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Editorial

Editorial: **Proteostenosis: cancer's Achilles heel?**

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SEE CORRESPONDING ARTICLE ON PAGE 921

Deciphering how genetic information is translated into proteins is one of first and greatest triumphs of molecular biology. Protein translation remained a central topic of investigation until the introduction of gene cloning, when focus shifted to understanding the expression and function of individual gene products. Abandoning translation was shortsighted. As the mother of all proteins and the greatest consumer of cellular energy (expended synthesizing proteins and also in generating the protein-synthesizing machinery itself), translation is arguably the most important cellular process [1].

Inevitably, translation has returned to the main stage of biomedical research. The tremendous advances in understanding protein degradation mediated by the Ub-proteasome system has led to the central concept of proteostasis [2], defined as the balance cells must achieve between synthesizing and degrading individual gene products to maintain desired levels of expression. A critical yet largely ignored factor in proteostasis is integrating the overall protein synthesis rate with the degradative capacity of cells. This is particularly important given evidence that 20–40% of nascent proteins are degraded within minutes of their synthesis [3, 4] (termed RDPs [5]), creating a substantial pool of proteasome substrates that will compete with proteins as they naturally "retire" [every protein has a characteristic degradation rate, often obeying first-order kinetics (for reasons that are not understood), so it can be expressed accurately as a half-life]. The RDP pool consists of an unknown fraction of truly short-lived proteins and DRiPs, aberrant or excess forms of proteins that do not achieve a final, stable conformation in an appropriate time window [6]. DRiPs provide a significant source of MHC class I-associated peptides for immunosurveillance from otherwise stable proteins, accounting for the rapid recognition of virus-infected cells by $CD8^+$ T cells [6], and represent a potential source for other biologically active proteasome products.

The RDP fraction of nascent proteins has only been studied for a few cells lines, so there is limited information about how it might vary between cell lines or be influenced by changes in cell status [7, 8]. Enter Cenci et al. [9], whose interest in the sensitivity of MM cells to proteasome inhibitors stems from the introduction of the proteasome inhibitor BtZ as a standard second-line treatment for this highly lethal tumor. Despite the clear effect of BtZ in a significant fraction of MM patients, other patients have no benefit, prompting the obvious question: why?

Insightfully, Cenci et al. [9] reasoned, on first principles, that cell sensitivity to proteasome inhibitors relies on two variables: total proteasome activity and proteasome demand. Activity is based largely (but probably not exclusively) on cell proteasome copy number, whereas demand is based on the number of synthesized proteins that are not secreted or degraded in lysosomes, as nearly every other protein that is made will be degraded eventually by proteasomes. Synthetic rate, in turn, is dictated by cell size (as the protein fraction of cells is relatively constant), division time (as every protein must be duplicated for cells to make a faithful copy), RDP fraction, and amount of proteins released or secreted from cells. Cenci et al. [9] provide the first comparative measurements among cell lines of cell size and division rate, proteasome copy number and activity, rates of overall protein and RDP synthesis, and steady-state poly-Ub protein conjugates. This careful accounting reveals a number of surprises (**Table 1**).

First, whereas each of the MM cell lines expresses a similar amount of standard proteasomes, immunoproteasomes vary from a number equal to standard proteasomes to more than fivefold higher. The result is a threefold spread in overall proteasome number that parallels total proteasome enzymatic activity. The number of proteasomes/cell is in the same ballpark as in Princiotta et al. [10] (6×10^5) and similar to numbers of individual proteasome subunits determined by global mass spectrometric studies [11, 12]. Why only immunoproteasomes vary between the cells is intriguing and is consistent with recent findings, suggesting that immunoprotea-

Abbreviations: $^{[35]}$S-Met=$^{[35]}$S-methionine, BtZ=bortezomib, DRiP=defective ribosomal product, MM=multiple myeloma, RDP=rapidly degraded polypeptide, Ub=ubiquitin

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TABLE 1. Proteostenosis by the Numbers

Cell	BtZ EC_{50}	Ps/cell × 10^6	Ps act	Prot syn	% RDP	# RDPs	RDP/Ps
MM	6	10	30	=100	18	7200	275
KMS	6	14	30	87	16	5500	210
RPMI	46	28	75	35	2	280	2
U266	79	27	=100	25	1	=100	=1
	LC EC_{50}						
L29	100		=100	=100	4	=100	=1
+ LPS	3		40	250	30	=7500	40

See Cenci et al. [9] for details. Cell, Cell line studied; EC_{50}, given in nM for each proteasome inhibitor; Ps, proteasome; Ps act, proteasome activity; Prot syn, relative amount of protein synthesized/min; % RDP, fraction of protein degraded 30 min following pulse labeling that is blocked by proteasome inhibitors; # RDPs, calculated from Prot syn · % RDP; RDP/Ps, ratio of # RDPs:# Ps/cell; LC, lactacystin.

somes have unique functions in stressed cells [13–15].

Second, although the cells are of similar size, protein content, and division time, the amount of protein synthesized/unit time, as measured by incorporation of $^{[35]}$S-Met, varies fourfold. Given the enormous amount of cellular energy consumed by protein synthesis, this is astonishing.

Third, the fraction of RDPs varies over nearly 100-fold, with some cell lines demonstrating a miniscule rate, whereas for others, the rate approaches 20% of total synthesis. (Note that the 5-min labeling period used by Cenci et al. [9] likely underestimates the RDP fraction by approximately one-third, as a result of degradation during the labeling period itself [3]. Further, the 15-min Met starvation step is likely to induce changes in cell physiology that affect the RDP fraction [16]).

Fourth, the fraction of RDPs was proportional to the amount of protein synthesized, providing an explanation (or a partial explanation, depending on the average half-life of the stable protein pool) for the differences in protein synthesis rates between the cells.

Fifth, dividing the "proteasome load", the total amount of RDPs (the product of the RDP fraction and the protein synthesis rate), by the cellular proteasome activity (or "proteasome capacity") results in nearly 300-fold differences between the cells. This load-versus-capacity imbalance, artfully termed proteostenosis [17], correlates well with the differential sensitivity of cells to proteasome inhibitors.

These may be beautiful numbers indeed to cell accountants (tears in the scribes' eyes), but how do they predict reality? Rapid BtZ-induced increases in poly-Ub proteins are inhibited if cycloheximide is used to block protein synthesis simultaneously. This extends similar findings made in other cell types [4] and is consistent with a recent mass-spectrometric study concluding that RDPs are the major source of ubiquitylated proteins [18]. If RDPs represent the major source of proteasome substrates, and proteasome overload is responsible for MM sensitivity to BtZ, then reducing protein synthesis should protect cells from BtZ-induced apoptosis. Most importantly, despite cycloheximide having a proapoptotic effect on its own, moderate doses that diminish protein synthesis by ~50% protect cells from BtZ-induced apoptosis (but not apoptosis induced by other drugs), consistent with the importance of RDPs in pushing MMs to the edge of survival as a result of proteostenosis.

Extending this approach to cellular differentiation states, Cenci et al. [9] expose the L29 B cell line to LPS to induce plasma cell-like differentiation. This increases the protein synthesis rate 2.5-fold and the RDP fraction 7.5-fold to 30% of nascent proteins, whereas reducing proteasome capacity by 2.5-fold, resulting in a 40-fold increase in load:capacity ratio. Concomitantly, differentiation increases sensitivity to proteasome inhibitors by 33-fold, completely consistent with the proteostenosis hypothesis.

Proteostenosis represents an important, new parameter that must be considered in proteostatic analysis, as it offers the promise of predicting cell sensitivity to increases in the absolute number of RDPs, as well as their sensitivity to proteasome inhibitors and likely, other inhibitors that interfere with the Ub-proteasome pathway or other processes that prevent RDP disposal. It is of obvious importance to extend these findings to other cell types, including normal, as well as cancer cells, and to incorporate other methods of measuring proteasome activity and protein synthesis. These include exciting methods to visualize these processes in cells by proteasome activity probes and the ribopuromycylation method of measuring localized translation [19, 20].

As with all outstanding studies, Cenci et al. [9] raises many new questions. To what extent do RDP fractions account for the differences observed in protein synthesis rates? Is all translation sampled equally by the labeling technique used, or are there cell-based differences in the accessibility of $^{[35]}$S-Met to various tRNA pools? Given the immense energy requirement of protein synthesis, how do changes in protein synthesis activity integrate with the Warburg effect, i.e., the switch commonly observed in cancer cells (and immune and virus-infected cells), to use of glycolysis as an (less efficient) energy source? What accounts for the large differences in RDP fractions observed in the different cells examined? What fraction of RDPs is truly DRiPs, and how do these differences correlate with generation of class I peptide ligands? Why don't cells avoid proteostenosis simply by synthesizing more proteasomes? Would this entail too great an investment in all other accoutrements of the Ub-proteasome pathway when all

of the chaperones, ligases, hydrolases, etc., are tallied? And the mother of all questions: how can proteostenosis be exploited to treat cancer?

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Editorial: **Once more unto the breach, dear friends: CMV reactivates when the walls come down**

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SEE CORRESPONDING ARTICLE ON PAGE 933

In healthy individuals, CMV establishes an asymptomatic but persistent or latent infection. It is clear that continuous surveillance by T cells and NK cells keeps CMV in check and that loss of immune surveillance allows viral recrudescence. Thus, CMV is a major pathogen for immune-suppressed patients (recently reviewed in ref. [1]). Less clear is how CMV manages to reactivate in immune-competent individuals, many of whom maintain large populations of functional virus-specific T cells. Indeed, CMV infection elicits such exceptionally large numbers of virus-specific T cells [2] that one might think of them as an impenetrable barrier against viral recrudescence. Nevertheless, CMV reactivation and replication occur in

Abbreviation: MCMV = murine CMV

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