Clarifying the Cardiac Proteasome Paradox
Protein Quality Control

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Correct folding of proteins is crucial for proper protein, cell, and organ function.[1] 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, then this loss of protein function, along with the toxicity associated with the accumulation of misfolded proteins in cells, results in eventual organ failure.[2] 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 proteins.[3] For example, in the heart, accumulation of misfolded proteins has been associated with hypertrophic and dilated cardiomyopathies as well as ischemic heart disease.[4]

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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 on 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 (UPS). If the UPS is sufficient to remove terminally misfolded proteins, then proteotoxicity can be averted; however, if the UPS is insufficient, then the resulting proteotoxicity can contribute to organ dysfunction. Accordingly, the UPS aspect of protein quality control is essential for normal cell and organ function.[8]

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 proteins[9] (Figure, A). The resulting polyubiquitinated proteins are then transferred to the 26S proteasome, where they are degraded (Figure, B). The 26S proteasome, composed of as many as 34 polypeptides in the heart,[10,11] is located in the cytosol (Figure, C) and nucleus (Figure, D) 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) (Figure, E). Proteasomes also colocalize with sarcomeric z-disc proteins, such as α-actinin[12] (Figure, F).

The 26S proteasome (Figure, G) is composed of a 20S proteolytic core made of four stacked rings of α subunits and β subunits flanked by two 19S regulatory caps composed of a base and a lid. Polyubiquitinated proteins access the interior of the 20S core, where they are degraded into 3-AA-long to 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, chyotrypsin-like, trypsin-like, and caspase-like protease activities reside in the $\beta5$, $\beta2$, and $\beta1$ subunits of the 20S proteasome, respectively.[13] The $\beta5$ subunit is the most important for the assembly of active proteasomes.[17]

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 proteins,[13] any or all of which may contribute to myocardial injury. Studies designed to test this hypothesis have used proteasome gain-of-function 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] whereas 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 al[25] 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.

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 $\beta5$ subunit of the 20S proteasome was expressed in a cardiac-specific manner (T60A-$\beta5$ tg).[25] Transgene expression of T60A-$\beta5$ was relatively mild as a result of using an attenuated version of the α-myosin heavy chain promoter, which drove cardiac myocyte-specific expression but at relatively low levels, compared with the α-myosin heavy chain promoter used in most heart transgenic mouse models. This relatively mild transgene expression was probably a critical aspect of the study, because it was shown that under basal conditions, the transgene had no deleterious effects on cardiac structure or

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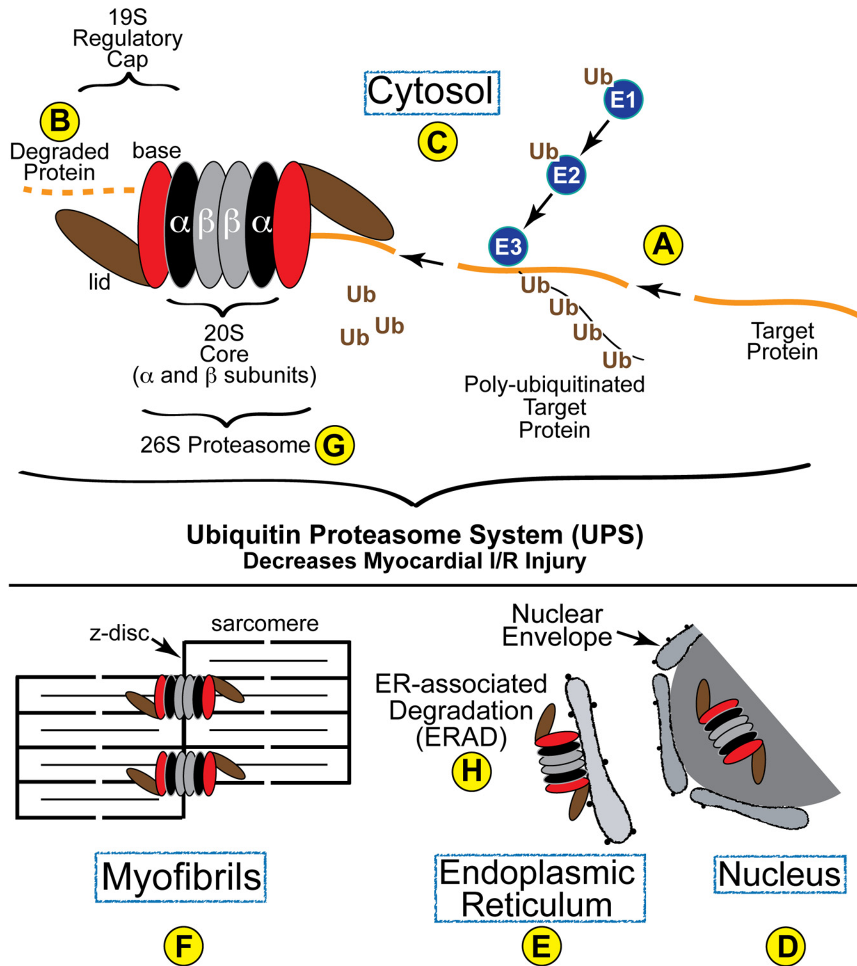

Figure. Diagram of the ubiquitin proteasome system in the heart. Proteins are targeted for degradation and then ubiquitin is conjugated to them by the concerted transfer of ubiquitin from E1 to E2 and, finally, to E3 ubiquitin ligases, the latter of which can polyubiquitinate proteins, as shown (**A**). Polyubiquitinated 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-rings (black) and β-rings (gray), proteins are degraded (**B**) by the proteolytic activities resident in the $\beta 5$, $\beta 2$, and $\beta 1$ subunits. In cardiac myocytes 26S, proteasomes (**G**) have been found in the cytosol (**C**), nucleus (**D**), and have been associated with the z-disc region of sarcomeres (**F**) and intracellular membranes, such as the sarcoplasmic reticulum and endoplasmic reticulum (ER) (**E**), where they are involved in ER-associated degradation (**H**).

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 green fluorescent protein that is susceptible to misfolding facilitated an assessment of proteasome function in the heart in vivo. Using this mouse model, they found that on I/R, proteasome-mediated degradation of the misfolded green fluorescent protein 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 laboratory, wherein a transgenic mouse model of proteasome activation showed decreased I/R damage,[21] 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 damage,[21] whereas loss-of-function studies using T60A-$\beta 5$ transgenic mice with decreased proteasome activity showed increased I/R damage.[25]

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. Tian et al found that cardiac-restricted inhibition of proteasome function increased I/R injury, whereas 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 because of 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 noncardiac 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.

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, although 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 hypertrophy,[26] whereas others find that it promotes hypertrophy.[27] Accordingly, future studies examining the effects in genetically modified mouse models of proteasome gain-of-function 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.

Tian et al found that a c-Myc-tagged version of T60A-b5 was located in the nucleus and cytosol of cardiac myocytes. For the most part, T60A-b5 in the cytosol adopted a striated pattern similar to α-actinin, which is localized to the z-disc region of sarcomeres (Figure, F). These results correlate with those of another study that also found that some cytosolic proteasomes localize to intracellular membranous structures, such as the sarco/endoplasmic reticulum of cardiac myocytes.[12] 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 myocytes.[28] 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 calcinuerin 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 sarco/endoplasmic reticulum, is consistent with their involvement in ER-associated degradation (Figure, H). Whereas ER-associated degradation 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 manner.[31]

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-β5 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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Disclosures

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