Immunoproteasomes: Regulating the regulator

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If proteins were people, half of the world's population (3×10^9) would cohabit, cheek by jowl, the typically sized vertebrate cell. Proteins are the most abundant macromolecular constituent of cells and certainly the most important and interesting. Because the interactions of proteins with each other and other cellular components are governed by the laws of mass action, the concentration of a protein greatly influences its function. With the ascendance of molecular genetics in the late 1960s, interest in cellular control of the copy number of individual proteins focused nearly exclusively on transcription. But protein expression is determined not only by the rate of synthesis but also by the rate of degradation. Indeed, it was recognized at least five decades ago that nimble control of protein levels can only be achieved by maintaining the capacity for rapid degradation (1). With the awarding of the 2004 Nobel Prize in Chemistry to Ciechanover, Hershko, and Rose for "their discovery of ubiquitin-mediated protein degradation," protein degradation finally achieved equal footing with transcription in studying protein expression. Ubiquitin covalently marks proteins for degradation by the proteasome, a ubiquitous, abundant, multicatalytic protease that has the unique ability to degrade virtually any protein to oligopeptides. Although much has been learned about the enzymes that control protein ubiquitylation, relatively little is known about how proteasomes themselves are regulated. In this issue of PNAS, Heink *et al.* (2) report a significant advance in understanding how cells regulate proteasome levels in cells. This finding has broad implications for the possible functions of immunoproteasomes, a form of the proteasome intimately involved with the immune system of jawed vertebrates.

Proteasomes are composed of 14 distinct subunits (seven α and seven β) arrayed in four symmetrical rings ($\alpha_7\beta_7$–$\beta_7\alpha_7$) to form a barrel with closed ends that can be opened to create a narrow entry channel for substrates. Just a few of the 14 different subunits exhibit protease activity [β1, β2, and β5; β7 may also be a protease (3)]. Such "20S" proteasomes are capable of degrading unfolded proteins *in vitro*, and there is increasing evidence that 20S proteasomes function in cells to degrade nonubiquitylated substrates (4). Degradation of ubiquitylated substrates requires the addition of 19S regulators to the ends of 20S proteasomes to create 26S proteasomes. 19S regulators are complex multisubunit structures in their own right, with multiple tasks that include binding and deubiquitylating substrates, unfolding, and threading substrates through the aperture they create in the end of the 20S barrel. Because of the ATP-dependence of 19S regulator function, 26S proteasome-mediated degradation is energy-dependent, which provided one of the initial clues to its complexity (5).

Because cellular proteins are degraded with an average of ≈2 days, proteasomes are fed a steady supply of slowly degraded proteins (SDPs)

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amounting to $\approx 5 \times 10^5$ substrates per min per cell (6). Furthermore, ≈30% of newly synthesized proteins in mammalian cells are degraded with a half-life of <10 min. A significant fraction of these rapidly degraded polypeptides (RDPs) are defective ribosomal products (DRiPs), defective forms of gene products that are generated because of imperfections in the process of converting genetic information into proteins. In actively dividing cells (1 day doubling time), RDPs provide an additional 1.3×10^6 substrates per min per cell. Together with SDPs, this amounts to nearly 2×10^6 proteins degraded per min per cell, generating $\approx 10^8$ oligopeptides per min per cell. That cells do not simply fill up with proteasome products is a testimony to the activities of endopeptidases and aminopeptidases, which degrade oligopeptides into free amino acids within seconds (6).

A few peptides manage to avoid the proteolytic buzz saw, however, to wind up on major histocompatibility complex class I molecules. Nearly all cell types in jawed vertebrates constitutively express class I molecules, which exploit DRiPs as a source of peptides to enable the immune system to monitor what gene products individual cells are translating (6). This system is particularly useful for detecting viruses, which are obliged to use host cell ribosomes to propagate. Indeed, host CD8 T cells (T_{CD8^+}) can recognize cells rapidly after infection (sometimes within an hour), despite the fact that viral proteins typically are extremely stable once they have achieved a native structure.

The immune system is nothing if not complex, and the class I antigen presentation system is no exception. In addition to standard proteasomes, cells are capable of producing immunoproteasomes, in which the three catalytic subunits are replaced by homologous subunits (β1i, β2i, and β5i). Immunoproteasomes generate a different spectrum of peptides from standard proteasomes, which influences T_{CD8^+} responses at the level of the repertoire of T_{CD8^+} available for antiviral responses and the viral peptides that are presented to T_{CD8^+}. Immunoproteasomes are constitutively expressed in immune tissues (although their expression in the myriad types of immune cells is not well characterized). Immunoproteasomes are expressed at much lower levels in other cell types but can be induced by exposing cells to IFN-γ or TNF-α, cytokines that are released in the early stages of viral infections. With the need for rapid expression of immunoproteasomes, it could be guessed that cells might have some tricks up their sleeves for regulating proteasome assembly and disassembly. Indeed, they must have a trick or two, given that within a week of virus infection livers replace nearly all of their proteasomes with immunoproteasomes, despite the fact that the proteasome half-life in livers is >1 week (7).

Given the broad proteolytic specificities of the three catalytic subunits, assembling proteasomes, like the mating of porcupines, must be done with extreme care. To prevent premature proteolysis, the catalytic subunits are inactive until a NH_2-terminal propeptide is removed to create the NH_2-terminal active site threonine residue that is eponymous of this unique class of proteases. Final activation is not achieved until the final 20S structure is assembled.

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It is at this point that Heink *et al.* (2) enter the picture. This group has long studied proteasome assembly and has provided one of the original descriptions of POMP (proteasome maturation protein). POMP is essentially a dedicated molecular chaperone for proteasome assembly and a disposable one at that, because its degradation accompanies the final assembly of standard proteasomes. In their present article, Heink *et al*. carefully examine POMP function in the assembly of immunoproteasomes.

Treating cells with IFN-γ induces immunoproteasomes and POMP mRNA but curiously results in a decrease in POMP levels. Pulse-chase metabolic radiolabeling reveals that, although POMP synthesis is enhanced, its degradation is increased to a greater extent, accounting for the decrease in steady-state POMP levels. As observed previously with standard proteasomes, immunoproteasome assembly accompanied POMP degradation. Importantly, immunoproteasomes are assembled four times faster than standard proteasomes (21 min vs. 82 min half-time of assembly), raising the issue of why standard proteasomes aren't assembled faster.

The decrease in POMP levels is strictly due to induction of a functional β5i subunit and not, as might have been expected, to other IFN-γ-induced factors. Indeed, POMP could be shown to physically interact with β5i, consistent with a recent report that POMP also interacts with β5 (8). The essential role of POMP in proteasome assembly was demonstrated by RNA silencing of POMP, which resulted in a commensurate decrease in proteasome expression and proteasome function as measured by accumulation of polyubiquitylated substrates, decreased MHC class I expression, and, ultimately, cell death.

Importantly, Heink *et al*. (2) also show that immunoproteasomes exhibit much reduced stability relative to standard proteasomes, with respective $t_{1/2}$ of 27 h vs. 133 h. For both proteasome classes, stability was not affected by exposing cells to IFN-γ, so this appears to be an intrinsic property of the proteasomes themselves. The extent to which these findings can be extrapolated *in vivo* remains to be established, because the rapid replacement of standard proteasomes with immunoproteasomes in infected liver (7) implies accelerated degradation of standard proteasomes.

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These findings demonstrate that in response to cytokines typically unleashed in the first day of viral infection by innate immune mechanisms, cells shift into high gear to synthesize immunoproteasomes, which are then assembled as soon as possible, implying an urgent need for immunoproteasomes. Moreover, in demonstrating a decreased lifespan relative to standard proteasomes, these data suggest that immunoproteasomes may begin to stink after a few days.

The malodor of immunoproteasomes may have little to do with the processing of viral determinants. Although immunoproteasomes clearly alter the peptide repertoire presented by class I molecules, the effect is relatively subtle. Moreover, immunoproteasomes are likely to be induced in both virus-infected and uninfected cells in a given tissue due to the general availability of released cytokines. This scenario raises the possibility that immunoproteasomes generate new self-determinants in uninfected cells that elicit autoimmune tissue-specific responses. Perhaps in the battle against viruses, damage from friendly fire is an acceptable price for destruction of the enemy's sanctuary. On the other hand, the increased turnover of immunoproteasomes would appear to have little purpose if cells are ultimately fated for a T_{CD8^+}-mediated death.

These ruminations lead to the prospect that immunoproteasomes have important functions with little or nothing to do with antigen processing. Proteasomes are known to perform endoproteolytic cleavages that generate biologically active proteins. Moreover, it would be surprising if the massive amounts of oligopeptides generated by proteasomes were completely devoid of biological function. Thus, the different specificity of immunoproteasomes might serve to modify levels of biologically active fragments of proteins, particularly proteins induced by cytokines released in the inflammatory process.

There is indirect evidence for alternative immunoproteasome function. Targeted disruption of the β1i gene in mice impairs the ability of their T_{CD8^+} to compete with wild-type T_{CD8^+} in antiviral responses, suggesting a specialized role for immunoproteasomes in activated T cells (9). Intriguingly, immunoproteasomes appear to be constitutively expressed in ocular lens and brain (10). These tissues are immune privileged sites, with no apparent need to generate class I peptide ligands, suggesting an alternative function for immunoproteasomes.

Taken together, these observations imply that immunoproteasomes have important non-antigen-processing functions in normal and pathogen-infected tissues. Immunoproteasomes are hard to know. For sure, they are worth knowing much better.

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