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Clarifying the Cardiac Proteasome Paradox

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Protein quality control

Correct folding of proteins is crucial for proper protein, cell, and organ function¹. Mutations and cellular stresses, some of which occur during pathology, can lead to incorrect folding and subsequent loss of protein function. If it goes uncorrected, this loss of protein function, along with the toxicity associated with the accumulation of misfolded proteins in cells, results in eventual organ failure². Many diseases, including neurodegenerative, hepatic, endocrine, and cardiovascular disorders, are thought to be associated with, if not caused by the organ failure resulting from the accumulation of misfolded proteins³. For example, in the heart, accumulation of misfolded proteins has been associated with hypertrophic and dilated cardiomyopathies, as well as ischemic heart disease⁴.

Cells have evolved an elaborate protein quality control system, part of which involves organelle-initiated unfolded protein responses^{5–7}. The unfolded protein responses that are activated upon accumulation of misfolded proteins are designed to adjust the relevant cellular machinery to enhance protein folding capacity, and/or to degrade terminally misfolded, potentially proteotoxic proteins via the ubiquitin proteasome system, or UPS. If the UPS is sufficient to remove terminally misfolded proteins, proteotoxicity can be averted; however, if the UPS is insufficient, the resulting proteotoxicity can contribute to organ dysfunction. Accordingly, the UPS aspect of protein quality control is essential for normal cell and organ function⁸.

Proteasomes as Mediators of Intracellular Protein Degradation

Most intracellular protein degradation via the UPS is an ATP-dependent process that involves E1, E2, and E3 ubiquitin ligases, which function in concert with chaperones to identify and ubiquitinate appropriate target proteins⁹ (Fig. 1A). The resulting poly-ubiquitinated proteins are then transferred to the 26S proteasome, where they are degraded (Fig. 1B). The 26S proteasome, composed of as many as 34 polypeptides in the heart^{10, 11}, is located in the cytosol (Fig. 1C) and nucleus (Fig. 1D) of all eukaryotic cells. In the myocardium, a significant number of cytosolic proteasomes are membrane-bound, and are located in various places, such as the endoplasmic reticulum (ER) (Fig. 1E). Proteasomes also co-localize with sarcomeric z-disc proteins, such as α -actinin¹² (Fig. 1F).

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The 26S proteasome (Fig. 1G) is composed of a 20S proteolytic core made of four stacked rings of α - and β -subunits flanked by two 19S regulatory caps composed of a base and a lid. Poly-ubiquitinated proteins access the interior of the 20S core, where they are degraded into 3–20 AA long peptides by the proteolytic activities of the 20S proteasome^{13, 14}. The protease activities of the 20S proteasome reside in the β subunits^{15, 16}. Specifically, chymotrypsin-, trypsin-, and caspase-like protease activities, reside in the $\beta 5$, $\beta 2$, and $\beta 1$ subunits of the 20S proteasome, respectively¹³. The $\beta 5$ subunit is the most important for the assembly of active proteasomes¹⁷.

A Cardiac Proteasome Paradox

Numerous studies have demonstrated alterations in proteasome function in animal models of heart disease^{13, 14, 18}; proteasome functional insufficiency has been observed most consistently in myocardial ischemia/reperfusion (I/R) injury^{19, 20}. Such studies support the hypothesis that I/R decreases proteasome activity by reducing ATP levels, as well as oxidatively damaging and unfolding proteasome proteins¹³, any or all of which may contribute to myocardial injury. Studies designed to test this hypothesis have used proteasome gain- or loss-of-function in animal models of myocardial I/R injury. However, the results of these studies have generated the following paradox:

- Gain-of-function using transgenic mice with *increased proteasome activity showed protection from I/R injury*²¹.
- Loss-of-function using pharmacological means to *decrease proteasome activity also showed protection from I/R injury*^{22–24}.

This paradox has clouded our view of the roles played by proteasomes in cardiac pathology. In this issue of *Circulation Research*, Tian et al²⁵ have clarified this view and enhanced our understanding of UPS function in the heart by determining the effects of cardiac restricted proteasome inhibition on I/R damage in mouse hearts.

Cardiac-restricted Proteasome Inhibition in the Heart

To examine the effect of decreased cardiac myocyte proteasome activity on myocardial I/R injury, Tian et al generated a transgenic mouse line wherein a catalytically inactive, dominant interfering form of the $\beta 5$ subunit of the 20S proteasome was expressed in a cardiac specific manner (T60A- $\beta 5$ tg)²⁵. Transgene expression of T60A- $\beta 5$ was relatively mild as a result of using an attenuated version of the α MHC promoter, which drove cardiac myocyte expression, but at relatively low levels, compared to the α MHC promoter used in most heart transgenic mouse models. This relatively mild transgene expression was probably a critical aspect of the study, since it was shown that under basal conditions, the transgene had no deleterious effects on cardiac structure or function. Tian et al also used a transgenic mouse model they had previously generated in which the ability of proteasomes to degrade a mutant form of GFP that is susceptible to misfolding facilitated an assessment of proteasome function in the heart, *in vivo*. Using this mouse model, they found that, upon I/R, proteasome mediated degradation of the misfolded GFP was decreased in the area at risk and border zones, as well as the remote area. These results were confirmed using biochemical assays of proteasome activity from extracts of the same hearts. Then, using the T60A- $\beta 5$ tg mice, the authors found that cardiac myocyte-restricted proteasome inhibition resulted in increased I/R injury. When combined with a previous report from the same lab, wherein a transgenic mouse model of proteasome activation showed decreased I/R damage²¹, the results of Tian et al contribute to clarifying the cardiac proteasome paradox, as follows:

- Gain-of-function studies using genetically modified mice with increased proteasome activity showed decreased I/R damage²¹.
- Loss-of-function studies using T60A- β 5 transgenic mice with decreased proteasome activity showed increased I/R damage²⁵.

Implications of the Study

In addition to addressing the role of proteasome inhibition on myocardial I/R injury, the study by Tian et al may help us understand other aspects of proteasome function in the healthy and diseased heart.

Opposing Effects of Global and Cardiac Myocyte-Specific Proteasome Inhibition

Tian et al found that cardiac restricted inhibition of proteasome function increased I/R injury, while other studies showed that global inhibition of proteasome function using pharmacological proteasome inhibitors, *in vivo*, decreased I/R injury^{22, 23}. Caution should be exercised when comparing results from studies that used such different approaches due to a variety of caveats, including potential off-target effects of pharmacological proteasome inhibitors, as well as the potential for differential effects of chronic proteasome inhibition by transgenesis and acute inhibition by pharmacological approaches. However, at face value, these findings support the hypothesis that inhibiting proteasomes in all cell types in the heart results in a diametrically opposed response of the heart to I/R than cardiac restricted proteasome inhibition. Additional studies in which cardiac proteasomes are inhibited in a cell-specific manner in non-cardiac myocytes, such as endothelial cells, smooth muscle cells, or fibroblasts, will be needed to address this hypothesis, which will be required to determine whether there is a need to implement cell specificity in the future design proteasome-targeted therapies for ischemic heart disease.

Potential Therapeutic Effects of Preserving Proteasome Function in the Heart

The results of Tian et al suggest that preserving proteasome function in cardiac myocytes might decrease the effects of I/R injury, which could ultimately lead to decreasing the morbidity and mortality of myocardial infarction. This conclusion correlates with the current consensus that proteasome function is impaired in animal models of I/R injury. However, there is less consensus on the effects of other models of cardiac pathology on proteasome function^{13, 18}. Thus, while preserving proteasome function may be advantageous in the ischemic heart, it is not as clear whether it would be beneficial in hypertrophy, dilated cardiomyopathy, or heart failure. In fact, some studies suggest that pharmacological inhibition of proteasome function inhibits adaptive and maladaptive cardiac hypertrophy²⁶, while others find that it promotes hypertrophy²⁷. Accordingly, future studies examining the effects of genetically-modified mouse models of proteasome gain- and loss-of-function on cardiac diseases, in addition to I/R will be required to better assess the full potential of proteasome-targeted therapies.

Subcellular Localization of Proteasomes Suggests Regional-specific Functions

Using confocal immunocytofluorescence, Tian et al found that a c-Myc-tagged version of the transgene that they used to inhibit proteasome function was located to the nucleus and cytosol of cardiac myocytes isolated from transgenic mouse hearts. For the most part, c-Myc staining in the cytosol adopted a striated pattern that co-stained with α -actinin, which is localized to the z-disc region of sarcomeres (Fig. 1F). These results correlate with those of another study, which also found that some cytosolic proteasomes localize to intracellular membranous structures, such as the SR/ER of cardiac myocytes¹². These results are consistent with the hypothesis that the ability of proteasomes to selectively degrade certain proteins is partly a result of their organelle-specific localization in cardiac myocytes²⁸. For

example, numerous signal transduction proteins that facilitate biomechanical sensing and signaling in cardiac myocytes are localized to the z-disc^{29, 30}. Among these signaling molecules are components of the calcineurin A, NFAT, PKC, ERK and p38 MAP kinase signaling systems, the activities and levels of which can be regulated by the UPS. Thus, z-disc-associated proteasomes may participate in regulating the levels of such signaling proteins. Additionally, the localization of proteasomes to intracellular membranes of myocytes, such as the SR/ER, is consistent with their involvement in ER-associated degradation (ERAD) (Fig. 1H). While ERAD has not been studied in detail the heart, in other cell types it has been shown that proteasomes located on the cytosolic face of the ER are responsible for the degradation of misfolded ER proteins that are translocated from the ER lumen to the cytosolic face of the ER, where they are ubiquitinated and degraded in a proteasome-dependent manner³¹.

Conclusions

In conclusion, the results of the study by Tian et al have made a significant contribution toward furthering our understanding of the roles for proteasomes in the ischemic heart. Moreover, in addition to facilitating future studies that will address important questions about the function of proteasomes in other cardiac pathologies, the T60A- β 5 transgenic mouse model described by Tian et al provides proof-of-principle for a method that could be used to examine the effect of proteasome inhibition in other cell types in the heart, as well as other tissues. Such studies could extend the potential utility of proteasome-targeted therapies well beyond the heart to include numerous other tissues in which the accumulation of misfolded proteins is known to contribute to disease.

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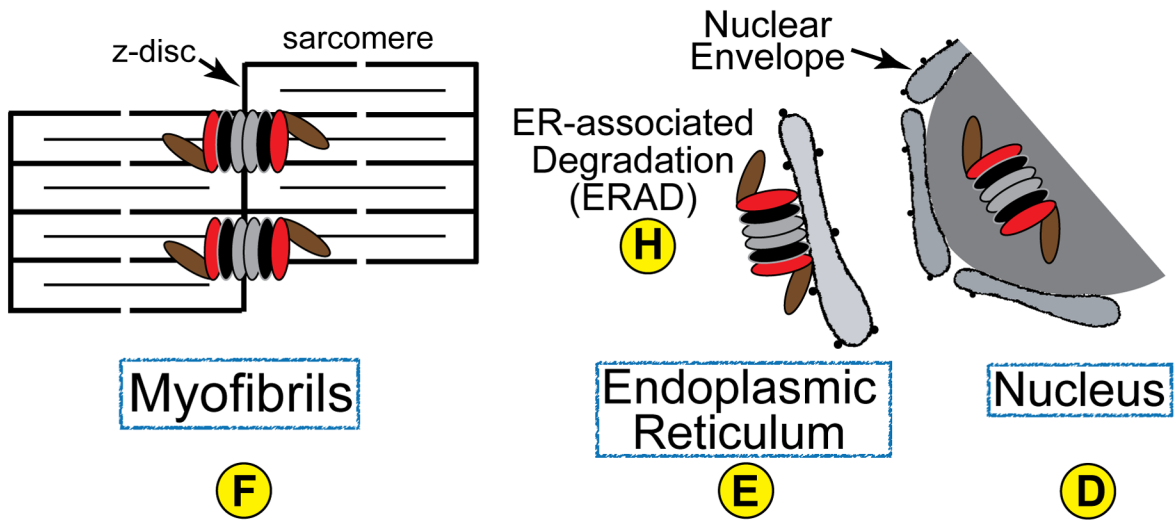
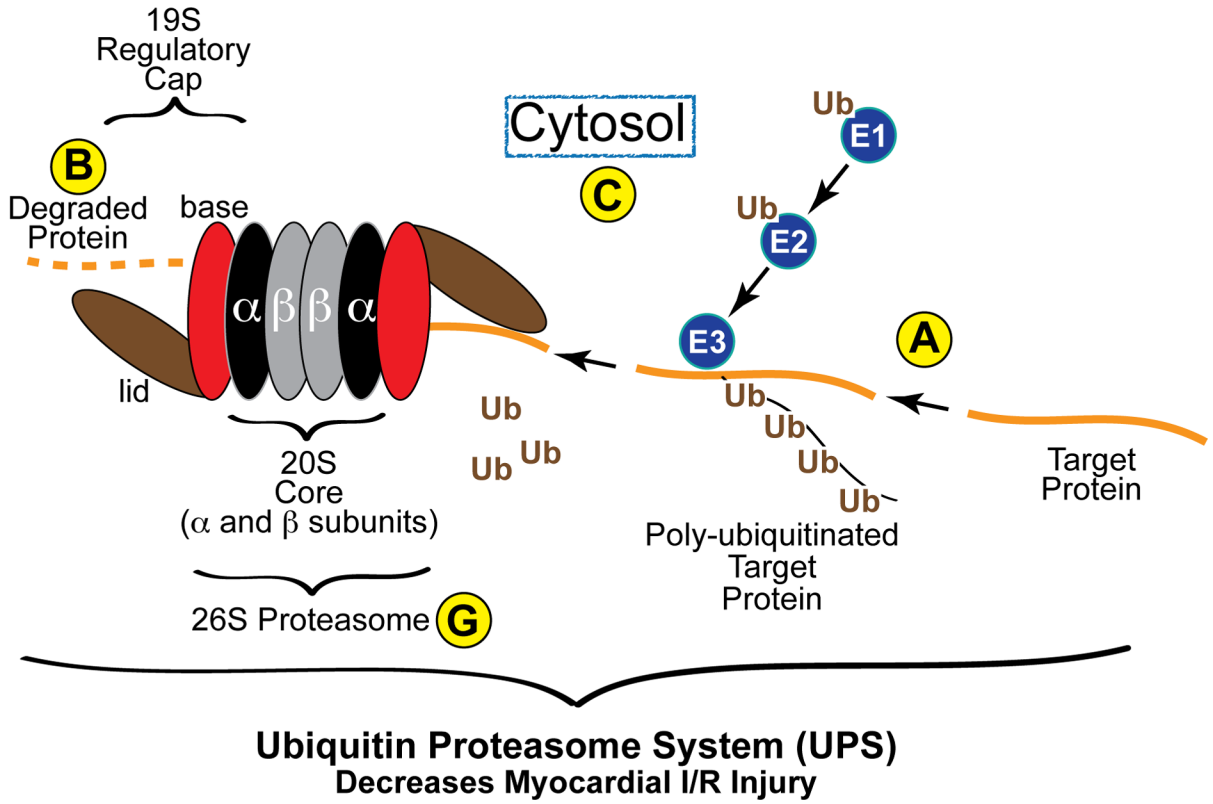


Figure 1. Diagram of the Ubiquitin Proteasome System in the Heart- Proteins are targeted for degradation and then ubiquitin is conjugated to them by the concerted transfer of ubiquitin from E1 to E2 and, finally, to E3 ubiquitin ligases, the latter of which can poly-ubiquitinate proteins, as shown (A). Poly-ubiquitinated proteins are then recognized by the 19S regulatory cap of the proteasome, which is composed of a base (red) and a lid (brown). Once within the barrel structure of the 20S portion of the proteasome, which is composed of α - (black) and β -rings (grey), proteins are degraded by the proteolytic activities resident in the β_5 , β_2 and β_1 subunits. In cardiac myocytes, proteasomes have been found in the cytosol (C), as well as associated with the z-disc region of sarcomeres (F)

and intracellular membranes, such as the SR and ER (E), where they are involved in ERAD (H).

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