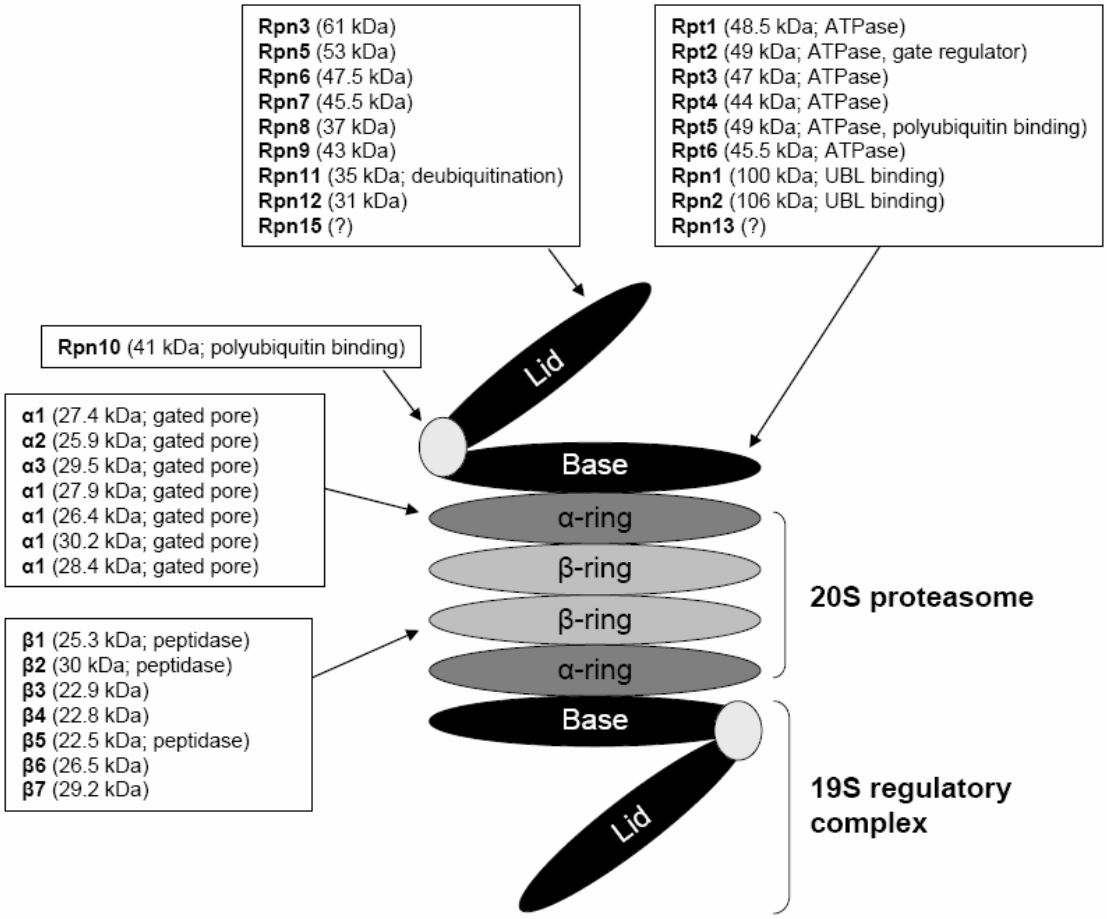

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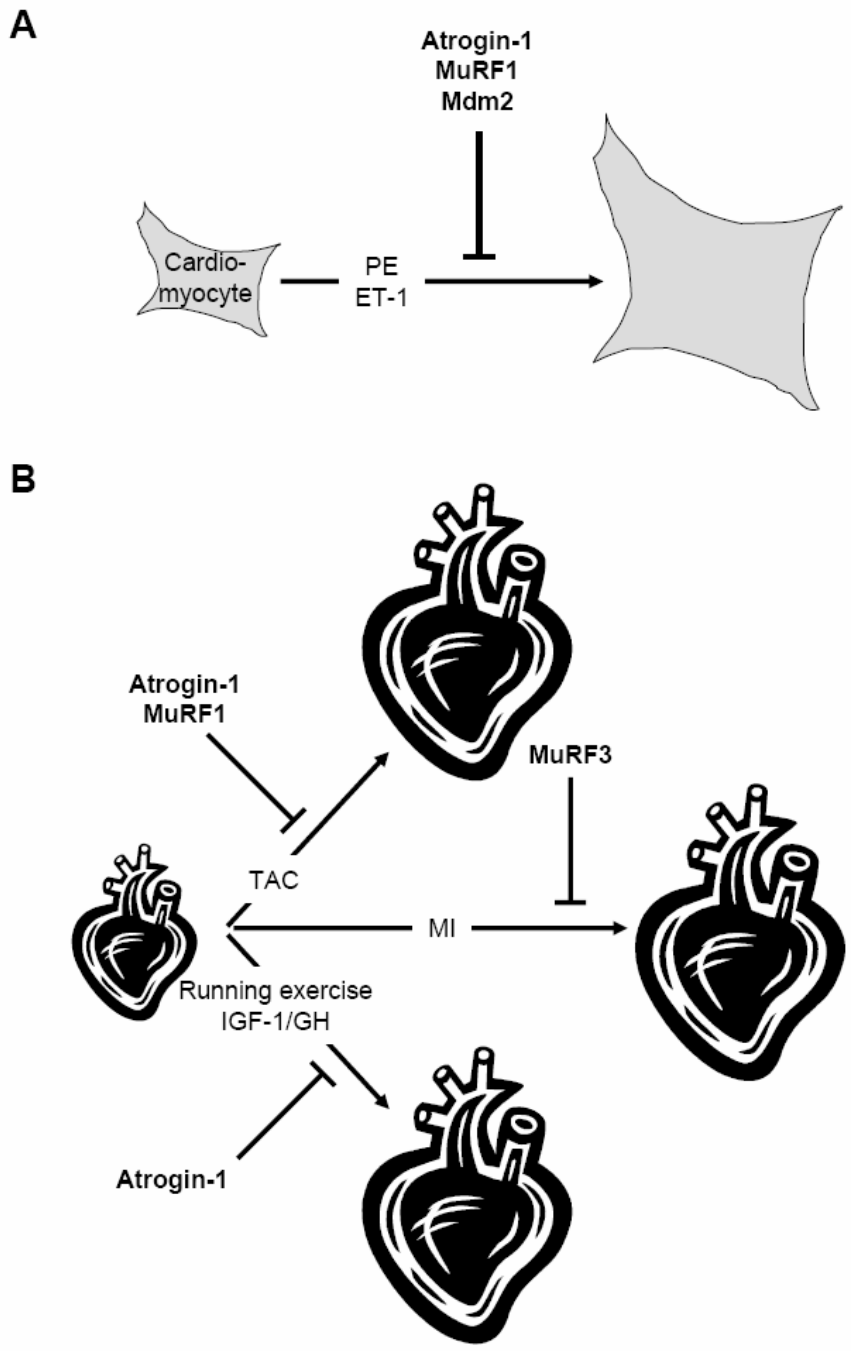

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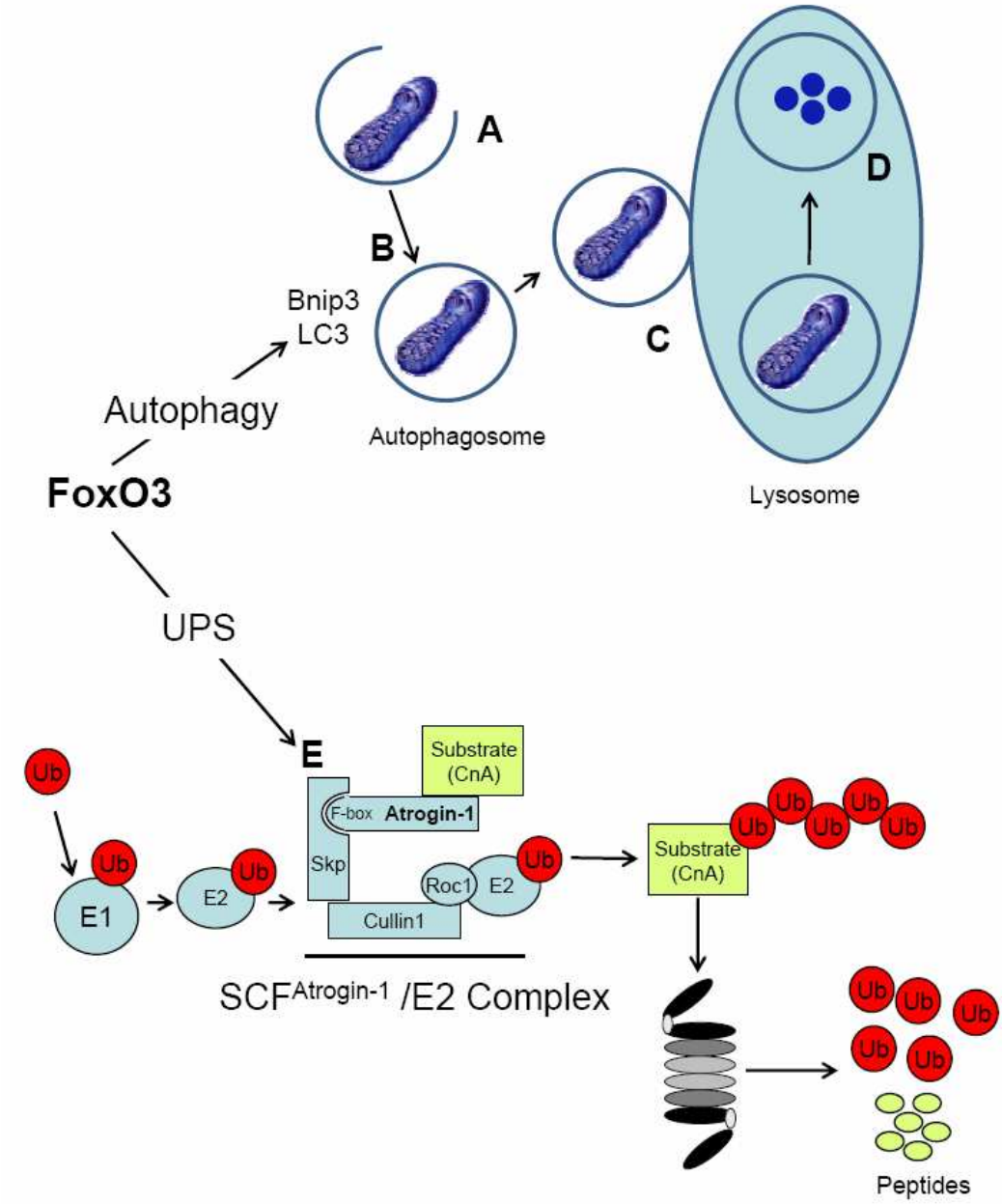

Mearini et al., Fig. 5