PKG Primes the Proteasome

Running title: Gillette et al.; PKG Primes the Proteasome

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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 post-mitotic 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\(^1,2\). Indeed in both dilated cardiomyopathy and ischemic heart disease, perturbations in proteostasis are evidenced by accumulation of poly-ubiquitinated proteins\(^3\). Additionally, genetic disorders that lead to aggregate formation can trigger cardiomyopathy\(^4\).

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.

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 two 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 which 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\(^5,6\).

In a model of desmin-related cardiomyopathy (CryAB\(^{R120G}\)), cardiomyocytes harbor increases in intracellular protein aggregates which correlate with increases in autophagic flux, both of which
arise prior to declines in contractile function\textsuperscript{7}. Mice that carry the CryAB\textsuperscript{R120G} transgene, but have a decreased ability to mount an autophagic response due to a haploinsufficiency of the key autophagic protein Beclin1, manifest enhanced accumulation of intracellular aggregates and accelerated pathology\textsuperscript{7}. Similar results have been observed in neonatal rat ventricular myocytes (NRVMs) infected with an adenovirus expressing CryAB\textsuperscript{R120G} and in which autophagy was either up- or down-regulated experimentally by a variety of means\textsuperscript{7-9}.

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 polyubiquinated 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 two copies of fourteen different subunits (α1–α7 and β1–β7) stacked in heptameric rings. The active sites of the catalytic subunits (β1, β2, and β5) line the central lumen of a chamber gated by an α subunit at either end of the cylinder\textsuperscript{10}. PA700 consists of a hexameric ring of six AAA-family ATPases (Rpt1–Rpt6) and three non-ATPase subunits (Rpn1, 2, and 13) that form a base that binds to the outer rings of the 20S proteasome\textsuperscript{11}. This ATP-dependent interaction results in opening of the gate to allow substrate access to the 20S 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, as well as 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 CryABR120G model of desmin-related cardiomyopathy. In earlier work, these investigators used cardiomyocyte-specific overexpression of the proteasome subunit 28a (PA28α) 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 unfoldase activity. In fact, the PA28 complex, initially identified for its role in processing peptides for MHC-class I antigen presentation, functions primarily in formation of the immunoproteasome. More recently, PA28 family complexes have been shown to play a larger role in both intracellular protein degradation and, intriguingly, in mitigating ER stress. Over-expression of PA28α in mouse hearts led to a decrease in the steady-state levels of GFPdgn, a model UPP substrate, and decreased CryABR120G-induced aggregate formation. Importantly, CryABR120G;11S double transgenic mice also manifested increased cardiac function and survival rates as compared with CryABR120G-only mice. In culture, PA28α expression promoted, and PA28α knockdown inhibited, CryABR120G aggregate formation.

In the current work, Ranek et al identify protein kinase G (PKG), a serine/threonine protein kinase activated by cGMP, as an activator of proteasomal proteolytic activity. Using NRVMs infected with an adenovirus expressing a constitutively active PKG mutant, or treated with the cGMP-specific phosphodiesterase type 5 inhibitor sildenafil, they observed increased turnover of the model UPP substrate GFPu and decreases in CryABR120G-induced aggregate accumulation and cell death. Inhibition of PKG activity by siRNA knockdown or using KT5823
resulted in an increase in both aggregate formation and the half-life of GFPu. Interestingly, these effects did not translate to other known targets of the UPP, suggesting the increase in proteolytic degradation is substrate specific.

PKG-dependent activation of the UPP was observed in vivo when the CryAB$^{R120G}$ mouse model of desmin-related cardiomyopathy was exposed to sildenafil. Treatment for 4 weeks resulted in robust decreases in the accumulation of CryAB aggregates in heart. Functionally, sildenafil-treated mice manifested modest slowing of CryAB$^{R120G}$-induced disease progression; at this point, it is unclear whether this change would lead to a long-term increase in survival. Finally, PKG activation had no impact on proteasome abundance. Rather, it enhanced the peptidase activity of the proteasome. This increase in activity correlated with an acidic shift in the isoelectric point of the PA700 subunit Rpt6 and the 20S subunit β5, suggestive of phosphorylation.

The discovery that PKG is capable of modulating proteasome peptidase activity, possibly via subunit phosphorylation, is consistent with that observed for a number of kinases, including calmodulin-dependent protein kinase II (CaMKII) and cAMP-dependent protein kinase (PKA), each of which has been shown to act similarly to enhance the degradation of model UPP substrates$^{18, 19}$ (Figure 2). In fact, as suggested for PKG, both CaMKII and PKA phosphorylate Rpt6, although in the case of PKG, it remains unclear as to whether this putative phosphorylation event is direct.

The mechanism underlying increased proteasomal activity by phosphorylation is unclear. De-phosphorylation of the proteasome by the ubiquitin-like domain-containing C-terminal domain phosphatase 1 (UBLCP1) results in a decrease in proteolytic activity by altering assembly of the PA700 and 20S$^{20}$. Interestingly, UBLCP1 is localized to the nucleus, providing
a way to modulate proteasome activity in different cellular compartments. Indeed, increases in peptidase activities 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 post-translational modifications as playing a role in regulating the rate of substrate translocation through modulation of 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 GFP model substrates are available with defined ubiquitin ligase pathways, such as the N-end rule (Ub-R-GFP) and Ub-fusion degradation pathway (Ub\textsuperscript{G76V}-GFP) or the ubiquitin-independent ornithine decarboxylase degradation (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. Also, is that phosphorylation mediated directly by PKG or via some intermediary protein?

Finally, by activating this fundamental mechanism of intracellular protein degradation, is therapeutic benefit observed in models of heart disease that involve protein aggregation and yet are clinically more prevalent (e.g. 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 post-translational 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.

Conflict of Interest Disclosures: None.

References:


Figure Legends:

**Figure 1.** Damaged or misfolded proteins are primarily targeted for degradation by the ubiquitin proteasomal pathway (UPP). When an excess of these proteins accumulates, they aggregate and are degraded by autophagic pathways. If misfolded proteins continue to accumulate, they can be ultimately sequestered by the cell into ubiquitin-rich cytoplasmic inclusions called aggresomes.

**Figure 2.** Multiple protein kinases have been identified that stimulate the peptidase activity of the 26S proteasome through posttranslational modification of either the 20S core or the PA700/19S regulatory subunits.
Figure 1

soluble unfolded proteins

protein aggregates

aggresome

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Figure 2
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