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SPOTLIGHT REVIEW

The ubiquitin-proteasome system in myocardial ischaemia and preconditioning

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The ubiquitin-proteasome system (UPS) represents the major pathway for degradation of intracellular proteins. This article reviews the major components and configurations of the UPS including the 26S proteasome and 11S activated proteasome relevant to myocardial ischaemia. We then present the evidence that the UPS is dysfunctional during myocardial ischaemia as well as potential consequences of this, including dysregulation of target substrates, many of them active signalling proteins, and accumulation of oxidized proteins. As part of this discussion, potential mechanisms, including ATP depletion, inhibition by insoluble protein aggregates, and oxidation of proteasome and regulatory particle subunits, are discussed. Finally, the evidence suggesting a role for the UPS in ischaemic preconditioning is presented. Much of this is inferential but clearly indicates the need for additional research.

Keywords Ubiquitin-proteasome system • Immunoproteasome • Myocardial ischaemia • Ischaemic preconditioning

This article is part of the Spotlight Issue on: The Role of the Ubiquitin-Proteasome Pathway in Cardiovascular Disease

1. Introduction

The ubiquitin-proteasome system (UPS) is the major non-lysosomal pathway for intracellular degradation of proteins and plays a major role in regulating many cellular processes. These include the cell cycle,[1,2] cell signalling,[3–6] apoptosis,[7–9] immune response and antigen presentation via the immunoproteasome,[10–12] and protein turnover under normal and pathologic conditions.[13–17] Regulation of cell functions through degradation of proteins prompted Ciechanover *et al.*[3] to term this 'biological regulation via destruction'. Moreover, the UPS plays key roles in protein quality control by removal of damaged, oxidized, and/or misfolded proteins.[18–22] Following is a relatively brief discussion of the forms of the proteasome thought to be involved in myocardial ischaemia.

1.1 Structure of the ubiquitin-proteasome system

The key components of the UPS are the 26S proteasome and ubiquitin. The 26S-proteasome is a macromolecular structure consisting of two subcomplexes, the 20S-proteasome, and one (mushroom configuration) or two (dumbbell configuration; actually 30S proteasome) 19S-regulatory particles (*Figure 1*). The 20S-proteasome is the proteolytic core and is a barrel-shaped structure consisting of two pairs of homologous rings each containing seven subunits. The proteolytic activity resides in the inner two rings which contain the β-type subunits, designated β1 through β7. The proteasome has three main proteolytic activities: 'trypsin-like', assigned to the β2 subunit; 'chymotrypsin-like', assigned to the β5 subunit; and 'caspase-like' activity assigned to the β1 subunit. Under certain conditions, the β1, 2, and 5 subunits can be replaced by immunoforms and are designated β1*i*, β2*i*, and β5*i* and the transformed 20S-proteasome called the immunoproteasome.[23] Replacement with the immunoforms has been associated with additional proteolytic activities, including BrAAP (cleavage after branched chain amino acids) and SNAAP activities (cleavage after small neutral amino acids) favouring formation of peptides consistent with the MHC class I antigens.[24] While this can occur in response to exposure to γ-interferon, in depth proteomic analysis of the cardiac proteasome has indicated that the presence of these immunoforms is more prevalent than previously thought and rather than being homogeneous, the proteasome is quite heterogeneous, and exists as a dynamic mixture of both constitutive and induced (immuno) catalytic β-type subunits.[25,26] Subunit heterogeneity is thought to account for the multitude of

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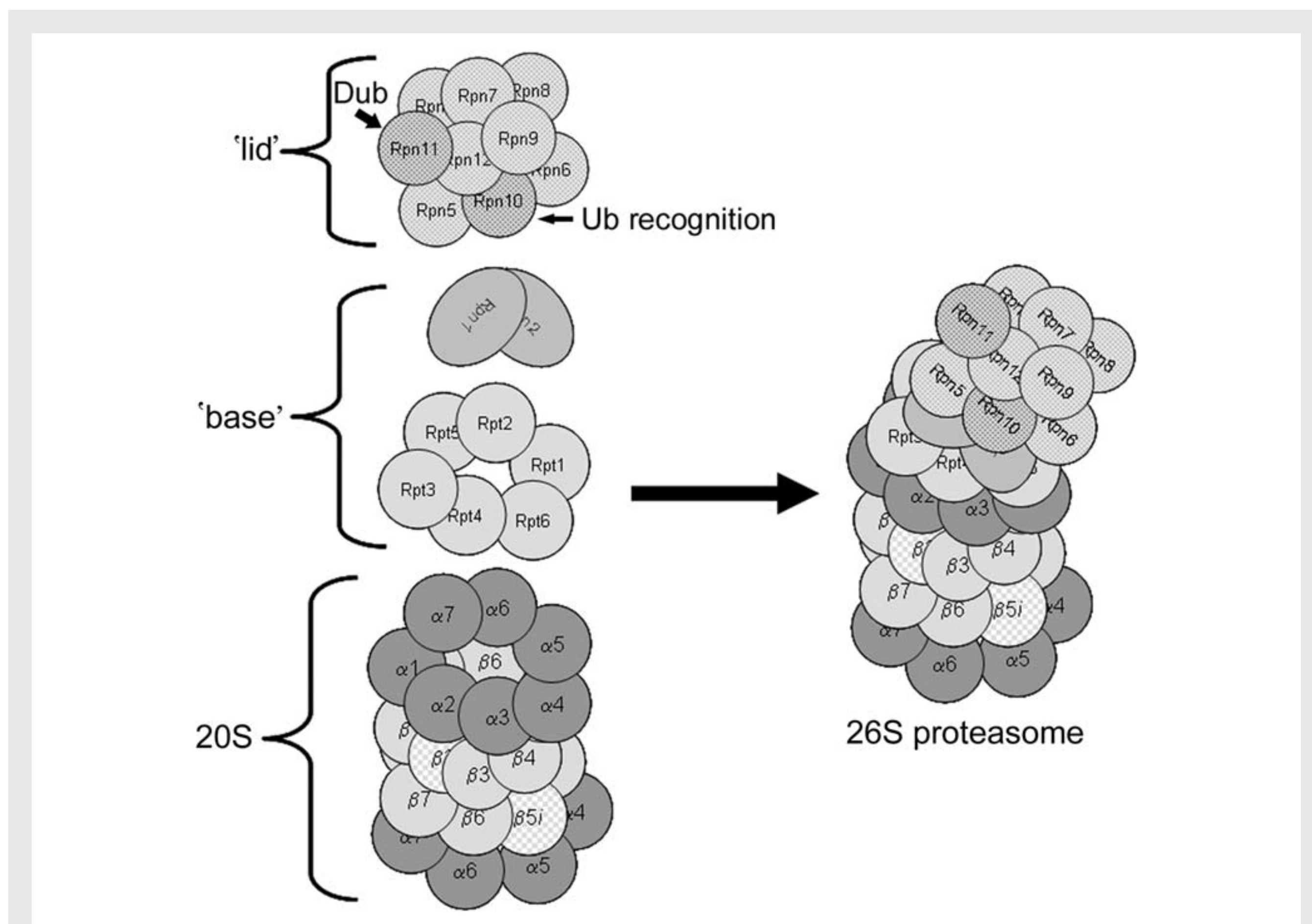

Figure 1 Structure of the 26S proteasome. The 26S-proteasome is composed of the 20S proteasome which is a barrel-shaped structure composed of four rings each consisting of seven subunits. Note that this 20S proteasome has immunoforms of the β2 (β2*i*) subunit in the upper β ring and the β5 (β5*i*) subunit in the lower β ring illustrating the heterogeneity of this structure. Docked at one or both ends is the 19S regulatory particle consisting of an additional 18 proteins. For the sake of clarity, the illustrated structure has only one 19S regulatory particle docked. This is the mushroom configuration and is actually 26S. When two 19S regulatory particles are docked at either end this is called the dumbbell configuration and is actually 30S but is also commonly called the 26S proteasome.

catalytic activities displayed by the proteasome allowing for cleavage of diverse substrates and production of diverse peptides. The outer rings contain the α-type subunits, designated α1 through α7. In the eukaryotic proteasome, these subunits have no direct proteolytic activity but play an important gating role in preventing access of folded and unfolded proteins to the central proteolytic chamber when proteasome is in the non-activated state.[27] X-ray crystallography indicates that the N-termini of subunits α1, α2, α3, α6, and α7 project into the openings at either end of the proteasome effectively sealing it and preventing access to the central chamber. Docking of the 19S regulatory particle to the 20S proteasome core activates the proteasome causing these subunits to rearrange allowing access to the proteolytic core.[28,29]

Docked at either end of the 20S-proteasome are the 19S regulatory particles containing an additional 18 subunits arranged in two distinct subcomplexes, the 'base' and the 'lid'. The base consists of the six ATPase subunits, Rpt1 through Rpt6, plus the two largest of the non-ATPase subunits, Rpn1 and Rpn2. The base activates the 20S-core by inducing a conformational change in the α-subunits opening up the entrance channel to the catalytic chamber, and also unfolds the substrate in an ATP requiring process.[22] The Rpt2, Rpt5, and Rpn2 subunits also appear to play pivotal roles in attachment of the 'base' to 20S proteasome α-rings and binding of the 19S particle 'lid' to the 'base'.[30–32] The 'lid' contains the remaining non-ATPase subunits, Rpn3 through Rpn12 whose functions are somewhat obscure. Rpn10 contains the main ubiquitin binding (or recognition) domain, and Rpn11 is one of the intrinsic deubiquitinating enzymes. The 26S-proteasome recognizes and cleaves polyubiquitinated substrates into peptides of 5–12 residues in length. The multi-ubiquitination of a protein by sequential addition of ubiquitins to the ε-NH_2 of a lysine residue is an energy requiring process involving: (i) activation of ubiquitin by a ubiquitin activating enzyme (E1); (ii) transfer of the activated ubiquitin to a protein substrate by a ubiquitin carrying or conjugating enzyme (E2); and (iii) addition of the ubiquitin to the substrate by a ubiquitin protein ligase (E3) which is the rate-limiting step (*Figure 2*). Specificity of the UPS

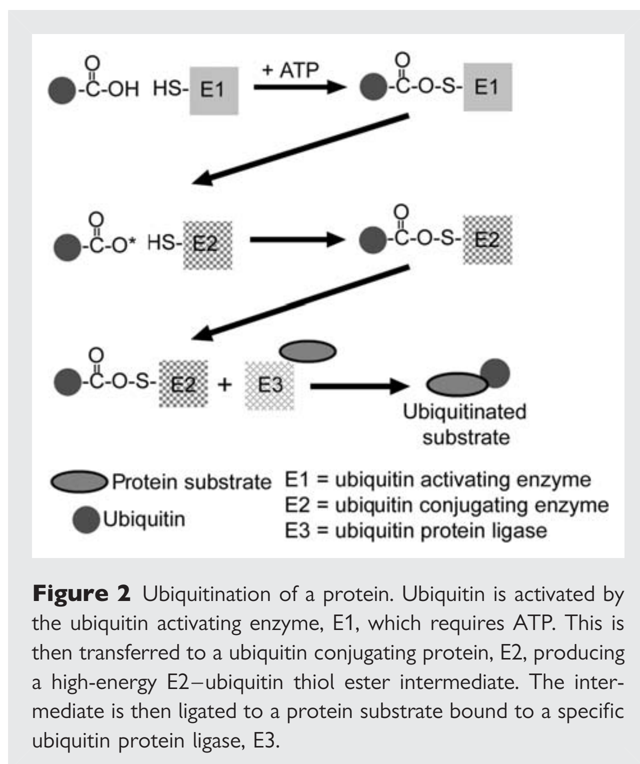

Figure 2 Ubiquitination of a protein. Ubiquitin is activated by the ubiquitin activating enzyme, E1, which requires ATP. This is then transferred to a ubiquitin conjugating protein, E2, producing a high-energy E2–ubiquitin thiol ester intermediate. The intermediate is then ligated to a protein substrate bound to a specific ubiquitin protein ligase, E3.

resides in the multitude of E3s which number in the 100s (possibly 1000s) and recognize specific motifs on target proteins.[24,30,33–37]

1.2 The 11S activated proteasome

The 11S activated proteasome is an alternate form of the proteasome ('zomes') that is regulated by a complex other than the 19S regulatory particle. The regulatory particle controls recognition and unfolding of the protein substrate with the possible exception being direct recognition of oxidized proteins by the 20S proteasome.[38] The regulatory particle for the 11S activated-proteasome is the 11S activator ring which is a heterohexamer or heteroheptamer consisting of three PA28α and three PA28β subunits or three PA28α and four PA28β subunits, respectively (*Figure 3*).[39,40] The 11S activated proteasome consists of a 20S proteasome that is docked with one or two 11S activator rings, or one 19S regulatory particle on one end and an 11S activator ring on the other end, called the hybrid proteasome. Docking of an 11S activator ring with the 20S proteasome appears to increase its proteolytic capacity without affecting overall catalytic subunit activity. This is thought to occur as a result of insertion of the carboxy-terminus tails of the PA28 subunits into pockets of the 20S proteasome resulting in conformational changes in its α-subunits. This in turn opens the access channel to a greater degree enhancing access to the catalytic chamber.[41,42] PA28 subunits are induced by interferon γ, and despite the fact that knockdowns have very little effect, the 11S activator ring is thought to play a role in antigen presentation, thus this 'zome' has been referred to as the immunoproteasome.[43–45] All of these subunits and regulatory particles can exist simultaneously and a study of Hela cells suggests that the free 20S proteasome predominates (31%), followed by the hybrid (18%), the double 11S activator ring configuration (15%), the 26S proteasome (11%), and the remainder a mixture of free regulatory subunits.[46] An *in vitro* study suggests dynamic interplay between the capping regulatory particles such that when function of the 19S regulatory particle is impaired, the 20S proteasome may actively recruit and exchange PA28 subunits.[47] Even though the function of the 11S activated proteasome is somewhat obscure, it is relevant to any discussion of the role of the proteasome in myocardial ischaemia as studies have suggested a role in removal of damaged or senescent proteins.[48,49] Indeed, we[50] have shown that this 'zome' may be upregulated in experimental hyperglycaemia associated with increased oxidative stress. An intriguing hypothesis is that the 11S activated proteasome, in one form or another, may be responsible for degradation of proteins oxidized during myocardial ischaemia.

2. The UPS in ischaemia/reperfusion

Ischaemia is defined as the condition of oxygen deprivation accompanied by inadequate removal of metabolites consequent to reduced perfusion. Since this spotlight issue is focused on the various pathophysiologic roles of the proteasome in cardiovascular disease, any discussion of the role of the UPS in ischaemia/reperfusion should be limited to the heart. However, not to include a discussion of UPS in brain ischaemia or stroke would be a serious omission from a historical perspective as all of the heart studies derive from the earlier brain studies demonstrating dysfunctional proteasome.

2.1 Brain ischaemia/reperfusion

Perhaps the earliest suggestion that the UPS may be dysfunctional following ischaemia/reperfusion was in 1992 with a report of an increase in insoluble ubiquitin-conjugates in the mitochondrial fraction of gerbil cortex and hippocampus following 5 min of transient forebrain ischaemia.[51] In 1996, this same group reported reversible decreased 26S proteasome activity in gerbil cortex following 10 min bilateral common carotid artery occlusion.[52] This was followed by a report in 2000 of reperfusion time-dependent proteasome dysfunction in both cortex and hippocampus of mice subjected to 1 h middle cerebral artery occlusion, also the first report to suggest that dysfunction may be secondary to oxidative stress.[53] Subsequent reports have confirmed and extended these earlier reports.[54] The first report[55] suggesting proteasome dysfunction as a result of myocardial ischaemia/reperfusion was not until 2001.

2.2 Myocardial ischaemia/reperfusion

In the following discussion, we examine reports indicating that proteasome is dysfunctional during myocardial ischaemia/reperfusion. These reports have raised several questions in regards to mechanism and consequences, if any. Further, some investigators have advocated the rather counter-intuitive use of proteasome inhibitors as a treatment modality for the ischaemic myocardium, a somewhat controversial issue described briefly further on.

The earliest report of decreased proteasome function in myocardial ischaemia/reperfusion was presented by Bulteau *et al.*[55] who

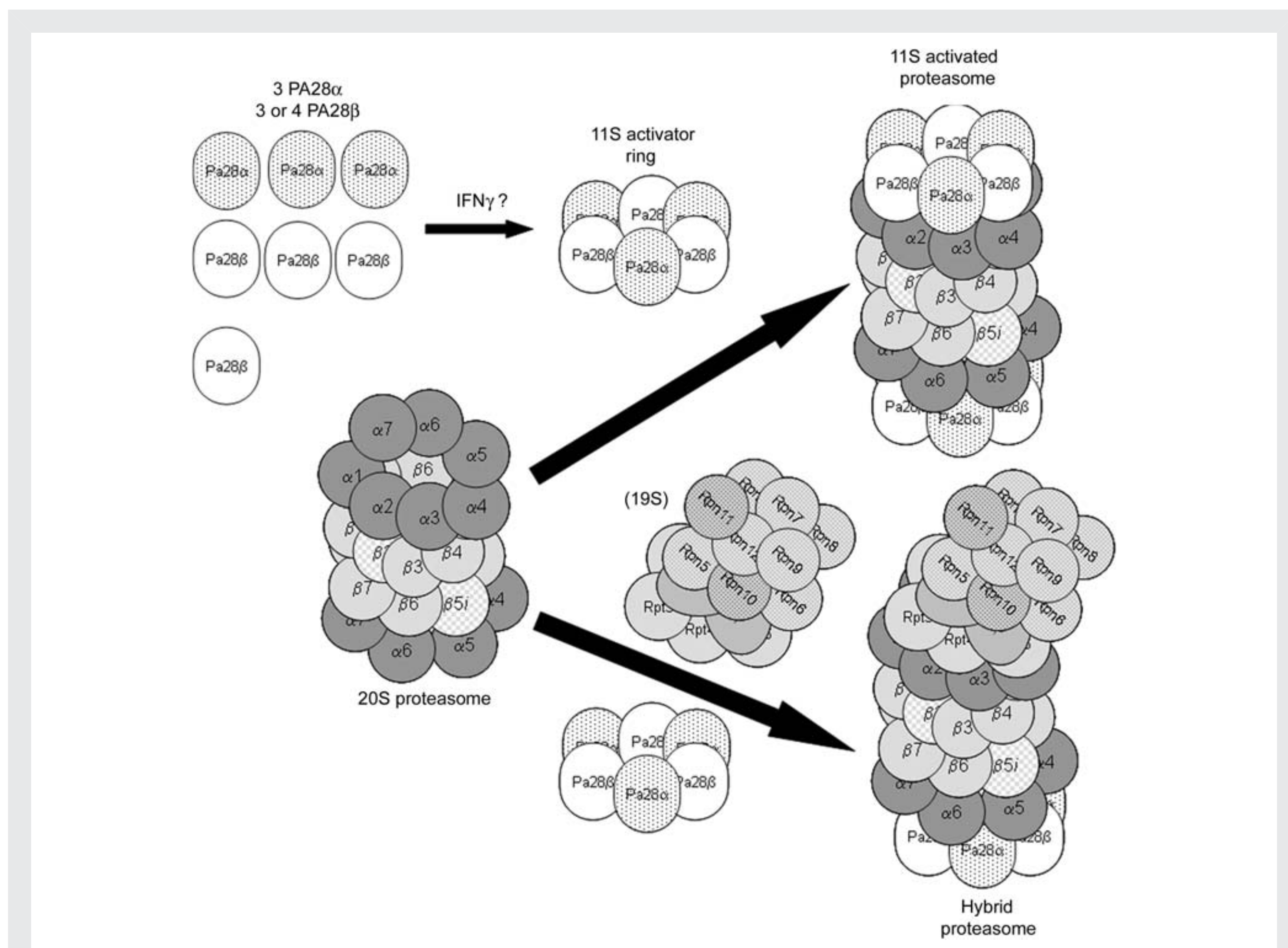

Figure 3 The 11S activated proteasome and the related hybrid proteasome. The 11S activated proteasome is formed when an 11S activator ring docks at one or both ends of a 20S proteasome. The 11S activator ring is composed of either three PA28α and three PA28β subunits (shown) or three PA28α and four PA28β subunits and may be induced by interferon γ. This form of the proteasome or 'zome' has also been called the immunoproteasome and invariably contains one or more of the immunoforms of the catalytic β-type subunits. Another 'zome' is the hybrid proteasome which has an 11S activator ring docked at one end of the 20S proteasome and a 19S regulatory particle docked at the end. This 'zome' has also been called the immunoproteasome.

showed loss of chymotrypsin-, caspase- and trypsin-like activity following 30 min of *in vivo* LAD artery occlusion. Following purification, only the decrease in trypsin-like activity was observable, yet ubiquitinated proteins were increased suggesting a functional loss of proteasome activity. Subsequently, we[56,57] confirmed this observation in the isolated perfused heart and also demonstrated that the ATP-dependent proteasome activity is decreased and preferentially affected suggesting defects in 26S-proteasome function consistent with increases in myocardial ubiquitinated proteins. While the Bulteau *et al.*[55] study and our studies[56,57] may suggest global cardiac proteasome dysfunction, a more recent study[58] suggests that at least short-term ischaemia/reperfusion may be more associated with selective dysfunction affecting degradation of specific proteins. However, this study[58] did not directly assess proteasome activity but rather took an indirect approach examining degradation (or lack thereof) of signalling proteins known to be subject to UPS-mediated degradation and also examined effects of proteasome inhibitors on these proteins as well. While this type of approach can yield some useful information, the lack of studies of ubiquitinated homologues of these signalling proteins makes it difficult to determine the role of the proteasome. Why proteasome becomes dysfunctional is not clear but could be the result of multiple processes which are discussed in the next section.

2.3 Possible mechanisms for proteasome dysfunction

Three possible mechanisms may explain dysfunction of the UPS during ischaemia/reperfusion: (i) ATP depletion; (ii) direct inhibition by protein aggregates; and (iii) oxidative damage to proteasome and/or regulatory subunits.

2.3.1 ATP depletion during ischaemia

The hypothesis that ATP depletion could be partially responsible for decreased proteasome activity in the ischaemic heart is based on the requirement for ATP by the 19S regulatory particle to unfold protein substrates for presentation to the 20S

proteasome.[59] It is known that ATP is depleted during ischaemia[60] and is likely a contributing factor to proteasome dysfunction during the ischaemic period; however, this is difficult to prove thus remains purely conjecture at this point.

2.3.2 Direct inhibition by protein aggregates

This hypothesis is based on the earlier studies of Sitte *et al*.[61] demonstrating that the incubation of fibroblasts with a synthetic lipofuscin-like material results in proteasome inhibition. These authors theorized that due to their inherent 'stickiness', lipofuscin or other crossed linked aggregates of oxidized proteins can physically 'plug' the chamber preventing substrate access.[62] Subsequently, we[63] showed that incubation of cardiomyocytes with a synthetic lipofuscin-like material derived from peroxidized liver mitochondria results in cell death secondary to proteasome inhibition and generalized accumulation of UPS degraded pro-apoptotic proteins. Later studies by several groups have confirmed that accumulation of misfolded or mutated proteins can inhibit the cardiac UPS and result in cardiomyopathy.[64–66] These cardiomyopathies are now grouped together in a class known as 'surplus mutant protein cardiomyopathies' and is the subject of other reviews in this issue (see Su and Wang[67]). During myocardial ischaemia/reperfusion, proteins are subject to oxidation[66] and these do accumulate in the heart through what is likely a combination of enhanced production[56] and decreased proteasome-mediated degradation.[68] An attractive hypothesis is that these modified proteins accumulate to a level favouring formation of insoluble aggregates capable of inhibiting the proteasome. In fact, accumulation of aggregates of oxidized proteins leading to impairment of proteasome function has been shown to occur following brain ischaemia/reperfusion.[69] To these authors' knowledge, the only example of such a process reported in heart was an increase in lipofuscin granules in atrial appendage following atrial fibrillation associated with cardiopulmonary bypass.[70] However, this was not linked to changes in proteasome activity. Whether or not protein aggregates accumulate as a general consequence of myocardial ischaemia/reperfusion is as of yet unknown.

2.3.3 Oxidation of proteasome and/or regulatory subunits

Oxidative modification of proteins affects their secondary and tertiary structures resulting in unfolding and exposure of hydrophobic patches leading to loss of function and enhanced degradation.[71,72] We and others have shown that during myocardial ischaemia/reperfusion, many cytosolic, myofibrillar, and mitochondrial proteins are subject to various oxidative modifications.[73–76] Since the proteasome is a macromolecular structure composed of multiple protein subunits, it stands to reason that it could be a potential target of the oxidative species produced during ischaemia/reperfusion. In fact, both 20S and 26S proteasome have been shown to be subject to oxidative inactivation. *In vitro* studies have shown that exposure of purified proteasome to oxidants, including 4-hydroxynonenal,[77,78] peroxynitrite,[79,80] hypochlorite, and hydrogen peroxide[81] leads to inactivation with the 26S configuration approximately 10-fold more sensitive.[81] Consistent with the view that proteasome can be damaged by oxidative phenomena, we[82] have shown that pre-treatment of isolated hearts with α-tocotrienol, a vitamin E analogue, preserves post-ischaemic proteasome function. Few studies have examined oxidative damage to proteasome subunits. In their original study, Bulteau *et al*.[55] observed 4-hydroxynonelation of several α-type subunits of the 20S proteasome following ischaemia/reperfusion, though these modifications could not be related to the decreases in proteasome activity. Subsequently, this group reported that proteasome purified from rat heart seems to be somewhat resistant to inactivation by 4-hydroxynonenal requiring concentrations in excess of 100 μM to observe loss of chymotryptic- and caspase-like activities possibly explained by the lack of modification of the catalytic β-type subunits although several α-type units were modified.[83] The higher vulnerability of the 26S proteasome to oxidative inactivation[84] and our study suggesting that the ATP-dependent activity of the proteasome is most affected by ischaemia/reperfusion,[57] has led to an intriguing hypothesis that perhaps subunits of the 19S regulatory particle are more sensitive to oxidative inactivation than subunits of the catalytic 20S proteasome. In fact, this has been demonstrated in SH-SY5Y cells exposed to an oxidative environment in which the Rpt3 (S6 ATPase) subunit, of all 26S proteasome subunits, was found to be uniquely sensitive to carbonylation reactions and that suppressing this subunit using RNAi diminishes 26S proteasome activity.[85] In ongoing studies in our laboratory, we have tentatively identified Rpt3/Rpt5 as the only 26S proteasome subunits significantly carbonylated during myocardial ischaemia/reperfusion.[86] While much work needs to be done on this promising line of research, we have come to the conclusion that proteasome dysfunction is probably not due to any one factor but rather a combination of multiple processes.

2.4 Consequences of UPS dysfunction

Since the UPS degrades numerous proteins and regulates multiple signalling pathways, it is reasonable to suggest that dysfunction of this complex during ischaemia/reperfusion could have profound effects on myocardial function. We[56] have observed that the degree of proteasome dysfunction during reperfusion is dependent on length of ischaemia and correlates with levels of oxidized and ubiquitinated proteins which tend to increase as proteasome activity decreases. Based on this study, we have proposed that *dysregulation* is occurring in which dysfunctional proteasome fails to degrade normal substrates. Keeping in mind that under normal circumstances, degradation by proteasome is not the rate-limiting step, d*ysregulation* would occur when there is insufficient proteasome activity to degrade ubiquitinated substrates allowing them to accumulate. At what level this occurs or whether dysregulation applies equally to all proteins degraded by the UPS is not known. Our initial study[56] suggests that a minimum of 50% dysfunction is necessary, but this was under conditions of high stress. There are several reports that support this hypothesis. For example, phosphorylation of c-SRC signals for ubiquitination and degradation by proteasome.[87] Phosphorylated c-SRC is increased during ischaemia and associated with poor outcomes.[88] We[89] have shown that tocotrienol pre-treatment preserves proteasome function and decreases post-ischaemic levels of phosphorylated c-SRC suggesting that the increase was related to UPS dysfunction. Other studies have suggested that post-ischaemic accumulation of phosphorylated-IκB is related to proteasome dysfunction[58] or that

dysfunction in some way interferes with UPS regulation of substrate availability for interaction with lipid rafts.[90] With respect to non-signalling proteins, we[68] have demonstrated that pre-treatment of isolated hearts with the proteasome inhibitor, lactacystin, results in a greater accumulation of oxidized proteins and diminished degradation of oxidized actin in the post-ischaemic heart, thus implying a role in their removal. Several studies report that pre-ischaemic treatment of the heart with a proteasome inhibitor either diminishes post-ischaemic function or has little effect,[56,68,91] yet others report just the opposite thus this is controversial (presented in the next section). In our original review on proteasome in the heart,[92] we proposed a model to illustrate the possible consequences of UPS dysfunction in the ischaemic heart. According to this model (*Figure 4*), in the non-ischaemic heart, the UPS functions to degrade oxidized, misfolded, and ubiquitinated proteins thus recycling the constituent amino acids, and maintaining a dynamic balance between pro-survival and pro-death signalling proteins. During ischaemia/reperfusion resulting in cell death or dysfunction, UPS function is inhibited leading to accumulation of oxidized and ubiquitinated proteins. D*ysregulation* may occur in which normal UPS-mediated degradation of pro-death proteins is depressed. Over the past few years, little has changed that would cause us to alter this model.

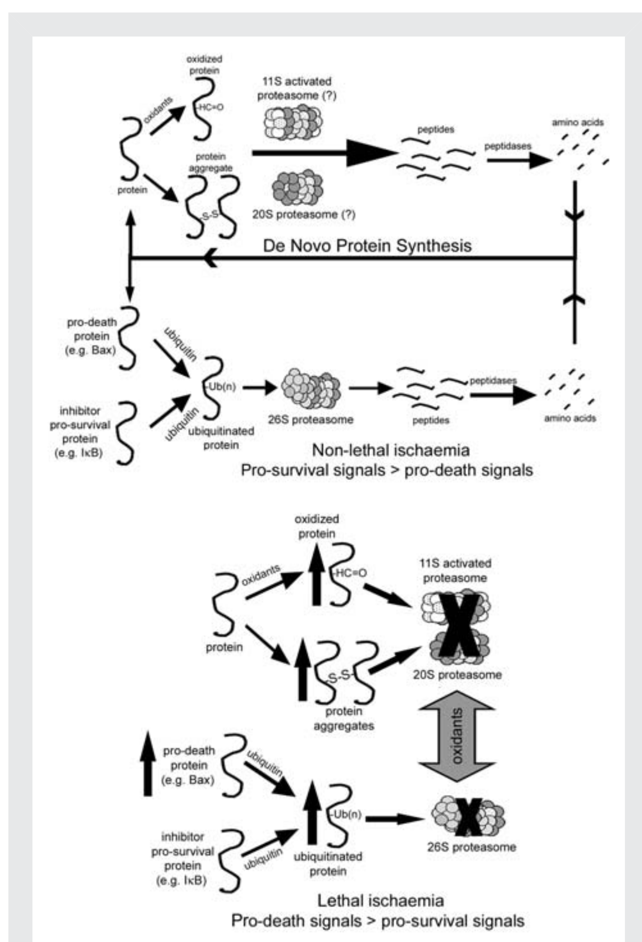

Figure 4 Potential roles for the UPS and 11S-activated proteasome in short and long duration ischaemia. In the non-ischaemic heart, oxidized, misfolded, and ubiquitinated proteins are degraded through both ubiquitin- and non-ubiquitin-mediated pathways, recycling the constituent amino acids, and maintaining a dynamic balance between pro-survival and pro-death signals. During an ischaemic insult resulting in cell death or dysfunction, UPS function is inhibited leading to accumulation of oxidized and ubiquitinated proteins. In addition, a condition known as dysregulation may occur in which normal UPS-mediated degradation of pro-death proteins is depressed.

2.5 Use of proteasome inhibitors to mitigate myocardial ischaemic injury

A handful of studies in the literature suggest that this strategy may be beneficial. Most of these studies examined the proteasome inhibitor, PS-519 (Millennium Pharmaceuticals), with the rationale that the inhibitor would either decrease leukocyte adhesion to endothelial cells or diminish nuclear translocation of NFκB thus limiting the inflammatory response.[93–95] The most recent of these studies[95] prompted us to respond with a letter[96] expressing concerns in light of several studies[97–99] reporting cardiac toxicity associated with administration of the proteasome inhibitor, bortizomeb (Velcade®), for the treatment of multiple myeloma. As stated previously,[92,96] under certain conditions, it is conceivable that a proteasome inhibitor may be of some value due to their potential anti-inflammatory properties. However, when proteasome may already be significantly dysfunctional, caution is advisable as additional inhibition may push the cell towards death. We do not envision global proteasome inhibitors as the future for this type of therapy, but rather foresee altering degradation of specific proteins through targeting of specific E3 ligases as a future viable therapeutic intervention. The use of proteasome inhibitors in myocardial ischaemia is discussed in depth in this issue by Yu and Kem.[100]

2.6 UPS dysfunction during ischaemia of other organs

Proteasome activity has been examined in one other organ, the kidney. Long-term renal ischaemia induced by permanent clipping of one renal artery[101] and reversible renal ischaemia followed by reperfusion[102] are associated with diminished proteasome activity. The observance of similar findings in multiple organ systems suggests broad relevance of these findings.

2.7 Autophagy and the UPS

Autophagy represents an additional cellular pathway for protein degradation. Studies have indicated that in certain pathological states, including some cardiac proteinopathies, autophagy and the UPS may be activated in parallel or alone to compensate if one or the other is inhibited (Su and Wang,[67] this issue). Although studies have indicated a role for autophagy in ischaemia/reperfusion[103] and adenosine-mediated preconditioning,[104] evidence for a similar link to the UPS under these conditions is lacking at this time.

3. Potential role for the UPS in ischaemic preconditioning

Ischaemic preconditioning (IPC) decreases vulnerability of the myocardium to longer durations of ischaemia as a result of pre-ischaemic exposure to short ischaemic bursts resulting in improved post-ischaemic haemodynamic function and reduced markers of myocardial injury.[105] The mechanisms by which IPC exert its protective effects appear to involve signalling changes resulting in opening of the inward mitochondrial K_{ATP} channels[106] and prevention of opening of the mitochondrial permeability transition pore.[107] Early effects of IPC include decreased release of cytochrome C, diminished cellular apoptosis,[107,108] and decreased production of oxidative species during the early phases of reperfusion.[109] One of the later effects of IPC is decreased levels of certain pro-apoptotic proteins, such as Bax.[110] For a more complete review of the signalling changes associated with IPC see Murphy and Steenbergen.[111] Given that the UPS degrades up to 70% of all intracellular proteins, including many signalling proteins,[92] a hypothesis has emerged that perhaps the UPS plays a role in IPC whereby this system facilitates some of the signalling changes associated with this protective manoeuvre. As discussed earlier, the UPS may become dysfunctional as a result of ischaemia so by necessity IPC must in some way preserve post-ischaemic proteasome function. In the following discussion, we review what is primarily inferential evidence in support of this hypothesis.

Like ischaemia/reperfusion, much of the inferential evidence can be derived from the ischaemic brain literature. For example, the pro-apoptotic protein, Bim, and its ubiquitinated homologues accumulate following simulated ischaemia (hypoxia and glucose deprivation) of cultured neurons, yet are rapidly degraded if ischaemia is preceded by a preconditioning stimulus[112] suggesting preservation of functional UPS. Other studies report that IPC prior to transient focal brain ischaemia results in diminished production of protein aggregates[113] or that prior administration of a proteasome inhibitor prevents IPC induced translocation of NFκB and diminishes protective effects.[114]

To date, most of the studies in heart parallel observations in the brain. At least two studies indicate that prior treatment with a proteasome inhibitor can prevent protective effects of myocardial IPC that include degradation of PTEN[91] and IκB;[58] and one study[115] that suggests a similar effect on postconditioning where the intermittent ischaemia is initiated within the first 10 min of reperfusion. A recent study presented the rather intriguing hypothesis that the immunoproteasome is in some way involved in IPC based on the observation that protection is lost in mice deficient in the β1*i* subunit (LMP2) and implicated pre-ischaemic changes in PTEN.[116] Given the diverse pathways regulated by the UPS, that while important it is unlikely that only changes in PTEN can account for the loss of IPC protection in this transgene. Further, this study focused on the immunoproteasome with no analysis of constitutive proteasome, did not assess global function or possible defective assembly of proteasome in the presence of the subunit knockout, and did not assess the effect of IPC on proteasome function, thus leaving many questions unanswered. The only data that IPC might actually preserve post-ischaemic function of the proteasome is presented in our original study[56] where preliminary results suggest that pharmacologic preconditioning with nicorandil may have some protective effects. Nicorandil is an anti-anginal drug thought to open the inward mitochondrial K_{ATP} channels and mimic the protective effects of IPC.[117] While these studies may suggest a role for the UPS in IPC, there have been no definitive studies clearly demonstrating a protective effect of IPC on post-ischaemic proteasome activity and the underlying ischaemia-induced defect or on UPS regulated signalling events.

4. Summary

This review has presented evidence that the UPS plays a role in myocardial ischaemia and IPC. The evidence that the UPS is dysfunctional during myocardial ischaemia/reperfusion was examined. Potential mechanisms for the dysfunction were discussed but it is clear that the actual mechanism(s) is not known indicating the need for more studies. Also, potential consequences of proteasome dysfunction were presented with the evidence suggesting that the process of *dysregulation* is occurring. However, even with this topic, many of the studies were inferential at best and none addressed the critical question as to the level of dysfunction necessary before regulation of a protein becomes *dysregulated*. Lastly, we examined the evidence that the UPS plays some role in IPC possibly by facilitating degradation of pro-apoptotic proteins in the post-ischaemic period, yet the evidence here is even more inferential. Clearly, additional studies are required to improve our understanding of UPS function during ischaemia/reperfusion so that viable therapeutic modalities can be developed that target specific proteins.

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