Proteostasis, the precisely orchestrated balance between protein synthesis and degradation, plays a critical role in the maintenance of cell function. This is particularly the case for postmitotic cells such as cardiomyocytes and neurons, which rarely re-enter the cell cycle to divide and hence survive for decades. Disease-related stress can promote dysregulation of proteostasis, leading to accumulation of damaged or misfolded proteins as toxic aggregates capable of triggering cell death. Indeed, in both dilated cardiomyopathy and ischemic heart disease, perturbations in proteostasis are evidenced by accumulation of polyubiquitinated proteins. Additionally, genetic disorders that lead to aggregate formation can trigger cardiomyopathy. For example, specific mutations in the coding region of the molecular chaperone \( \alpha \)-B-crystallin (CryAB) disrupt its chaperone function, leading to a distinctive myofibrillar myopathy characterized by accumulation of toxic aggregates in cardiac myocytes reminiscent of Alzheimer disease or polyglutamine expansion disorders.

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Cells can be protected from accumulation of abnormal protein aggregates by either a decrease in their formation or an increase in their clearance. Protein aggregate clearance is accomplished by 2 major catabolic processes: the ubiquitin proteasome pathway (UPP) or the autophagy-lysosomal pathway (Figure 1). Inhibition of either pathway provokes increases in toxic, intracellular aggregates that can promote disease pathogenesis.

Autophagy is a highly conserved catabolic process underlying bulk removal of cytoplasmic proteins and organelles. Autophagy acts as an adaptive response both under conditions of starvation, where recycling of intracellular contents is required to replenish life-sustaining nutrients, and in the setting of aggregate accumulation from disrupted proteostasis. In a model of desmin-related cardiomyopathy (CryAB\(^{R120G}\)), cardiomyocytes harbor increases in intracellular protein aggregates that correlate with increases in autophagic flux, both of which arise before declines in contractile function. Mice that carry the CryAB\(^{R120G}\) transgene but have a decreased ability to mount an autophagic response as a result of a haploinsufficiency of the key autophagic protein Beclin1 manifest enhanced accumulation of intracellular aggregates and accelerated pathology. Similar results have been observed in neonatal rat ventricular myocytes infected with an adenovirus expressing CryAB\(^{R120G}\) and in which autophagy was either upregulated or downregulated experimentally by a variety of means.

Genesis of insoluble protein aggregates is governed by targeting of misfolded or unfolded proteins for degradation via the UPP. The UPP comprises sequential steps in which an enzyme (E3 ligase) targets a protein for degradation through covalent attachment of ubiquitin followed by subsequent elongation into a polyubiquitin chain. The polyubiquitinated protein is then recruited to the 26S proteasome, deubiquitinated, and degraded. The 26S proteasome consists of a proteolytic 20S core particle flanked on either side by PA700 regulatory subunits. The 20S particle is a barrel-shaped structure formed by 2 copies of 14 different subunits (\( \alpha\)1–\( \alpha\)7 and \( \beta\)1–\( \beta\)7) stacked in heptameric rings. The active sites of the catalytic subunits (\( \beta\)1, \( \beta\)2, and \( \beta\)5) line the central lumen of a chamber gated by an \( \alpha\) subunit at either end of the cylinder. PA700 consists of a hexameric ring of 6 A6A family ATPases (Rpt1–Rpt6) and 3 non-ATPase subunits (Rpn1, 2, and 13) that form a base that binds to the outer rings of the 20S proteasome. This ATP-dependent interaction results in opening of the gate to allow substrate access to the 26S catalytic sites. The base also contains the ATPase-dependent chaperone-like activity that acts to unfold the substrate and feed it into the central lumen. The remaining PA700 subunits comprise the lid, which harbors sites for polyubiquitin chain binding and the ubiquitin peptidases responsible for deubiquitination activity. The process of protein degradation by the 20S proteasome is dependent on ATP hydrolysis and appears to couple translocation, deubiquitination, and protease activity.

In this issue of *Circulation*, Ranek et al build on earlier findings by this group that enhancement of proteasomal activities can protect the heart in the CryAB\(^{R120G}\) model of desmin-related cardiomyopathy. In earlier work, these investigators used cardiomyocyte-specific overexpression of the proteasome subunit 28A (PA28a) to stimulate proteasomal activity. Like the PA700 activator, the PA28 complex (11S) binds to the ends of the 20S proteasome to allow access of substrates into the proteolytic core. Unlike PA700, 11S function is independent of ATP, and it does not target the degradation of ubiquitinated substrates, nor does it manifest chaperone-like unfolding activity. In fact, the PA28 complex, initially identified for its role in processing peptides for major histocompatibility class I antigen presentation, functions primarily in formation of the immunoproteasome. More recently, PA28 family complexes have been shown to play a larger role both in intracellular protein degradation and intriguingly in mitigating endoplasmic reticulum stress. Overexpression of PA28a in mouse hearts led to a decrease in the steady-state levels of GFPdgn, a model of misfolded proteins.
manifested modest slowing of CryABR120G-induced disease aggregates in heart. Functionally, sildenafil-treated mice resulted in robust decreases in the accumulation of CryAB myopathy was exposed to sildenafil. Treatment for 4 weeks when the CryABR120G mouse model of desmin-related cardiolytic degradation is substrate specific.

The mechanism underlying increased proteasomal activity mediated by phosphorylation is unclear. Deyhosphorylation of the proteasome by the ubiquitin-like domain-containing C-terminal domain phosphatase 1 results in a decrease in proteolytic activity by altering assembly of the PA700 and 20S. Interestingly, ubiquitin-like domain-containing C-terminal domain phosphatase 1 is localized to the nucleus, providing a way to modulate proteasome activity in different cellular compartments. Indeed, the increases in peptidase activitiey reported here may stem from enhanced gating of the 20S by PA700, which would be consistent with the role of Rpt6 in maintaining the stability of the 26S proteasome. Recent structural studies have implicated the ATPase activity of Rpt1, Rpt2, and Rpt6 in substrate translocation into the proteolytic core. It is intriguing to consider posttranslational modifications as playing a role in regulating the rate of substrate translocation through the modulation of this ATPase activity.

As with all discoveries, this elegant study raises important new questions. It will be interesting to explore the suggested substrate selectivity of PKG-dependent activation of the UPP. A number of tagged green fluorescent protein (GFP) model substrates are available with defined ubiquitin ligase pathways such as the N-end rule (Ub-R-GFP) and Ub-fusion degradation pathway (Ub676-GFP) or the ubiquitin-independent ornithine decarboxylase degron (ODC-GFP); further studies may help define the scope of PKG activation of proteasomal proteolysis. How is it that this substrate selectivity is conferred? It will be interesting to determine whether the effects of PKG do, in fact, involve phosphorylation of proteasome components and, if so, at which residues. In addition, is that phosphorylation mediated directly by PKG or by some intermediary protein? Finally, by activating this fundamental mechanism of intra-cellular protein degradation, is therapeutic benefit observed in models of heart disease that involve protein aggregation and yet are clinically more prevalent (eg, ischemia/reperfusion injury or severe afterload stress)? Does this mechanism contribute to the established hypertrophy-regressing actions of sildenafil?

In summary, Ranek et al have uncovered a novel mechanism whereby the proteasome pathway can be titrated in heart.
Their findings add to a growing literature that suggests that posttranslational modification of proteasomal machinery may provide a tractable means to regulate proteostasis in disease states. Indeed, their work demonstrates that this novel mechanism is imminently druggable, raising the prospect of early translation to the bedside for potential therapeutic benefit.

Disclosures

None.

References


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PKG Primes the Proteasome
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