ANTIOXIDANTS & REDOX SIGNALING
Volume 8, Numbers 9 & 10, 2006

Forum Review

Protein Degradation by the 26S Proteasome System in the Normal and Stressed Myocardium

ALDRIN V. GOMES, CHENGGONG ZONG, and PEIPEI PING

ABSTRACT

The 26S proteasome is a multicatalytic threonine protease complex responsible for degradation of the majority of proteins in eukaryotic cells. In the last two decades, the ubiquitin proteasome system (UPS) has been increasingly recognized as an integral component in numerous biologic processes including cell proliferation, adaptation to stress, and cell death. The turnover of intracellular proteins inevitably affects the contributions of these molecules to cellular networks and pathways in any given tissue or organ, including the myocardium. Perturbations in the protein-degradation process have been shown to affect protein turnover and thereby affect the cardiac cell functions that these molecules are designated to carry out, engendering diseased cardiac phenotypes. Recent studies have implicated the role of proteasomes in stressed cardiac phenotypes including postischemia–reperfusion injury and cardiac remodeling (*e.g.*, heart failure). The 26S proteasomes also appear to be susceptible to modulation by stresses (*e.g.,* reactive oxygen species). This review focuses on roles of the 26S proteasome system in protein degradation; it provides an overview of the progress made in cardiac proteasome research as well as a discussion of recent controversies regarding the UPS system in diseased cardiac phenotypes. *Antioxid. Redox Signal.* 8, 1677–1691.

INTRODUCTION

The ubiquitin-proteasome system (UPS) is hypothesized to play a central role in governing the cellular adaptation processes to stress or injury via cellular quality-control and defense mechanisms (33, 43). Although the degradation of a protein by the 26S proteasome usually represents the end of the life of a protein, the UPS also plays an important role in eukaryotic transcription, which represents the beginning of the life of that protein (16). Most intracellular proteins, including misfolded, damaged, and mutated proteins, are specifically recognized and removed efficiently by the UPS. In addition to the UPS, the lysosomal degradation system also contributes significantly to intracellular proteolytic activities (Table 1). Other critical intracellular protein-degradation mechanisms in mammalian cells include specialized proteases compartmentalized in the mitochondria, nuclei, and/or endoplasmic reticulum (ER) (Table 1). The UPS is a highly complex system with two primary functional components; the first component achieves protein ubiquitination, and the second component accomplishes proteolysis of proteins (the proteasome) (Fig. 1).

Protein ubiquitination

The process of attaching ubiquitin (Ub) to target substrates is called ubiquitination, which is an essential reversible posttranslational modification that occurs in all eukaryotic cells. The reversible Ub⇔de-Ub process is highly dynamic, and its importance rivals that in phosphorylation⇔dephosphosphorylation in physiologic functions, as these processes are fundamental to ensure proper cell function (70). It is likely that every intracellular pathway uses the proteasome system for the turnover of either the partial or the full list of its protein components. The role of Ub in physiologic functions begins with the covalent attachment of the C-termi-

Departments of Physiology and Medicine, Cardiac Proteomics and Signaling Laboratory at CVRL, UCLA School of Medicine, Los Angeles, California.

TABLE 1. MAJOR PROTEASES INVOLVED IN PROTEIN DEGRADATIONS IN HEART AND OTHER CELL TYPES

Proteases involved	*Regulation features*	*Subcellular locations*	*References*
26S Proteasomes	ATP dependent Ubiquitin dependent	Cytosol Nuclear ER	Heart (36,37,84) yeast (46), liver (30)
20S Proteasomes	ATP independent Ubiquitin independent	Cytosol nuclear	Heart (84), yeast (46), liver (30) bacteria (77)
Lon Clp AAA proteases	ATP dependent Ubiquitin independent	Mitochondria	Heart (24), yeast (107) liver (24), bacteria [Lon homologues (3)]
Lysosomal enzymes and catepsins	ATP independent Ubiquitin independent	Lysosome	Heart (20), yeast (103) liver (19)
Nuclear Proteases	Various requirements *e.g.,* matrix metalloproteinase-2 is zinc dependent	Nuclear	Heart (57), yeast (102) liver (58)
Calpains	Calcium dependent	Cytosol Cytoskeleton Nucleus	Heart (104), yeast (99) liver (114), bacteria (99)
Caspases	Caspase activation is ATP dependent	Mitochondria Cytosol	Heart (18), liver (13) yeast [caspase-related (67)]

Multiple mechanisms of protein degradation in distinct subcellular organelles have been reported. Table 1 illustrates the potential protein degradation mechanisms that may be used by the heart and the proteases that are involved. References for the presence of the various kinds of proteases in different cell types are shown. AAA, ATPases associated with a wide variety of cellular activities; it is a superfamily of proteases.

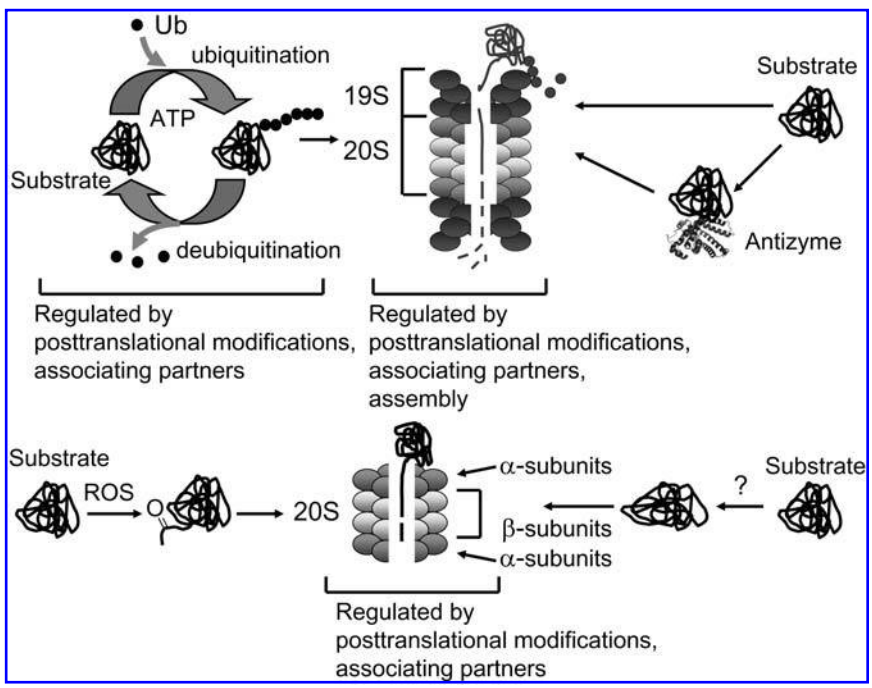

FIG. 1. Protein degradation by the ubiquitin-proteasome system (UPS). Top panel: The process of protein degradation by the UPS. A protein substrate is ubiquitinated, bound to the 19S complex, where it is unfolded, and subsequently is sent to the core 20S proteasome. Proteolytic digestion of the substrate takes place in the β rings of the core 20S proteasome, resulting in the release of small polypeptides. Ub is removed by deubiquitinases present in the 19S complex and recycled. In addition, some proteins can be degraded by the 26S proteasome in the absence of ubiquitination, whereas other nonubiquitinated proteins are degraded by the 26S proteasome after binding to another protein called antizyme. We hypothesize that both the ubiquitination process and the function of proteasome maybe regulated by multiple factors, including posttranslational modifications and associating partners. **Lower panel:** The 20S proteasome can also degrade proteins in a ATP- and ubiquitin-independent manner. Mildly oxidized proteins are preferentially degraded by the 20S proteasome. Some proteins are degraded by the 20S proteasome in vitro in the presence of fatty acids. It is likely that some proteins would be degraded by the 20S proteasome via mechanisms not currently known. ROS, Reactive oxygen species.

nal glycine 76 of Ub to the NH_2 group of a lysine residue on the protein substrate, forming an isopeptide bond. Ub itself has a compact globular shape containing α-helices and β sheets as well as an extended C terminus that facilitates the formation of a bond with substrates. This process is repeated to form a polyubiquitin chain that links glycine 76 on one Ub to specific lysine residues on the other attached Ub molecule. The actual addition of Ub to the protein substrate requires at least three classes of enzymes: E1, a ubiquitin-activating enzyme; E2, the ubiquitin-conjugating enzyme, and E3, the ubiquitin–protein ligase. Several recent reviews focus on these enzymes (2, 44, 81, 82). A key function of the ligase system is proposed to achieve substrate specificity; the specific mechanism remains to be fully understood but is known to depend on the interaction between an E2 (a cell contains various types E2s) and an E3 (possibly hundreds of different kinds in eukaryotic cells).

It also is important to note that ubiquitination has important roles independent of the 26S proteasome. Ubiquitination of histone results in heterochromatin relaxation and assembly of transcription complexes on the promoter. Other roles of ubiquitination include targeting of membrane proteins for degradation by the lysosome, protein activity modulation, changes in subcellular localization of proteins, and alterations in protein–protein interactions (106, 112).

The proteasomes

The second functional component of the UPS is the proteasome complexes, which constitute the proteolytic enzyme system; it catalyzes the degradation of intracellular proteins. The 26S proteasome is a large multiprotein complex (~2 MDa) with a 20S proteolytic core particle (CP) and one or two 19S regulatory particles (RP) located at the end of the core particle (Fig. 1). The 19S complex recognizes and deubiquitinylates; it unfolds the target proteins and aids their entry into the 20S catalytic core, where they are degraded. The mammalian 19S complex has been reported to contain between 16 and 20 subunits. Using a targeted organelle proteomic approach, recent investigations characterized the cardiac 19S complex be consist of six ATPase subunits (Rpt1–6) and at least 11 additional non-ATPase subunits (Rpns) (36). Degradation of proteins by the 26S proteasome requires energy in the form of adenosine 5′-triphosphate (ATP), which is used by the ATPases in the 19S.

The 26S proteasomes may be subject for modulation by a variety of regulatory mechanisms (37). Besides being subject to a number of posttranslational modifications, the 26S proteasome is constantly being assembled and disassembled; a number of associating partners are now known to interact with and regulate the 26S proteasome (119). Some associating proteins may tune the activity of the proteasome, whereas others may have more significant effects on the proteasome function (34, 96).

The 20S proteasomes are cylinder-shaped complexes composed of 28 subunits, which exist as two α and two β rings with α1–7, β1–7, β1–7, and α1–7 symmetry (Fig. 1). The seven α subunits align in precise order within the outer rings and share approximately 30% sequence identity with one another. The active sites of the three proteolytic β subunits (β1, β2, and β5) are all N-terminal threonine residues located inside the proteolytic chamber and are responsible for a broad specificity in hydrolyzing peptide bonds from the carboxyl end of acidic (postglumate peptide hydrolyzing, PGPH), basic (trypsin-like, T-L), or hydrophobic (chymotrypsin-like, CT-L) amino acids, respectively (90, 110). This arrangement of the active proteolytic sites inside the proteasome prevents unwanted random degradation of proteins. To reach the proteolytic sites, substrates must first pass through a narrow opening in the outer ring of α subunits called the α annulus (113). The α annulus is blocked by N-terminal residues of the α subunits in the absence of an activating complex such as the 19S complex (38). However, even when the α annulus is opened by activating complexes, the entry into the central proteolytic cavity may not be large enough for globular proteins. These large proteins must be unfolded before they can enter the central core of the proteasome. The 20S proteasome can potentially degrade any protein that enters the catalytic chamber.

The inefficiency of the 20S to degrade folded proteins is largely overcome when one or two 19S regulatory particles bind to one or both sides of the 20S complex, forming either 26S or 30S proteasomes, respectively. However, the 30S proteasomes are still commonly referred to as the 26S proteasomes. For the remainder of this article, the 20S bound to either one or two 19S complexes will both be called 26S proteasomes. Very few, if any, free 19S complexes float in mammalian cells; the majority of the 19S complexes are bound, as a two- to threefold excess of 20S proteasomes in molar amounts are observed both in rat liver and in cultured cell lines (9). The 20S proteasomes can function independent of the 19S proteasome by interacting with other complexes such as the 11S complex (45).

Before or during degradation of substrates by the proteasomes, the poly-Ub moieties on the substrate are removed by deubiquitinating enzymes while the substrate remains bound to the proteasome (108). The rate of subsequent degradation by the proteasome depends on the presence or absence of an unstructured region in the substrate (85). The degradation of tightly folded proteins by the proteasome is significantly accelerated when an unstructured region is present and it is therefore acting as the initiation site for degradation (85). This unstructured region is proteolyzed first, and then the rest of the protein is degraded. In the absence of unstructured regions in tightly packed proteasome substrates, degradation of these substrates occurs at a very slow rate. It is possible that other proteins may be involved in initially creating small unstructured regions, which act as initiation sites for degradation in proteasome substrates.

Proteins degraded by the proteasome are usually broken down into peptides of 4–25 residues in length. However, in some cases, the proteasome degrades proteins in a limited manner, resulting in larger polypeptides such as when NF-κB is cleaved from a p105 inactive precursor to yield a shorter p50 active subunit (80). The complete breakdown of proteins into free amino acids is not accomplished exclusively by the proteasome as other enzymes could act either upstream or downstream of the proteasome, depending on the protein substrate. In skeletal muscle wasting, some enzymes such as m-calpain, cathepsin L, and/or caspase 3 act upstream of the proteasome, whereas other enzymes such as tripeptidyl-

peptidase II and aminopeptidases further degrade peptides released from the proteasome.

The proteasome and antigen presentation

One of the key roles of the proteasome is the generation of peptide antigens that bind major histocompatibility complex (MHC) class I molecules (54). Other proteases, such as tripeptidyl peptidase II (TPPII), thimet oligoendopeptidase (TOP) or Trim-peptidases, also contribute antigenic peptides, but to a smaller extent than the proteasome. The generation of peptide antigens by the proteasome is an important event whereby three inducible proteolytic 20S subunits, β1i (LMP2), β2i (MECL-1), β5i (LMP7), have coevolved, which could replace β1, β2, and β5 subunits, respectively. These alterations in proteasome proteolytic components lead to optimization of the production of antigenic peptides for MHC class I presentation. Once bound to the MHC class I complex, the peptides are carried to the cell surface, where they are recognized by the cytotoxic T cells as a "self" or "nonself" antigen with the presenting cell of "nonself" antigens ultimately being eliminated by the T cell. The importance of the inducible β subunits in cardiac tissue remains to be investigated.

Extracellular degradation by the proteasome

Although the UPS has been well recognized as an intracellular degradation system, recent results suggest that the UPS is involved in extracellular degradation of a 70-kDa sperm receptor HrVC70 in the ascidian *Halocynthia roretzi* and, consequently, for sperm penetration of the vitelline coat during fertilization (93). This study carries tremendous significance both with respect to the role of UPS in extracellular protein degradations as well as in establishing investigative models to characterize the role of the UPS in intracellular protein degradation.

ROLE OF THE 26S PROTEASOME IN CARDIAC DISEASE

Similar to what has been observed in other mammalian cells, the UPS is important for normal heart muscle homeostasis (63). Protein synthesis as well as protein turnover are essential cellular processes required to maintain a dynamic steady state of cells. When protein-degradation rate is altered, either significantly increased or decreased, this physiologic steady state is disrupted; cellular functions designated by these proteins are affected; consequently, mild to lethal diseases may manifest in the heart. Despite advances in recent therapeutics, cardiovascular diseases still have high morbidity and high mortality in Western societies (50). Novel experimental and therapeutic approaches that target the UPS may offer potential new therapies for cardiovascular diseases.

Cardiac cells use the UPS as a major mechanism of protein turnover. Several studies suggest that the number of ubiquitinated proteins in the heart at any time point represents a significant population of proteins in cardiac cells (111). Because the reported methods used to determine global ubiquitination of proteins are limited to the detection of moderately abundant proteins, it is likely that a proportion of most if not all proteins that are degraded by the UPS are ubiquitinated at any time point. Other studies have documented the importance of the ubiquitin ligase system in degradation of key molecules in the heart (21, 52). Although it remains unclear how many E2s or E3s exist in cardiac cells; current evidence supports their roles in achieving selectivity.

The role of the second functional component of UPS, the proteasome system in cardiovascular disease is less understood. This topic of investigation has been gaining significant interest in the last few years (37, 42). Several lines of evidence demonstrated an association of proteasome dysfunction in the pathogenesis of heart disease, such as ischemia–reperfusion injury (10), atrophy (87), doxorubicin cardiotoxicity (56), transgenic mice models of mutant αB-crystallin (14) and desminopathy (62), and pressure-overload–induced heart failure (105). Dilated cardiomyopathy and ischemic cardiomyopathy are associated with increased Ub both at mRNA and protein levels (Table 2), and this increase in Ub is associated with an accumulation of ubiquitinated proteins. Certain families of E2s and E3s as well as 20S proteasome subunits are upregulated in cardiac hypertrophy (88) (Table 2). Table 2 provides an overview regarding proteins in the UPS reported to be altered in cardiac pathology.

Ischemia–reperfusion injury

Using an *in vivo* rat model, Bulteau *et al*. (10) found that 30 min of ischemia followed by 60 min of reperfusion resulted in declines in chymotrypsin-like, peptidylglutamyl-peptide hydrolase, and trypsin-like activities of the proteasome when assayed in cytosolic extracts; this change was associated with increases in cytosolic levels of oxidized and ubiquitinated proteins. In related studies, ischemia–reperfusion has been shown to increase the rate of free radical production in cardiac tissue (1,7). The increases in reactive free radicals are thought to produce oxidative damage by reacting with lipids, proteins, and DNA. The decrease in trypsin-like protease activity was associated with oxidative modification of three α subunits of the purified 20S (α1, α2, and α4) by the lipid peroxidation product 4-hydroxy-2-nonenal (HNE) (10).

The molecular mechanisms underlying the altered proteolytic activities in these studies remain unknown. Because the HNE-modified proteins are α subunits, which are known to interact with at least one of the catalytic β-like subunits, β1, β2, and/or β5, potentially, HNE modifications to α subunits may affect the accessibility of the catalytic core to proteasome substrates and/or catalytic activities by altering interaction(s) between other α subunits and β subunits. The modifications of the 20S subunits by HNE are also interesting because oxidatively modified proteins are themselves preferentially degraded *in vitro* by the 20S proteasome in an ATP-independent fashion (97). Many of the proteins that are oxidized during myocardial ischemia–reperfusion are degraded by the proteasomes (such as oxidized actin) (25). Nevertheless, 20S purified from the ischemia–reperfusion injury heart tissue was reported to have no significant difference in chymotrypsin-like and peptidylglutamyl-peptide hydrolase activities when compared with 20S isolated from control rat hearts

(10). These data are consistent with the notion that cytosolic proteasome inhibitors may have been increased or that cytosolic proteasome activators may have been decreased by ischemia–reperfusion injury. The possibility of increased proteasome inhibition is evident by the fact that HNE cross-linked proteins (and proteins cross-linked by other free radical processes) have been shown to be resistant to proteolysis and can act as proteasome inhibitors (31, 32, 98). The possibility of decreased proteasome activation in ischemic injury remains to be investigated.

In recent studies, addition of HNE to purified rat cardiac 20S proteasomes resulted in modification of specific 20S subunits (α1, α2, α4, α5, α6, and β6) and changes in proteasome activities (28). These results suggest that HNE modification of 20S subunits are likely to account for some of the changes observed during cardiac ischemia–reperfusion. Interestingly, purified 20S proteasome from rat liver showed a different susceptibility to HNE modification under the same conditions used for the cardiac 20S (28). Although α1, α6, and β6 are not modified in the liver 20S proteasome, other subunits α3, β1i, β3, and β4, which are not modified in the heart proteasome, are modified in the liver proteasome. These results suggest that the subunit composition and/or posttranslational modifications on the 20S proteasome may affect HNE accessibility. Interestingly, whereas the chymotrypsin-like activity of the liver 20S proteasome is the most sensitive to HNE inactivation, the trypsin-like activity of the heart 20S proteasome is the most susceptible to inactivation by HNE (29). The inactivation of the trypsin-like activity in the heart 20S proteasome by HNE can be prevented by addition of Hsp 90 (17).

The past several years of proteasome investigations in the heart have yielded conflicting results. Rat hearts subjected to 15 min of ischemia had no effect on the 26S and 20S β5 proteasome activities (83). However, rat hearts subjected to 30 min of ischemia and 60 min of reperfusion showed significantly decreased 26S and 20S β5 proteasome activities. Preischemic treatment of hearts with the proteasome inhibitor, MG132, resulted in dose-dependent decreases in recovery of postischemic function. Preconditioning with repetitive ischemia or preischemic treatment with nicorandil resulted in a significant increase in postischemic 20S-proteasome activity after 60 min of reperfusion (83). Other studies suggest that compounds that affect the proteasome could have cardioprotective effects (23). Figure 2 shows a summary of the different ischemia–reperfusion protocols on the activity of the proteasome. Isolated rat hearts pretreated with palm tocotrienol (isomer of vitamin E) and then subjected to 30 min of global ischemia followed by 2 h of reperfusion showed higher 20S and 26S proteasome activities than did hearts that received vehicle. The cardioprotective effects of palm tocotrienol was shown to be due to inhibition of c-Src activation and proteasome stabilization (23).

Atrophy

In a rat model of atrophic remodeling induced by heterotrophic heart transplantation, the levels of polyUb and UbcH2 (E2 enzyme) increased after 2–7 days and then decreased 28 days after transplantation (87). Although the level of UbcH2 after 28 days was not significantly different from that in the control rats, the level of polyUb after 28 days was higher than that the control mice but significantly lower than 7 days after transplantation (87). Cardiac atrophy activated two downstream components of the mammalian target of rapamycin (mTOR), p70S6K and 4EBP1. Atrophy was acceler-

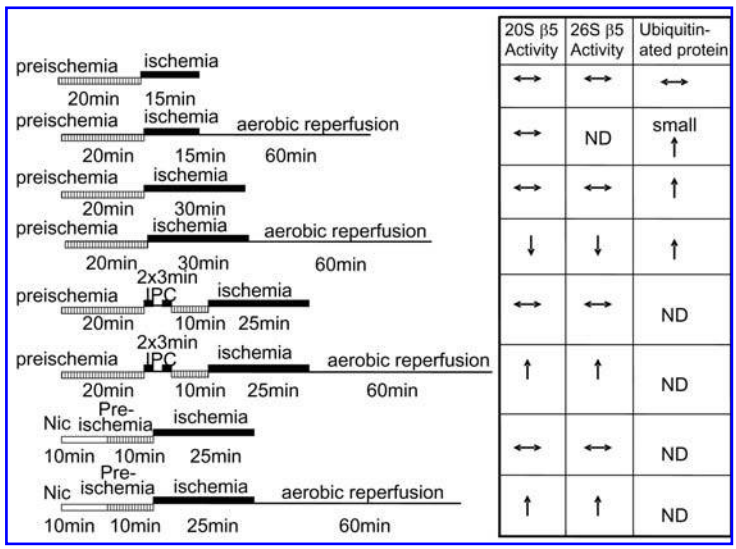

FIG. 2. Schematic overview of experimental protocols used for the global rat heart ischemia model and results obtained from these studies. ND, not determined; IPC, ischemic preconditioning; Nic, nicorandil. All the results are stated relative to their respective controls, based on Powell *et al*., 2005 (83).

TABLE 2. PROTEINS ASSOCIATED WITH THE UBIQUITIN-PROTEASOME SYSTEM THAT ARE AFFECTED IN CARDIAC DISEASES

Protein	*Disease phenotypes*	*Species*	*Expression*	*Ref.*
Ubiquitin	End-stage heart failure due to idiopathic dilated cardiomyopathy (DCM)	Human	mRNA expression in left ventricular myocardium is 1.8-fold upregulated	(55)
	End-stage heart failure due to DCM	Human	Protein expression in left ventricular myocardium is upregulated	(111)
	End-stage heart failure due to DCM	Human	Protein expression in heart is upregulated	(105)
	End-stage heart failure due to DCM	Human	mRNA and protein expression in heart is upregulated	(115)
	End-stage heart failure due to ischemic cardiomyopathy	Human	mRNA expression in heart is 2.2-fold upregulated	(115)
	Pressure-overload heart caused by thoracic aortic constriction	Mouse	Protein expression in heart is upregulated	(105)
	Streptozotocin-induced diabetes	Rat	mRNA expression in heart is 1.5-fold upregulated	(63)
UbB (polyubiquitin gene)	Cardiac hypertrophy (caused by constriction of the ascending aorta)	Rat	mRNA expression in heart is upregulated	(88)
	Hypoxemic (exposure to hypobaric hypoxia) heart	Rat	mRNA expression in left ventricle is upregulated	(88)
	Cardiac atrophy	Rat	mRNA expression in heart is upregulated	(88)
E2s				
Ubc2	End-stage heart failure due to idiopathic DCM	Human	Protein expression in left ventricular myocardium is 2.3-fold upregulated	(55)
UbcH2	Cardiac hypertrophy	Rat	mRNA expression in heart is upregulated	(88)
	Hypoxemic heart	Rat	mRNA expression in left ventricle is upregulated	(88)
	Cardiac atrophy	Rat	mRNA expression in heart is upregulated	(88)
E2–14kDa	Hypoxemic heart	Rat	mRNA expression in left ventricle is upregulated	(88)
UBE2G2	End-stage heart failure due to idiopathic DCM	Human	mRNA expression in left ventricular myocardium is 1.8-fold upregulated	(48)
	End-stage heart failure due to hypertrophic cardiomyopathy (disease caused by Arg719Gln β-myosin heavy-chain mutation)	Human	mRNA expression in left ventricular myocardium is 1.9-fold upregulated	(48)
UBE2D2	Atrial fibrillation	Human	mRNA expression in right atrial appendages is upregulated	(53)
UBE3C	End-stage heart failure due to DCM	Human	mRNA expression in atrium is downregulated	(51)
E3s				
MuRF-1	Cardiac hypertrophy	Rat	mRNA expression in heart is upregulated	(88)
	Hypoxemic heart	Rat	mRNA expression in left ventricle is upregulated	(88)
	Cardiac atrophy	Rat	mRNA expression in heart is downregulated	(88)
Mafbx/ Atrogin-1	Cardiac hypertrophy			
	Cardiac hypertrophy	Rat	mRNA expression in heart is upregulated	(88)
	Hypoxemic heart	Rat	mRNA expression in left ventricle is upregulated	(88)
	Cardiac atrophy	Rat	mRNA expression in heart is downregulated	(88)

Continued

TABLE 2. CONTINUED

Protein	*Disease phenotypes*	*Species*	*Expression*	*Ref.*
Deubiquitinases				
USP2	End-stage heart failure due to DCM	Human	mRNA expression in atrium is downregulated	(51)
USP5	End-stage heart failure due to idiopathic DCM	Human	Protein expression in left ventricular myocardium is 2.3-fold upregulated	(55)
USP8	Atrial fibrillation	Human	mRNA expression in right atrial appendages is 2.3-fold upregulated	(53)
USP20	End-stage heart failure due to ischemia	Human	mRNA expression in left ventricular myocardium is ~3.5-fold upregulated	(111)
	End-stage heart failure due to DCM	Human	mRNA expression in left ventricular myocardium is 5.4-fold upregulated	(51)
Proteasome subunits				
α6	Desmin-related cardiomyopathy	Mouse	Protein expression in heart is upregulated	(62)
β2	Desmin-related cardiomyopathy	Mouse	Protein expression in heart is upregulated	(62)
β3	End-stage heart failure due to DCM	Human	mRNA expression in atrium is downregulated	(51)
β5	Desmin-related cardiomyopathy	Mouse	Protein expression of pre-PSMB5 in heart is upregulated	(62)
β6	End-stage heart failure due to DCM	Human	mRNA expression in atrium is downregulated	(51)
β7	End-stage heart failure due to DCM	Human	mRNA expression in atrium is downregulated	(51)
	Cardiac hypertrophy	Rat	mRNA expression in heart is upregulated	(88)
	Hypoxemic heart	Rat	mRNA expression in left ventricle is upregulated	(88)
Rpt3	Desmin-related cardiomyopathy	Mouse	Protein expression in heart is downregulated	(62)
Rpt5	Desmin-related cardiomyopathy	Mouse	Protein expression in heart is downregulated	(62)
S5b	End-stage heart failure due to DCM	Human	mRNA expression in atrium is downregulated	(51)
11Sβ	End-stage heart failure due to DCM	Human	mRNA expression in left ventricular myocardium is downregulated	(51)

Altered UPS transcripts or proteins in cardiac diseases reported in the literature; a number of changes were identified in the UPS, including E2s (Ub-conjugation enzymes), E3s (Ub ligases), deubiquitinases, 19S proteasome subunits, 20S proteasome subunits, and ubiquitin.

ated if the activation of p70S6K and 4EBP1 was blocked with rapamycin, suggesting that the mTOR pathway is important for protein synthesis during cardiac atrophy. This study using the rat model demonstrated that cardiac atrophy is associated with early upregulation of the UPS. However, this upregulation is transient, whereas the simultaneous activation of the mTOR pathway is more sustained and may be important for preventing further decreases in the size of cardiomyocytes. Skeletal muscle atrophy has also been shown to be associated with increases in mRNA levels of Ub, E2s, and E3s and proteasome components (6, 72).

Doxorubicin cardiotoxicity

Doxorubicin is a potent anticancer agent; doxorubicin carries cardiac toxicity, as it engenders cardiomyopathy (74). Using a transgenic mouse model that ubiquitously expresses a surrogate protein substrate (GFP carrying a constitutively active degradation signal) for the UPS, Kumarapeli and colleagues (56) found that doxorubicin significantly enhanced the proteolytic function of the UPS in cardiomyocytes and in the heart. The chymotrypsin-like proteolytic activity of the proteasome was decreased 6 and 24 h after doxorubicin treatment, whereas the caspase-like proteolytic activity was decreased 24 h after doxorubicin treatment. The reduction in the proteolytic activity of the proteasome is intriguing, as the overall effect of doxorubicin treatment on the cardiac UPS seemed to be an enhanced protein degradation when judged by the degradation of the surrogate protein substrates. Enhanced protein degradation was abolished by proteasomal inhibition, suggesting that doxorubicin cardiotoxicity may be due to enhanced UPS function.

Transgenic mouse model of mutant αB-crystallin

αB-crystallin is the most abundant small heat-shock protein in the heart and is important in protecting the intermediate filaments. Chen *et al*. (14) recently reported that the cardiac proteasome function was severely impaired in a mouse model of intrasarcoplasmic amyloidosis caused by expression of mutant αB-crystallin (Arg120Gly). The Arg120Gly αB-crystallin mutant is unable to stabilize desmin resulting in the aggregation of desmin. Although all three proteolytic activities of the proteasome were significantly increased in 6-month-old hearts from mice containing mutant αB-crystallin (Arg120Gly), the amount of ubiquitinated proteins also drastically increased when compared with nontransgenic littermates. The likely cause of impairment of the proteasome is insufficient delivery of substrate proteins into the core proteasome because of reductions in the 19S proteasome complexes (14). Although the mutant αB-crystallin induces cardiac hypertrophy, the impairment of the UPS occurs before the hypertrophy, suggesting that a defective UPS is an important contributor to cardiac failure. Other results suggest that heat-shock proteins may ameliorate UPS malfunction induced by the Arg120Gly αB-crystallin mutant in cultured cardiomyocytes (14).

Transgenic mouse model of desminopathy

Desmin is the main intermediate filament in mature cardiac cells. In desmin-related cardiomyopathy (cardiomyopathy caused by mutant desmin, which forms aggregates in the cytoplasm of cardiac cells), the expression of two 19S subunits (Rpt3 and Rpt5) are decreased, whereas the expression of three 20S subunits investigated (α6, β2, pre-β5) were all increased (62). Overexpression of mutant desmin (but not normal desmin) in the heart impairs the proteolytic function of the proteasome (62). This impairment of the proteolytic function of the proteasome was not due to reductions in the proteolytic activity of the proteasome but more likely due to reduced entry of ubiquitinated proteins into the core proteasome (62). Another important finding obtained using these desminopathy hearts was that the malfunction of proteasomes was not secondary to cardiac malfunction or to the disruption of desmin filaments (62). Therefore, impairment of the UPS may be an important pathogenic mechanism directly involved in cardiac diseases, such as those associated with aberrant protein aggregation.

Pressure overload–induced heart failure

The UPS was recently shown to be involved in heart failure associated with a pressure-overloaded heart model in mice (105). Pressure overload is a well-established model that results in left ventricular remodeling and the subsequent development of heart failure (39, 47). In these heart-failure models, cardiac dysfunction occurs only after a reduction of proteasome activities and accumulation of ubiquitinated proteins, suggesting that the decrease in proteasome activities was not a consequence of heart failure but was one of the causes of heart failure. An increase in Ub-positive cardiomyocytes is also observed in patients with heart failure (69).

Other cardiac diseases associated with proteasome dysfunction

Cardiac myosin binding protein C (cMyBP-C) is one of several sarcomeric proteins that has been shown to be associated with familial hypertrophic cardiomyopathy (FHC) (35). cMyBP-C is a major component of the thick filament and has both structural and regulatory roles in cardiac muscle contraction. Recombinant adenovirus expressing wild-type and two different C-terminal truncated cMyBP-Cs (FHC-causing mutations) in neonatal rat cardiomyocytes showed that the truncated cMyBP-Cs were present in low amounts because of accelerated preferential degradation by the UPS (91). The larger cMyBP-C truncation (80% truncation) formed aggregates and also showed misincorporation into the sarcomere. The colocalization of aggregated cMyBP-C and Ub suggests that the aggregate formation is due to impaired proteasomal degradation of ubiquitinated mutant cMyBP-C. Significant increases in ubiquitinated proteins would disturb intracellular homeostasis and may be responsible for late-onset cardiomyopathy that is characteristic for FHC mutations in cMyBP-C. Although it is not clear whether the proteolytic activities of the proteasome are directly affected by the aggregated cMyBP-C, it is apparent that the UPS is involved in familial hypertrophic cardiomyopathy.

A potential role of UPS in cardiac hypertrophy was suggested in the study by Li *et al.* (60). Cardiac-specific overexpression of activated calcineurin A in transgenic mice results

in cardiac hypertrophy, which develops into heart failure and death (75). The muscle-specific F-box protein, atrogin-1, directly interacted with calcineurin A and α-actinin-2 at the Z-disc of cardiomyocytes. Atrogin-1 associated with three other proteins to form a SCFatrogin-1 complex, which displayed ubiquitin ligase activity. Neonatal rat cardiomyocytes overexpressing atrogin-1 attenuated calcineurin A signaling by promoting ubiquitination and degradation of calcineurin A. Transgenic mice overexpressing atrogin-1 attenuated cardiac hypertrophy associated with banding of the thoracic aorta.

A possible link between the UPS and cardiac apoptosis was identified through the dual function of cochaperone/ubiquitin ligase, called carboxyl terminus of Hsp70-interacting protein (CHIP). CHIP is a physiologic regulator of stress-dependent apoptosis in the heart (4). Mice deficient in CHIP were associated with more frequent reperfusion arrhythmias, 50% greater infarct size, and higher mortality rates after infarction (LAD ligation) when compared with wild-type mice (117). Cardiomyocytes from mice hearts deficient in CHIP were more prone to develop apoptosis than were cardiomyocytes from wild-type mice. These results imply that CHIP plays an important role in regulating the response to cardiac ischemia–reperfusion injury (22).

EFFECT OF STRESS ON PROTEASOMES

Exposure of mammalian cells to toxic environmental stresses results in biologic responses of cellular adaptation. Many cellular factors are found to participate in this stress response, including components of stress sensors that transmit this information to transcriptional factors, which then induce and regulate programmed gene expression of cell remodeling and adaptation (79). It is broadly accepted that oxidative stress may exert a direct effect on the UPS. Interestingly, the UPS is also involved in the degradation of key enzymes involved in oxidative stress. For example, the Nrf2-Keap1 system serves as an oxidative stress sensor (79); Nrf2 is an important transcriptional factor that regulates genes encoding detoxifying enzymes and antioxidant stress proteins; and Nrf2 itself was degraded by the UPS (71).

In young healthy mammalian cells, oxidatively damaged proteins were rapidly degraded by the proteasome (41). However, oxidatively damaged proteins accumulated in aging mammalian cells and were associated with decreased proteasome activities and a number of age-related pathologies (100). The loss of proteasome function was due to lower levels of β-type subunits (the "rate-limiting" subunits), whereas the α-type subunits occurred in excess as "free" subunits in senescent cells (15). Oxidized proteins did not require Ub conjugation or ATP for degradation by the proteasome (97). The use of proteasome inhibitors or antisense oligonucleotides suggests that the proteasome is the major degradation system for oxidized proteins in mammalian cells (40, 41). The 26S proteasome exhibited more susceptibility to oxidative stress than did the 20S form (89), suggesting that oxidized proteins may have been digested by the 20S proteasome. The transcriptional complex hypoxia-inducible factor (HIF) is a key player in the signaling pathway that controls the hypoxic response of mammalian cells. Many transcription factors such as the HIF-1α or tumor suppressor p53 have low protein abundance in unstressed cells because of efficient degradation of these proteins by the 26S proteasome (118). Interestingly, stability of these proteins increased significantly on hypoxic stress, although it remains to be determined whether ROS, in this case, was one of the mediators of an inhibitory effect of the proteasome function (118).

The 26S proteasome contains different posttranslational modifications that may regulate the function of the proteasome (8, 92). Environmental stress may also affect the posttranslational modifications associated with the 26S proteasome. Few posttranslational modifications on 19S subunits are reported because of the lack of investigation of this complex. The presence of posttranslational modifications (other than HNE modification of 20S proteasomes) on cardiac proteasomes has not been previously reported, but results from our laboratory suggest that several cardiac proteasome subunits including α7 are phosphorylated in the normal adult mouse heart (120).

EFFECT OF PROTEASOME INHIBITORS ON CARDIAC FUNCTION

Proteasome inhibitors block activities of the 20S and 26S proteasomes. In general, inhibition of UPS-mediated protein degradation by proteasome inhibitors leads to accumulation of proteasome substrates, including cyclins, transcriptional factors, tumor-suppressor proteins, and protooncogenes. Depending on the degree of proteasome inhibition, different levels of cellular dysfunction may occur, ranging from impaired mitochondrial function to cell-cycle arrest and activation of an apoptotic pathway. Once protein aggregates are formed, these aggregates exert further negative impact on the proteasome, leading to a perpetual cycle and the development of pathogenesis (5). Depending on the amount of aggregate formation, it is hypothesized that different severities of cardiac disease could occur.

Many proteins are shown to be ubiquitinated and degraded by proteasomes in mammalian cells, including transcription factors, signaling regulators, cell-cycle regulators, pro- and antiapoptotic proteins, channels, and receptors such as inositol 1,4,5-trisphosphate receptor, estrogen receptor α (intracellular receptors), and other receptors such as the platelet-activating factor receptor (26, 42). Another physiologically important cardiac protein known to be degraded by the proteasome is myosin heavy chain (MHC) (27). MHC is the major thick-filament protein and is needed for both the structural integrity of the myofibril and for the critical physiologic process of muscle contraction. Cardiac MHC is a relatively stable protein with a half-life of 22 hours in cultured neonatal rat ventricular myocytes. Three different proteasome inhibitors including lactacystin hindered sarcomeric cardiac MHC degradation and increased MHC half-life to 43 hours in cultured cardiac myocytes (27). Inhibition of contractile activity with the L-type Ca^{2+} channel blocker nifedipine resulted in sarcomeric disassembly, with MHC staining (observed by confocal microscopy) lacking a clear striated pattern. Although lactacystin treatment alone for 48 h had little effect on MHC sarcomeric staining in most myocytes, the

use of both lactacystin and nifedipine resulted in more intense staining of MHC because of a reduction in the rate of MHC degradation (27). However, in the presence of nifedipine and lactacystin, MHC accumulated in the sarcoplasm of cardiac myocytes showed a nonsarcomeric staining pattern, suggesting that MHC degradation by the proteasome occurs after the disassembly of MHC from myofibrils.

Several molecules involved in programmed cell death have been identified as substrates of proteasome, including p53, Bax, and Smac/DIABLO, Bid, and NF-κB (49, 59, 66). In the presence of proteasome inhibitors, the utilization of the RNA interference method to knock down the proapoptotic proteins, p53 or Bax, stopped the accumulation of these proapoptotic proteins and significantly prevented cardiomyocyte apoptosis. In pressure-overloaded mouse hearts, the levels of the proapoptotic proteins p53 and Bax increased 2 weeks after thoracic aortic constriction and increased further after 4 weeks; the antiapoptotic proteins Bcl-2 and Bcl-XL decreased 4 weeks after thoracic aortic constriction but not after 2 weeks. This imbalance between proapoptotic and antiapoptotic proteins may contribute to the development of heart failure (18). It is interesting that other substrates of the proteasome, Bcl-2 and Bcl-XL (both antiapoptotic proteins), are decreased when the proteasome is inhibited in cardiomyocytes and lung cancer cells (76). Other studies also suggest that inhibition of the UPS induces an imbalance of various apoptosis-regulating proteins (78). The reduced levels of proteasome substrates, Bcl-2 and Bcl-XL, in the presence of proteasome inhibitors strongly suggest that other factors such as oxidative stress may contribute to the induction of significant imbalance between proapoptotic and antiapoptotic proteins (109). In cultured cardiomyocytes, pharmacologic inhibition of the proteasome resulted in the accumulation of proapoptotic proteins such as p53 and Bax (105). Inhibition of the proteasome results in impairments of the mitochondrial electron-transport system, increased the formation of mitochondrial reactive oxygen species, and decreased energy production in mitochondria (61, 68, 101).

Contrary to this, the use of the proteasome inhibitor PS-519 in an isolated perfused rat heart model of ischemia (12) and in a porcine model of myocardial reperfusion injury (86) suggests that inhibition of the proteasome may be cardioprotective. In the perfused rat heart model, PS-519 was cardioprotective after ischemia and reperfusion in the presence of polymorphonuclear leukocytes (12). PS-519 reduced polymorphonuclear leukocyte accumulation in the ischemic myocardium by >70%, showing that this proteasome inhibitor is cardioprotective against neutrophil-mediated cardiac dysfunction in ischemia–reperfusion (12). In a porcine model of myocardial reperfusion injury (1-h ischemia and 3-h reperfusion) PS-519 decreased the size of myocardial infarction and reduced troponin I and creatine kinase release from the myocardium (86). Inhibition of the proteasome with MG132 in cultured neonatal rat cardiomyocytes results in p38 MAP kinase–dependent induction of Hsp72 and Hsp32 and protection from hyperthermic (46°C) or oxidative injury (H_2O_2) (65). The proteasome inhibitor dipeptide boronic acid (DPBA) was found to repress allograft rejection in mice (64).

Although not significantly used in cardiac studies, the best-studied proteasome inhibitor, bortezomib (PS-341), is used for the treatment of multiple myeloma. Bortezomib was the first proteasome inhibitor to be evaluated in human studies and is now being investigated as a potential treatment for non–small-cell lung cancer and small-cell lung cancer. Bortezomib is a boronic acid dipeptide that binds directly with and inhibits the proteasome, resulting in modulation of the expression of cyclins, p27kip1, p53, nuclear factor-κB, Bcl-2, and Bax (94). Bortezomib has antitumor activity *in vivo* and causes growth inhibition and apoptosis in numerous non–small-cell lung cancer cell lines *in vitro* (95). The role of the UPS in cancer has been well investigated, and results suggest that the UPS activities are critical to cell transformation and cancer progression. Pretreatment with bortezomib gave significant cardioprotection against ventricular tachyarrhythmias in a canine model of myocardial infarction (116). In dogs, 6 to 24 h after coronary artery ligation is generally characterized by tachyarrhythmias, subsequently triggering a sustained ventricular tachycardia and ventricular fibrillation. Bortezomib prevented rapid (>300 beats/min) and very rapid (>360 beats/min) ventricular triplets when compared with nonpretreated infarcted dogs.

Other reports suggest that inhibition of the cardiac proteasome may be associated with cardiac dysfunction. Pretreatment of isolated rat hearts with the 2 μ*M* of the proteasome inhibitor lactacystin resulted in 51 and 42% decreases in 20S and 26S proteasome activities, respectively, but did not have any effect on postischemic function (30-min global ischemia and 60-min reperfusion) (25). Preischemic treatment of isolated rat hearts with the proteasome inhibitor, MG132, resulted in dose-dependent decreases in the recovery of postischemic function (83). The decreases in the proteolytic activity of the proteasome, together with increased levels of oxidized and ubiquitinated proteins observed with age in rat hearts (11, 73), also suggest that inhibition of the proteasome may be cardioprotective only under certain conditions.

In view of this information, unfortunately, no unifying notion of whether inhibition of proteasomes is beneficial or detrimental to cardiac function exists. Although it is clear that proteasome dysfunction is concomitant with various cardiac diseased phenotypes, pretreatment of proteasome inhibitors may aid the adaptation of myocardium to stress and injury. Inevitably, the choice of experimental models (cultured cardiomyocytes, isolated heart, *in vivo* heart model) used, the chemical structures of proteasome inhibitor, the efficacy and cellular targets of the various inhibitors, as well as the temporal profile of inhibition may greatly contribute to the contrasting phenotypes observed. It should be noted that most of the proteasome inhibitors used for these studies are also known to affect other proteases in the cell (84), which may further complicate data interpretation.

Ostensibly, resolving this controversy is of great clinical significance to the cardiovascular community; this information is essential to design therapies aimed at regulation of proteasome functions. Recent studies delineating the dynamics and complexity of cardiac proteasomes with respect to their molecular composition, function, and regulation have presented exciting new data (36, 37). The cardiac proteasome complexes possess unique features that display multiple posttranslational modifications and associating partners, enabling a variety of complex assemblies (36, 37). Our working hypothesis is that distinct subpopulations of cardiac proteasomes exist, delineated by differences in their subunit com-

position, posttranslational modification, and associating partners. Therefore, a subpopulation may be targeted for specific functions and thus be amendable to distinct regulatory mechanisms of their proteolytic activities. This concept introduces a tantalizing possibility that, perhaps, by gaining further structure and molecular information of these distinct subpopulations, we may be able to exert a higher degree of control as well as sophistication in regulation of proteasome functions in the heart; consequently, the design of therapies targeting subpopulations of proteasomes will provide higher efficacy with minimized side effects. Tremendous investigations are in demand to test these hypotheses and to realize them in a practical manner.

CONCLUSION AND FUTURE INVESTIGATIONS

The UPS serves as a primary intracellular protein-degradation machinery; malfunction of the UPS has been found to be associated with a number of cardiovascular diseases including cardiomyopathies, ischemic injury, pressure overload–induced heart failure, and atrophy. Although remarkable progress has been made in our understanding regarding the role of UPS in many cellular processes, the molecular components, function, and regulation of the proteasome complexes in the heart remain virtually unknown (Fig. 3). Investigations in the past decade on UPS in the heart have been fruitful; however, controversies exist with respect to whether proteasome inhibition is beneficial or detrimental to cardiac function. Despite the conflicting data reported, consensus agrees that the proteasome system contributes significantly to the regulation of cardiac function in normal and stressed myocardium; delineation of the UPS system will be critical to advance our understanding of the pathogenesis of cardiovascular diseases.

ACKNOWLEDGMENTS

This work was supported, in part, by NIH grants HL63901, HL65431, HL76526, and HL080111 to Dr. Peipei Ping, and by an endowment from Theodore C. Laubisch to Dr. Ping.

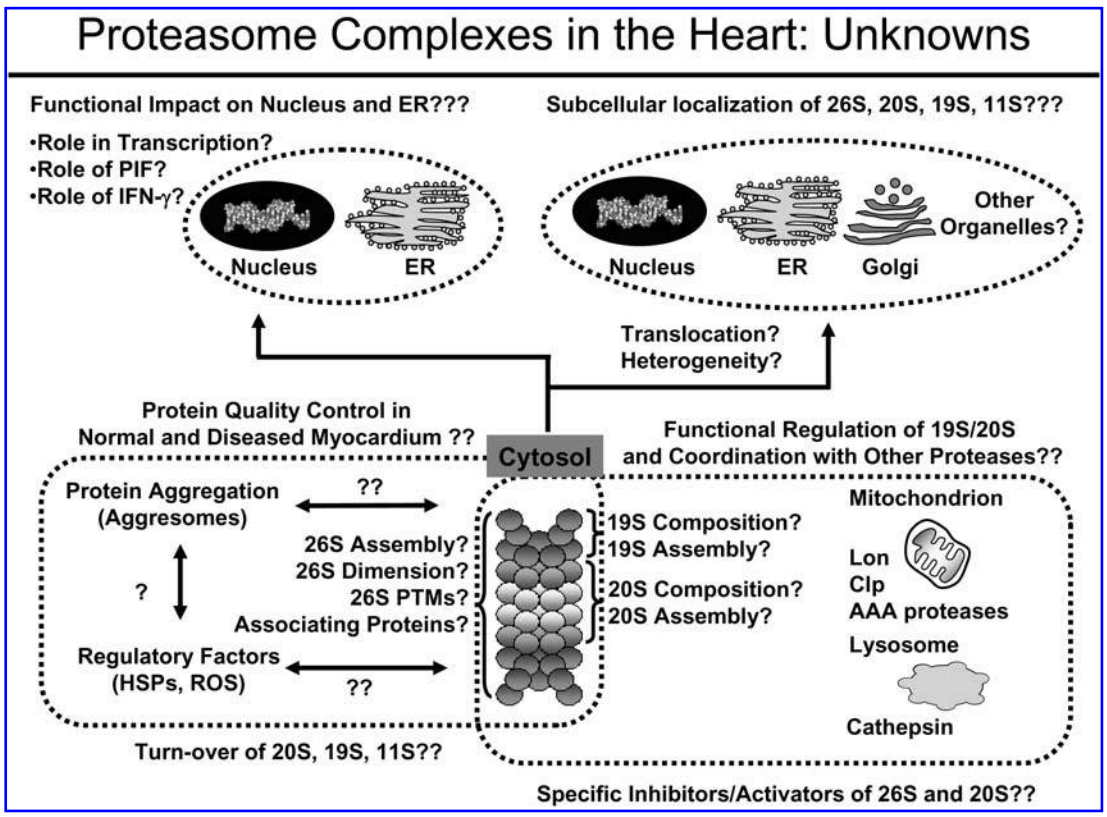

FIG. 3. Proteasome in the heart: unknowns. This figure provides an overview on the "Unknowns" in cardiac proteasome research today. The role of proteasomes in cardiac function is increasingly recognized in the cardiovascular community; nearly a decade of investigations has brought much new information as well as controversies surrounding the functional role of this organelle in the field. The tremendous unknowns of this organelle now limit our abilities to address carefully the conflicting data reported. A full delineation of the molecular composition and assembly of the proteasome complexes will aid our understanding of the molecular mechanisms responsible for their regulation. HSPs, heat-shock proteins; PIF, proteasome inducing factor; PTMs, posttranslational modifications; ROS, reactive oxygen species.

ABBREVIATIONS

ATP, adenosine 5′-triphosphate; CHIP, carboxyl terminus of HSP70-interacting protein; cMyBP-C, cardiac myosin-binding protein C; CP, core particle; CT-L, chymotrypsin-like; DPBA, dipeptide boronic acid; E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; E3, ubiquitin ligase; ER, endoplasmic reticulum; HIF, hypoxia-inducible factor; HNE, 4-hydroxy-2-nonenal; LAD, left anterior descending artery; MHC, major histocompatibility complex; PGPH, peptidyl-glutamyl-peptide hydrolyzing; ROS, reactive oxygen species; RP, regulatory particle; T-L, trypsin-like; Ub, ubiquitin; UPS, ubiquitin proteasome system.

REFERENCES

1. Ambrosio G, Flaherty JT, Duilio C, Tritto I, Santoro G, Elia PP, Condorelli M, and Chiariello M. Oxygen radicals generated at reflow induce peroxidation of membrane lipids in reperfused hearts. *J Clin Invest* 87: 2056–2066, 1991.
2. Ardley HC and Robinson PA. E3 ubiquitin ligases. *Essays Biochem* 41: 15–30, 2005.
3. Arribas J and Castano JG. A comparative study of the chymotrypsin-like activity of the rat liver multicatalytic proteinase and the ClpP from *Escherichia coli*. *J Biol Chem* 268: 21165–21171, 1993.
4. Ballinger CA, Connell P, Wu Y, Hu Z, Thompson LJ, Yin LY, and Patterson C. Identification of CHIP, a novel tetratricopeptide repeat-containing protein that interacts with heat shock proteins and negatively regulates chaperone functions. *Mol Cell Biol* 19: 4535–4545, 1999.
5. Bence NF, Sampat RM, and Kopito RR. Impairment of the ubiquitin-proteasome system by protein aggregation. *Science* 292: 1552–1555, 2001.
6. Bodine SC, Latres E, Baumhueter S, Lai VK, Nunez L, Clarke BA, Poueymirou WT, Panaro FJ, Na E, Dharmarajan K, Pan ZQ, Valenzuela DM, DeChiara TM, Stitt TN, Yancopoulos GD, and Glass DJ. Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science* 294: 1704–1708, 2001.
7. Bolli R and Marban E. Molecular and cellular mechanisms of myocardial stunning. *Physiol Rev* 79:609–634, 1999.
8. Bose S, Stratford FL, Broadfoot KI, Mason GG, and 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* 378: 177–184, 2004.
9. Brooks P, Fuertes G, Murray RZ, Bose S, Knecht E, Rechsteiner MC, Hendil KB, Tanaka K, Dyson J, and Rivett J. Subcellular localization of proteasomes and their regulatory complexes in mammalian cells. *Biochem J* 346: 155–161, 2000.
10. Bulteau AL, Lundberg KC, Humphries KM, Sadek HA, Szweda PA, Friguet B, and Szweda LI. Oxidative modification and inactivation of the proteasome during coronary occlusion/reperfusion. *J Biol Chem* 276: 30057–30063, 2001.
11. Bulteau AL, Szweda LI, and Friguet B. Age-dependent declines in proteasome activity in the heart. *Arch Biochem Biophys* 397: 298–304, 2002.
12. Campbell B, Adams J, Shin YK, and Lefer AM. Cardioprotective effects of a novel proteasome inhibitor following ischemia and reperfusion in the isolated perfused rat heart. *J Mol Cell Cardiol* 31: 467–476, 1999.
13. Chandler JM, Cohen GM, and MacFarlane M. Different subcellular distribution of caspase-3 and caspase-7 following Fas-induced apoptosis in mouse liver. *J Biol Chem* 273: 10815–10818, 1998.
14. Chen Q, Liu JB, Horak KM, Zheng H, Kumarapeli AR, Li J, Li F, Gerdes AM, Wawrousek EF, and Wang X. Intrasarcoplasmic amyloidosis impairs proteolytic function of proteasomes in cardiomyocytes by compromising substrate uptake. *Circ Res* 97: 1018–1026, 2005.
15. Chondrogianni N, Stratford FL, Trougakos IP, Friguet B, Rivett AJ, and Gonos ES. Central role of the proteasome in senescence and survival of human fibroblasts: induction of a senescence-like phenotype upon its inhibition and resistance to stress upon its activation. *J Biol Chem* 278: 28026–28037, 2003.
16. Conaway RC, Brower CS, and Conaway JW. Emerging roles of ubiquitin in transcription regulation. *Science* 296: 1254–1258, 2002.
17. Conconi M, Petropoulos I, Emod I, Turlin E, Biville F, and Friguet B. Protection from oxidative inactivation of the 20S proteasome by heat-shock protein 90. *Biochem J* 333: 407–415, 1998.
18. Crow MT, Mani K, Nam YJ, and Kitsis RN. The mitochondrial death pathway and cardiac myocyte apoptosis. *Circ Res* 95: 957–970, 2004.
19. Cuervo AM, Palmer A, Rivett AJ, and Knecht E. Degradation of proteasomes by lysosomes in rat liver. *Eur J Biochem* 227: 792–800, 1995.
20. Cuervo AM. Autophagy: many paths to the same end. *Mol Cell Biochem* 263: 55–72, 2004.
21. Cyr DM, Hohfeld J, and Patterson C. Protein quality control: U-box-containing E3 ubiquitin ligases join the fold. *Trends Biochem Sci* 27: 368–375, 2002.
22. Dai Q, Zhang C, Wu Y, McDonough H, Whaley RA, Godfrey V, Li HH, Madamanchi N, Xu W, Neckers L, Cyr D, and Patterson C. CHIP activates HSF1 and confers protection against apoptosis and cellular stress. *EMBO J* 22: 5446–5458, 2003.
23. Das S, Powell SR, Wang P, Divald A, Nesaretnam K, Tosaki A, Cordis GA, Maulik N, and Das DK. Cardioprotection with palm tocotrienol: antioxidant activity of tocotrienol is linked with its ability to stabilize proteasomes. *Am J Physiol Heart Circ Physiol* 289: H361–H367, 2005.
24. Delaval E, Perichon M, and Friguet B. Age-related impairment of mitochondrial matrix aconitase and ATP-stimulated protease in rat liver and heart. *Eur J Biochem* 271: 4559–4564, 2004.
25. Divald A and Powell SR. Proteasome mediates removal of proteins oxidized during myocardial ischemia. *Free Radic Biol Med* 40: 156–164, 2006.
26. Dupre DJ, Chen Z, Le Gouill C, Theriault C, Parent JL, Rola-Pleszczynski M, and Stankova J. Trafficking, ubiquitination, and down-regulation of the human platelet-activating factor receptor. *J Biol Chem* 278: 48228–48235, 2003.
27. Eble DM, Spragia ML, Ferguson AG, and Samarel AM. Sarcomeric myosin heavy chain is degraded by the proteasome. *Cell Tissue Res* 296: 541–548, 1999.

28. Farout L, Mary J, Vinh J, Szweda LI, and Friguet B. Inactivation of the proteasome by 4-hydroxy-2-nonenal is site specific and dependant on 20S proteasome subtypes. *Arch Biochem Biophys* (in press).
29. Ferrington DA and Kapphahn RJ. Catalytic site-specific inhibition of the 20S proteasome by 4-hydroxynonenal. *FEBS Lett* 578: 217–223, 2004.
30. French SW, Mayer RJ, Bardag-Gorce F, Ingelman-Sundberg M, Rouach H, Neve And E, and Higashitsuji H. The ubiquitin-proteasome 26s pathway in liver cell protein turnover: effect of ethanol and drugs. *Alcohol Clin Exp Res* 25: 225S–229S, 2001.
31. Friguet B, Stadtman ER, and Szweda LI. Modification of glucose-6-phosphate dehydrogenase by 4-hydroxy-2-nonenal: formation of cross-linked protein that inhibits the multicatalytic protease. *J Biol Chem* 269: 21639– 21643, 1994.
32. Friguet B and Szweda LI. Inhibition of the multicatalytic proteinase (proteasome) by 4-hydroxy-2-nonenal cross-linked protein. *FEBS Lett* 405: 21–25, 1997.
33. Glickman MH and Ciechanover A. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. *Physiol Rev* 82: 373–428, 2002.
34. Glickman MH and Raveh D. Proteasome plasticity. *FEBS Lett* 579: 3214–3223, 2005
35. Gomes AV and Potter JD. Molecular and cellular aspects of troponin cardiomyopathies. *Ann N Y Acad Sci* 1015: 214–224, 2004.
36. Gomes AV, Edmondson RD, Zong C, Young G, Liem DA, Wang GW, Jones RC, Thyparambil S, Zhang J, Pantaleon D, and Ping P. Biochemical and proteomic characterization of the murine cardiac 26S proteasome. *Circ Res* 97: E36, 2005.
37. Gomes AV, Zong C, Edmondson RD, Berhane BT, Wang GW, Le S, Young G, Zhang J, Vondriska TM, Whitelegge JP, Jones RC, Joshua IG, Thyparambil S, Pantaleon D, Qiao J, Loo J, and Ping P. The murine cardiac 26S proteasome: an organelle awaiting exploration. *Ann N Y Acad Sci* 1047: 197–207, 2005.
38. Groll M, Ditzel L, Lowe J, Stock D, Bochtler M, Bartunik HD, and Huber R. Structure of 20S proteasome from yeast at 2.4 A resolution. *Nature* 386: 463–471, 1997.
39. Grossman W, Jones D, and McLaurin LP. Wall stress and patterns of hypertrophy in the human left ventricle. *J Clin Invest* 56: 56–64, 1975.
40. Grune T, Reinheckel T, and Davies KJ. Degradation of oxidized proteins in K562 human hematopoietic cells by proteasome. *J Biol Chem* 271: 15504–15509, 1996.
41. Grune T, Reinheckel T, and Davies KJ. Degradation of oxidized proteins in mammalian cells. *FASEB J* 11: 526–534, 1997.
42. Herrmann J, Ciechanover A, Lerman LO, and Lerman A. The ubiquitin-proteasome system in cardiovascular diseases: a hypothesis extended. *Cardiovasc Res* 61: 11–21, 2004.
43. Hershko A and Ciechanover A. The ubiquitin system. *Annu Rev Biochem* 67: 425–479, 1998.
44. Hershko A. The ubiquitin system for protein degradation and some of its roles in the control of the cell division cycle. *Cell Death Differ* 12: 1191–1197, 2005.
45. Hill CP, Masters EI, and Whitby FG. The 11S regulators of 20S proteasome activity. *Curr Top Microbiol Immunol* 268: 73–89, 2002.
46. Hilt W, Heinemeyer W, and Wolf DH. The proteasome and protein degradation in yeast. *Adv Exp Med Biol* 389: 197–202, 1996.
47. Huber D, Grimm J, Koch R, and Krayenbuehl HP. Determinants of ejection performance in aortic stenosis. *Circulation* 64: 126–134, 1981.
48. Hwang JJ, Allen PD, Tseng GC, Lam CW, Fananapazir L, Dzau VJ, and Liew CC. Microarray gene expression profiles in dilated and hypertrophic cardiomyopathic end-stage heart failure. *Physiol Genomics* 10: 31–44, 2002.
49. Jesenberger V and Jentsch S. Deadly encounter: ubiquitin meets apoptosis. *Nat Rev Mol Cell Biol* 3: 112–121, 2002.
50. Jessup M and Brozena S. Heart failure. *N Engl J Med* 348: 2007–2018, 2003.
51. Kaab S, Barth AS, Margerie D, Dugas M, Gebauer M, Zwermann L, Merk S, Pfeufer A, Steinmeyer K, Bleich M, Kreuzer E, Steinbeck G, and Nabauer M. Global gene expression in human myocardium-oligonucleotide microarray analysis of regional diversity and transcriptional regulation in heart failure. *J Mol Med* 82: 308–316, 2004.
52. Kedar V, McDonough H, Arya R, Li HH, Rockman HA, and Patterson C. Muscle-specific RING finger 1 is a bona fide ubiquitin ligase that degrades cardiac troponin I. *Proc Natl Acad Sci U S A* 101: 18135–18140, 2004.
53. Kim YH, Lim DS, Lee JH, Shim WJ, Ro YM, Park GH, Becker KG, Cho-Chung YS, and Kim MK. Gene expression profiling of oxidative stress on atrial fibrillation in humans. *Exp Mol Med* 35: 336–349, 2003.
54. Kloetzel PM and Ossendorp F. Proteasome and peptidase function in MHC-class-I-mediated antigen presentation. *Curr Opin Immunol* 16: 76–81, 2004.
55. Kostin S, Pool L, Elsasser A, Hein S, Drexler HC, Arnon E, Hayakawa Y, Zimmermann R, Bauer E, Klovekorn WP, and Schaper J. Myocytes die by multiple mechanisms in failing human hearts. *Circ Res* 92: 715–724, 2003.
56. Kumarapeli AR, Horak KM, Glasford JW, Li J, Chen Q, Liu J, Zheng H, and Wang X. A novel transgenic mouse model reveals deregulation of the ubiquitin-proteasome system in the heart by doxorubicin. *FASEB J* 19: 2051–2053, 2005.
57. Kwan JA, Schulze CJ, Wang W, Leon H, Sariahmetoglu M, Sung M, Sawicka J, Sims DE, Sawicki G, and Schulz R. Matrix metalloproteinase-2 (MMP-2) is present in the nucleus of cardiac myocytes and is capable of cleaving poly (ADP-ribose) polymerase (PARP) in vitro. *FASEB J* 18: 690–692, 2004.
58. Kwo P, Patel T, Bronk SF, and Gores GJ. Nuclear serine protease activity contributes to bile acid-induced apoptosis in hepatocytes. *Am J Physiol* 268: G613–G621, 1995.
59. Li B and Dou QP. Bax degradation by the ubiquitin/proteasome-dependent pathway: involvement in tumor survival and progression. *Proc Natl Acad Sci U S A* 97: 3850–3855, 2000.
60. Li HH, Kedar V, Zhang C, McDonough H, Arya R, Wang DZ, and Patterson C: Atrogin-1/muscle atrophy F-box inhibits calcineurin-dependent cardiac hypertrophy by participating in an SCF ubiquitin ligase complex. *J Clin Invest* 114: 1058–1071, 2004.
61. Ling YH, Liebes L, Zou Y, and Perez-Soler R. Reactive oxygen species generation and mitochondrial dysfunction in the apoptotic response to bortezomib, a novel pro-

teasome inhibitor, in human H460 non-small cell lung cancer cells. *J Biol Chem* 278: 33714–33723, 2003.
62. Liu J, Chen Q, Huang W, Horak KM, Zheng H, Mestril R, and Wang X. Impairment of the ubiquitin-proteasome system in desminopathy mouse hearts. *FASEB J* 20: 362–364, 2006.
63. Liu Z, Miers WR, Wei L, and Barrett EJ. The ubiquitin-proteasome proteolytic pathway in heart vs skeletal muscle: effects of acute diabetes. *Biochem Biophys Res Commun* 276: 1255–1260, 2000.
64. Luo H, Wu Y, Qi S, Wan X, Chen H, and Wu J. A proteasome inhibitor effectively prevents mouse heart allograft rejection. *Transplantation* 72: 196–202, 2001.
65. Luss H, Schmitz W, and Neumann J. A proteasome inhibitor confers cardioprotection. *Cardiovasc Res* 54: 140–151, 2002.
66. MacFarlane M, Merrison W, Bratton SB, and Cohen GM. Proteasome-mediated degradation of Smac during apoptosis: XIAP promotes Smac ubiquitination in vitro. *J Biol Chem* 277: 36611–36616, 2002.
67. Madeo F, Herker E, Maldener C, Wissing S, Lachelt S, Herlan M, Fehr M, Lauber K, Sigrist SJ, Wesselborg S, and Frohlich KU. A caspase-related protease regulates apoptosis in yeast. *Mol Cell* 9: 911–917, 2002.
68. Marshansky V, Wang X, Bertrand R, Luo H, Duguid W, Chinnadurai G, Kanaan N, Vu MD, and Wu J. Proteasomes modulate balance among proapoptotic and antiapoptotic Bcl-2 family members and compromise functioning of the electron transport chain in leukemic cells. *J Immunol* 166: 3130–3142, 2001.
69. Martinet W, De Meyer GR, Andries L, Herman AG, and Kockx MM. In situ detection of starvation-induced autophagy. *J Histochem Cytochem* 54: 85–96, 2006.
70. McBride WH, Iwamoto KS, Syljuasen R, Pervan M, and Pajonk F. The role of the ubiquitin/proteasome system in cellular responses to radiation. *Oncogene* 22: 5755–5773, 2003.
71. McMahon M, Itoh K, Yamamoto M, and Hayes JD. Keap1-dependent proteasomal degradation of transcription factor Nrf2 contributes to the negative regulation of antioxidant response element-driven gene expression. *J Biol Chem* 278: 21592–21600, 2003.
72. Medina R, Wing SS, and Goldberg AL. Increase in levels of polyubiquitin and proteasome mRNA in skeletal muscle during starvation and denervation atrophy. *Biochem J* 307: 631–637, 1995.
73. Merker K and Grune T. Proteolysis of oxidised proteins and cellular senescence. *Exp Gerontol* 35: 779–786, 2000.
74. Minotti G, Menna P, Salvatorelli E, Cairo G, and Gianni L. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. *Pharmacol Rev* 56: 185–229, 2004.
75. Molkentin JD, Lu JR, Antos CL, Markham B, Richardson J, Robbins J, Grant SR, and Olson EN. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell* 93: 215–228, 1998.
76. Mortenson MM, Schlieman MG, Virudachalam S, Lara PN, Gandara DG, Davies AM, and Bold RJ. Reduction in BCL-2 levels by 26S proteasome inhibition with bortezomib is associated with induction of apoptosis in small cell lung cancer. *Lung Cancer* 49: 163–170, 2005.
77. Nagy I, Tamura T, Vanderleyden J, Baumeister W, and De Mot R. The 20S proteasome of *Streptomyces coelicolor*. *J Bacteriol* 180: 5448–5453, 1998.
78. Naujokat C and Hoffmann S. Role and function of the 26S proteasome in proliferation and apoptosis. *Lab Invest* 82: 965–980, 2002.
79. Nguyen T, Sherratt PJ, and Pickett CB. Regulatory mechanisms controlling gene expression mediated by the antioxidant response element. *Annu Rev Pharmacol Toxicol* 43: 233–260, 2003.
80. Palombella VJ, Rando OJ, Goldberg AL, and Maniatis T. The ubiquitin-proteasome pathway is required for processing the NF-kappa B1 precursor protein and the activation of NF-kappa B. *Cell* 78: 773–785, 1994.
81. Pickart CM. Back to the future with ubiquitin. *Cell* 116: 181–190, 2004.
82. Pickart CM and Eddins MJ. Ubiquitin: structures, functions, mechanisms. *Biochim Biophys Acta* 1695: 55–72, 2004.
83. Powell SR, Wang P, Katzeff H, Shringarpure R, Teoh C, Khaliulin I, Das DK, Davies KJ, and Schwalb H. Oxidized and ubiquitinated proteins may predict recovery of postischemic cardiac function: essential role of the proteasome. *Antioxid Redox Signal* 7: 538–546, 2005.
84. Powell SR. The ubiquitin proteasome system in cardiac physiology and pathology. *Am J Physiol Heart Circ Physiol* (in press).
85. Prakash S, Tian L, Ratliff KS, Lehotzky RE, and Matouschek A. An unstructured initiation site is required for efficient proteasome-mediated degradation. *Nat Struct Mol Biol* 11: 830–837, 2004.
86. Pye J, Ardeshirpour F, McCain A, Bellinger DA, Merricks E, Adams J, Elliott PJ, Pien C, Fischer TH, Baldwin AS, Jr., and Nichols TC. Proteasome inhibition ablates activation of NF-kappa B in myocardial reperfusion and reduces reperfusion injury. *Am J Physiol Heart Circ Physiol* 284: H919–H926, 2003.
87. Razeghi P, Sharma S, Ying J, Li YP, Stepkowski S, Reid MB, and Taegtmeyer H. Atrophic remodeling of the heart in vivo simultaneously activates pathways of protein synthesis and degradation. *Circulation* 108: 2536–2541, 2003.
88. Razeghi P, Baskin KK, Sharma S, Young ME, Stepkowski S, Faadiel Essop M, and Taegtmeyer H. Atrophy, hypertrophy, and hypoxemia induce transcriptional regulators of the ubiquitin proteasome system in the rat heart. *Biochem Biophys Res Commun* 342: 361–364, 2006.
89. Reinheckel T, Sitte N, Ullrich O, Kuckelkorn U, Davies KJ, and Grune T. Comparative resistance of the 20S and 26S proteasome to oxidative stress. *Biochem J* 335: 637–642, 1998.
90. Rivett AJ. Proteasomes: multicatalytic proteinase complexes. *Biochem J* 291: 1–10, 1993.
91. Sarikas A, Carrier L, Schenke C, Doll D, Flavigny J, Lindenberg KS, Eschenhagen T, and Zolk O. Impairment of the ubiquitin-proteasome system by truncated cardiac myosin binding protein C mutants. *Cardiovasc Res* 66: 33–44, 2005.
92. Satoh K, Sasajima H, Nyoumura KI, Yokosawa H, and Sawada H. Assembly of the 26S proteasome is regulated by phosphorylation of the p45/Rpt6 ATPase subunit. *Biochemistry* 40: 314–319, 2001.

93. Sawada H, Sakai N, Abe Y, Tanaka E, Takahashi Y, Fujino J, Kodama E, Takizawa S, and Yokosawa H. Extracellular ubiquitination and proteasome-mediated degradation of the ascidian sperm receptor. *Proc Natl Acad Sci U S A* 99: 1223–1228, 2002.
94. Schenkein DP. Use of proteasome inhibition in the treatment of lung cancer. *Clin Lung Cancer* 6(suppl 2): S89–S96, 2004.
95. Schenkein DP. Preclinical data with bortezomib in lung cancer. *Clin Lung Cancer* 7(suppl 2): S49–S55, 2005.
96. Schmidt M, Hanna J, Elsasser S, and Finley D. Proteasome-associated proteins: regulation of a proteolytic machine. *Biol Chem* 386: 725–737, 2005.
97. Shringarpure R, Grune T, Mehlhase J, and Davies KJ. Ubiquitin conjugation is not required for the degradation of oxidized proteins by proteasome. *J Biol Chem* 278: 311–318, 2003.
98. Sitte N, Huber M, Grune T, Ladhoff A, Doecke WD, Von Zglinicki T, and Davies KJ. Proteasome inhibition by lipofuscin/ceroid during postmitotic aging of fibroblasts. *FASEB J* 14: 1490–1498, 2000.
99. Sorimachi H, Suzuki K. The structure of calpain. *J Biochem (Tokyo)* 129: 653–664, 2001.
100. Stadtman ER. Protein oxidation in aging and age-related diseases. *Ann N Y Acad Sci* 928: 22–38, 2001.
101. Sullivan PG, Dragicevic NB, Deng JH, Bai Y, Dimayuga E, Ding Q, Chen Q, Bruce-Keller AJ, and Keller JN. Proteasome inhibition alters neural mitochondrial homeostasis and mitochondria turnover. *J Biol Chem* 279: 20699–20707, 2004.
102. Takahashi Y, Mizoi J, Toh EA, and Kikuchi Y. Yeast Ulp1, an Smt3-specific protease, associates with nucleoporins. *J Biochem (Tokyo)* 128: 723–725, 2000.
103. Teichert U, Mechler B, Muller H, and Wolf DH. Lysosomal (vacuolar) proteinases of yeast are essential catalysts for protein degradation, differentiation, and cell survival. *J Biol Chem* 264: 16037–16045, 1989.
104. Toyo-oka T, Morita M, Shin WS, Okaimatsuo Y, and Sugimoto T. Contribution of calcium-activated neutral protease to the degradation process of ischemic heart. *Jpn Circ J* 55: 1124–1126, 1991.
105. Tsukamoto O, Minamino T, Okada K, Shintani Y, Takashima S, Kato H, Liao Y, Okazaki H, Asai M, Hirata A, Fujita M, Asano Y, Yamazaki S, Asanuma H, Hori M, and Kitakaze M. Depression of proteasome activities during the progression of cardiac dysfunction in pressure-overloaded heart of mice. *Biochem Biophys Res Commun* 340: 1125–1133, 2006.
106. Urbe S. Ubiquitin and endocytic protein sorting. *Essays Biochem* 41: 81–98, 2005.
107. Van Dyck L and Langer T. ATP-dependent proteases controlling mitochondrial function in the yeast *Saccharomyces cerevisiae*. *Cell Mol Life Sci* 56: 825–842, 1999.
108. Verma R, Aravind L, Oania R, McDonald WH, Yates JR 3rd, Koonin EV, and Deshaies RJ. Role of Rpn11 metalloprotease in deubiquitination and degradation by the 26S proteasome. *Science* 298: 611–615, 2002.
109. Vogelstein B, Lane D, and Levine AJ. Surfing the p53 network. *Nature* 408: 307–310, 2000.
110. Voges D, Zwickl P, and Baumeister W. The 26S proteasome: a molecular machine designed for controlled proteolysis. *Annu Rev Biochem* 68: 1015–1068, 1999.
111. Weekes J, Morrison K, Mullen A, Wait R, Barton P, and Dunn MJ. Hyperubiquitination of proteins in dilated cardiomyopathy. *Proteomics* 3: 208–216, 2003.
112. Welchman RL, Gordon C, and Mayer RJ. Ubiquitin and ubiquitin-like proteins as multifunctional signals. *Nat Rev Mol Cell Biol* 6: 599–609, 2005.
113. Wenzel T and Baumeister W. Conformational constraints in protein degradation by the 20S proteasome. *Nat Struct Biol* 2: 199–204, 1995.
114. Yadav SS, Sindram D, Perry DK, and Clavien PA. Ischemic preconditioning protects the mouse liver by inhibition of apoptosis through a caspase-dependent pathway. *Hepatology* 30: 1223–1231, 1999.
115. Yang J, Moravec CS, Sussman MA, DiPaola NR, Fu D, Hawthorn L, Mitchell CA, Young JB, Francis GS, McCarthy PM, and Bond M. Decreased SLIM1 expression and increased gelsolin expression in failing human hearts measured by high-density oligonucleotide arrays. *Circulation* 102: 3046–3052, 2000.
116. Yu X, Huang S, Patterson E, Garrett MW, Kaufman KM, Metcalf JP, Zhu M, Dunn ST, and Kem DC. Proteasome degradation of GRK2 during ischemia and ventricular tachyarrhythmias in a canine model of myocardial infarction. *Am J Physiol Heart Circ Physiol* 289: H1960–H1967, 2005.
117. Zhang C, Xu Z, He XR, Michael LH, and Patterson C. CHIP, a cochaperone/ubiquitin ligase that regulates protein quality control, is required for maximal cardioprotection after myocardial infarction in mice. *Am J Physiol Heart Circ Physiol* 288: H2836–H2842, 2005.
118. Zhou J, Schmid T, and Brune B. HIF-1alpha and p53 as targets of NO in affecting cell proliferation, death and adaptation. *Curr Mol Med* 4: 741–751, 2004.
119. Zong C, Gomes AV, Qiao J, Berhane BT, Pantaleon D, Loo J, and Ping P. Phosphorylation is a major regulator of the proteasome activity. *Circ Res* 97: E40, 2005.
120. Zong C, Gomes AV, Drews O, Li X, Young GW, Berhane B, Qiao X, French SW, Bardag-Gorce F, and Ping P. Regulation of murine cardiac 20S proteasomes. Role of associating partners. *Circ Res* 99: 372–380, 2006.

Address reprint requests to:
Peipei Ping
Department of Physiology
David Geffen School of Medicine at UCLA
MRL Building, Suite 1609 CVRL
675 CE Young Dr.
Los Angeles, CA 90095

E-mail: peipeiping@earthlink.net

Date of first submission to ARS Central, April 30, 2006; date of acceptance, May 2, 2006.

This article has been cited by:

1. Saskia Schlossarek, Friederike Schuermann, Birgit Geertz, Giulia Mearini, Thomas Eschenhagen, Lucie Carrier. 2011. Adrenergic stress reveals septal hypertrophy and proteasome impairment in heterozygous Mybpc3-targeted knock-in mice. *Journal of Muscle Research and Cell Motility* . [CrossRef]
2. Albert Mulenga, Kelly Erikson. 2011. A snapshot of the Ixodes scapularis degradome. *Gene* **482**:1-2, 78-93. [CrossRef]
3. Yi-Fan Li, Xuejun Wang. 2011. The role of the proteasome in heart disease. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms* **1809**:2, 141-149. [CrossRef]
4. Paola Marambio, Barbra Toro, Carlos Sanhueza, Rodrigo Troncoso, Valentina Parra, Hugo Verdejo, Lorena García, Clara Quiroga, Daniela Munafo, Jessica Díaz-Elizondo, Roberto Bravo, María-Julieta González, Guilermo Diaz-Araya, Zully Pedrozo, Mario Chiong, María Isabel Colombo, Sergio Lavandero. 2010. Glucose deprivation causes oxidative stress and stimulates aggresome formation and autophagy in cultured cardiac myocytes. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* **1802**:6, 509-518. [CrossRef]
5. Xuejun Wang, Huabo Su, Mark J. Ranek. 2008. Protein quality control and degradation in cardiomyocytes. *Journal of Molecular and Cellular Cardiology* **45**:1, 11-27. [CrossRef]
6. Junichi Sadoshima . 2006. Redox Regulation of Growth and Death in Cardiac Myocytes. *Antioxidants & Redox Signaling* **8**:9-10, 1621-1624. [Citation] [Full Text PDF] [Full Text PDF with Links]