At the crossroads of cell biology and immunology: DRiPs and other sources of peptide ligands for MHC class I molecules

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Summary

CD8$^+$ T cells are a critical element of vertebrate immune responses to viruses and other intracellular parasites. They roam the body, monitoring cells for the presence of foreign peptides associated with MHC class I molecules of the major histocompatibility complex (MHC). Although it is clear that most of these peptides are generated through the action of proteasomes, the nature of the substrates degraded by proteasomes is an open question. Recent findings indicate that the major pool of substrates consists of a heterogeneous subset of proteins that are degraded within minutes of their synthesis. Evidence suggests that the fraction of newly synthesized proteins targeted for destruction is remarkably high – 30% or more, depending on cell type – possibly because they are defective in some way and cannot reach their intended conformation or location cellular in a time frame deemed appropriate by cells.

Key words: CD8 cell, T cell, Proteasome, Defective ribosomal product (DRiP), Major histocompatibility complex (MHC), Antigen presentation, Antigenic peptide, Ubiquitin, Protein synthesis

Biological context of the problem: viruses vs vertebrates

The world teems with viruses awaiting the opportunity to tap the inviting resources of unsuspecting host organisms (Flint et al., 2000). So much so, that in every host strategies for minimizing the effects of viruses on its reproductive potential have evolved. Vertebrates are particularly susceptible to viruses for at least two reasons. First, we are highly mobile, which maximizes virus transmission. Second, we have an unusually limited capacity for reproduction, which keeps our overall capacity to mutate at rock-bottom levels, which minimizes our ability to outrun viruses genetically. Extraordinary problems require creative solutions, and one of the great creations of evolution is the vertebrate immune system, which levels the playing field in the ongoing battle between vertebrates and viruses (Janeway and Travers, 1994).

The principal method used by the immune system to combat viruses is based on the recognition of small peptide fragments of viral proteins by roving thymus-derived cells (T cells) that express a clonally restricted T cell receptor (TCR) specific for the peptide. Peptides are derived from proteins synthesized within infected cells and are displayed on the surface of infected cells by MHC class I molecules of the major histocompatibility complex (MHC) (Townsend et al., 1986a). In this way, the immune system can monitor the presence of all viruses actively expressing their gene products, and not just those that conveniently express gene products on the cell surface. A common misconception is that MHC class I ligand peptides are derived only from viral proteins. In fact, the antigen-processing system does not discriminate between cell and foreign proteins, and cellular proteins provide *all* of the peptide ligands for MHC class I molecules present on uninfected cells (Rammensee et al., 1995; Engelhard, 1994). Because most cell types in the body constitutively express MHC class I molecules, this is extremely relevant biologically, and even clinically, since this (along with the MHC polymorphism described below) is responsible for the rejection of transplanted tissues.

The TCR predominantly interacts with residues from MHC class I molecules, but it is the relatively limited interaction with the peptide that boosts its affinity to a level that triggers T cell activation (Garcia et al., 1999). In addition, MHC-class-I-specific T cells express another cell surface protein, CD8, that interacts more weakly with a conserved portion of MHC class I molecules. CD8 is used to identify (literally and figuratively) such T cells, which are known as T_{CD8+}. T_{CD8+} combine exquisite sensitivity with exquisite specificity. At their best they can recognize <10 copies of a given peptide–MHC-class-I complex on a cell surface that displays ~10^5 MHC class I molecules bound to other peptides (Sykulev et al., 1996), and they can discriminate the presence of a single hydrogen atom in a contact region extending over ~2000 Å^2.

Genes that encode MHC class I proteins are highly polymorphic. This is probably the evolutionary solution to the problem created by the sky-high mutation rates of viruses. In humans, hundreds of alleles are possible at each of three loci, making MHC class I genes the most polymorphic human genes (along with MHC class II genes, which function similarly). Allelic differences between MHC class I genes are largely restricted to alterations in the peptide-binding region of the molecule; consequently each allomorph binds a distinct spectrum of peptides, >90% of which are 8-11 residues in length. The rules for peptide binding are complicated, but are largely based on the interaction between pockets in the MHC

class I peptide-binding groove and two or three residues in the peptide, one of which is always at the C terminus (Falk et al., 1991). These allelic differences place a high hurdle in the path of viruses bent on avoiding T_{CD8+} recognition, since each individual in a host population potentially presents new selection criteria for avoiding antigen presentation.

Consider for a moment the costs of this system. As a result of the variability of the MHC, the specificity of T cell receptors cannot be hard wired, but must be determined for each individual in a population. The solution to this problem has three parts. First, generate huge numbers of T cells clones, each expressing a unique TCR. Second, evolve an organ (the thymus) dedicated to killing the useless or dangerous T cells (negative selection) and facilitating the development of the few T cells that have just the right affinity for the MHC class I molecules expressed by the individual (positive selection). Third, evolve the TCR in such a way to make it likely that substitution of self-peptides with foreign peptides in the MHC molecule has a fair chance of increasing the affinity of the interaction to the threshold required for T cell activation. The breathtaking complexity and sophistication of the system is perhaps the most impressive testimony to its evolutionary value.

The proteasome: chamber of protein death and peptide birth

In the end, this system is only as good as the peptides it produces. Since the system cannot know in advance the nature of the virus it needs to control, it must be able to generate peptides from virtually any type of protein that a virus could make. Since there are so many MHC class I alleles, which exhibit different peptide specificities, the system has to generate a highly diverse set of ligands. Because it is difficult for a cell to know that it has been infected, the system should operate constitutively. This is a demanding set of design criteria, but it was solved *prior* to the evolution of the immune system and indeed, probably provided the impetus for the evolution of the MHC class I antigen-presentation system.

At the heart of this system is a remarkable peptide-producing machine, the proteasome (Voges et al., 1999). Proteasomes are abundant (~5×10^5 copies per HeLa cell; Hendil, 1988) macromolecular assemblies present in the nucleus and cytosol and are the major protease used by all eukaryotic cells to degrade unwanted proteins, usually those that are damaged or misfolded. These include nuclear, cytosolic and even proteins in the endoplasmic reticulum (ER), which are re-exported into the cytosol (Romisch, 1999) for degradation by proteasomes, possibly those decorating the cytosolic surface of the ER. Proteasomes consist of two major structural assemblies: a catalytic core known as the 20S proteasome, and regulatory subunits that attach to each end of the 20S proteasome. 20S proteasomes are barrel-shaped structures that comprise 14 distinct subunits arrayed in a four-ring structure of the type $\alpha_7\beta_7\beta_7\alpha_7$ (Voges et al., 1999). Three of the β subunits in each inner ring are catalytically active, their active sites facing a central chamber in which substrates are degraded. 20S proteasomes are closed at both ends, and must bind to a regulatory particle if protein substrates are to gain access to the central chamber. The major regulatory particle is the 19S regulator, which recognizes potential substrates bearing polyubiquitin (Ub) chains, unfolding the substrate and feeding it, spaghetti-like, into the barrel of the 20S proteasome, which opens as part of the process (Glickman et al., 1999).

Ubiquitin (Ub) is a 76-residue protein that is remarkably well conserved among eukaryotes. It is extremely abundant in cells (~10^8 copies per HeLa cell; Haas and Bright, 1985) and has many known cellular functions, which revolve around its covalent conjugation to the ε-NH_2 groups of lysine residues via its C terminus (Hershko and Ciechanover, 1998). Ub itself is ubiquitinated, and, when polyUb ‘trees’ containing four or more Ubs formed in this way are present on proteins, they bind to 19S regulators that deliver the protein to proteasomes for destruction, while the Ub is recycled (Thrower et al., 2000). Given its dire consequences, polyubiquitination of proteins would be expected to be a highly regulated and complicated affair, and, indeed, numerous gene products are devoted to Ub conjugation. For obscure reasons, cells are not content to add a single Ub tree to proteins destined for destruction. Rather, polyubiquitination is highly heterogeneous, and trees of different sizes are added to multiple substrate sites in what appears to be a highly irregular manner, such that polyubiquinated proteins usually migrate as a ladder (or smear) in SDS-PAGE. Moreover, the process is not irreversible, and enzymes capable of removing Ub from proteins (Ub-hydrolases) are highly active in cells (Wilkinson, 2000). Polyubiquitination is not an absolute prerequisite for protein degradation by proteasomes. There are only a few specific examples of such Ub-independent targeting at present, but this could well prove to be a major source of proteasome substrates (Verma and Deshaies, 2000).

The contributions of proteasomes to mammalian cell physiology have been defined largely through the use of membrane-permeant low-molecular-mass inhibitors of the proteolytic activities of 26S proteasomes (Bogyo et al., 1997). These include natural products of microorganisms and synthetic oligopeptide-based compounds. Although the value of proteasome inhibitors is indisputable, the secondary effects of blocking proteasomes on cell physiology can seriously confound experimental analysis. These effects include rapid depletion of the pool of free Ub available for conjugation, induction of molecular chaperones, and a general inhibition of protein synthesis. That some or all of these effects are likely to be caused by an accumulation of ubiquitinated substrates awaiting destruction does not simplify matters.

Proteasomes come in multiple forms. Indeed, the initial evidence implicating proteasomes in antigen processing was the discovery that the MHC encodes two proteasome subunits and that their expression (and that of a third subunit) is controlled by cytokines released by activated T cells (Monaco and Nandi, 1995). When induced, these subunits replace constitutively expressed subunits in newly assembled proteasomes to create 20S ‘immunoproteasomes’, which appear to be better at producing peptides favored by MHC class I molecules (Tanaka and Kasahara, 1998). Cytokine-exposed cells also produce 11S regulators, which take the place of 19S regulators at one or both ends of the 20S proteasome and favor antigen presentation through an undefined mechanism (Rechsteiner et al., 2000).

Proteasomes are the principal cytosolic protease used for generating MHC class I peptide ligands (Rock et al., 1994; Rock and Goldberg, 1999). Proteasomes can generate the

precise peptides presented by MHC class I molecules, but they also produce precursors of MHC class I ligands that are trimmed by other proteases. A considerable body of evidence indicates that peptides can be trimmed by aminopeptidases in the cytosol (York et al., 1999) and ER (Yewdell et al., 1999), whereas carboxypeptidase trimming seems to be highly unusual. ER trimming is possible because TAP, the MHC-encoded heterodimer that transports peptides across the ER membrane to nascent MHC class I molecules, transports peptides of between eight and ~17 residues with similar efficiency (Elliott, 1997; Momburg and Hammerling, 1998). An important question is the fraction of proteasome-generated peptides that must be further trimmed either in the cytosol to be transported by TAP and/or in the ER to generate peptides that bind MHC class I molecules with requisite affinity to trigger the export of MHC class I molecules from the ER (Pamer and Cresswell, 1998).

Where do antigenic peptides come from? The DRiP hypothesis

Here's the problem in a nutshell. Given that a virus infecting an epithelial cell can replicate in four hours, how can the infected cell produce sufficient viral peptides to enable T_{CD8+} recognition in a timely fashion in the face of an overwhelming number of cellular peptides? But first, can infected cells really do this? The answer is a resounding yes, at least for cultured cells, which can be recognized by T_{CD8+} <60 minutes after viral penetration (Esquivel et al., 1992). Since MHC class I molecules take 10-15 minutes to reach the cell surface once they are loaded with peptides in the ER, this is impressively rapid.

One solution could be that peptides are derived from viral proteins that are much less stable than cellular proteins with which they compete for access to MHC class I molecules. But, in fact, antigenic peptides are commonly derived from viral proteins that are extremely stable when measured by standard means. Another solution, originally proposed by van Pel and Boon (Van Pel and Boon, 1989), is that antigenic peptides are derived from short-lived, misbegotten out-of-frame polypeptides resulting from errors in transcription or translation. This probably occurs (Mayrand and Green, 1998), but it does not account for the vast majority of antigenic peptides, which are translated in the correct reading frame.

An alternative solution is that protein biosynthesis, like all biological processes, isn't perfect. A certain percentage of ribosomal products must not clear all the hurdles and arrive at the finish line in a stable conformation. It was appreciated years ago that introducing alterations in proteins that increases their degradation in cells increases their presentation to T_{CD8+} (Tevethia et al., 1983; Townsend et al., 1986b; Townsend et al., 1988), the implication being that imperfect forms of proteins generated from wild-type genes would be a preferred source of antigenic peptides. Many different types of error can be envisaged in mRNA generation or in the process of protein synthesis itself that result in the mis-incorporation of amino acids, premature termination, or deletion of residues. In addition, many proteins are members of multisubunit complexes, and there must be imbalances in synthesis that result in an excess of certain subunits that are unstable in the absence of their normal partners. Furthermore, mistakes must be made in protein targeting, such that proteins are delivered to the wrong organelle, where they are targeted for destruction. The folding of nascent proteins in the crowded environment of the cytosol is more difficult than folding in a homogeneous solution in vitro, and there are ample opportunities for the process to go awry, particularly for large multidomain proteins.

The products of these errors can be grouped together as a source of antigenic peptides, since they all will presumably be degraded by proteasomes and other cellular proteases involved in quality control. We termed such substrates defective ribosomal products (DRiPs) (Yewdell et al., 1996). The beauty of DRiPs for the immune system is that they enable MHC class I molecules to monitor protein synthesis rates in cells and not protein concentrations (Fig. 1). In this way, viral infections could be detected as soon a possible, particularly since many viruses rapidly monopolize the protein synthesis machinery to produce their own gene products. This would explain the rapid detection of virus-infected cells by T_{CD8+} and also the generation of antigenic peptides from a huge variety of viral proteins regardless of their apparent metabolic stability or intracellular targeting.

Note that we do not propose that DRiPs are exclusively limited to viral proteins – but rather that DRiPs are an inevitable consequence of synthesizing *any* protein. Indeed, the average viral protein may be less prone to DRiP formation than is the average cellular protein. An additional strength of the DRiP hypothesis is that it provides an explanation for the lack of bias of cellular peptides towards metabolically unstable proteins.

Putting a number on DRiPs

Given that DRiPs must exist at some level, the key question is how common they are. But how to find DRiPs in the first place? For sure, there is no substitute for serendipity in research (where it is often as good to be lucky as smart). In the course of studying the degradation of a defined proteasome substrate through metabolic radiolabeling of HeLa cell protein with [^{35}S]methionine (30 second pulse), we noted that up to three-fold more radioactive total cell protein can be recovered from cells treated with proteasome inhibitors (Schubert et al., 2000). This effect is immediate, and continues through the chase required to complete the incorporation of [^{35}S]Met into protein. Subcellular fractionation revealed that all of the additional label was present in proteins in the insoluble fraction; the marked bias in labeling of more slowly migrating proteins indicates that the increase in radioactivity cannot be trivially attributed to increased charging of tRNA with [^{35}S]Met or an increase in the rate of protein synthesis. Rather, the findings suggested that, under some conditions, up to 75% of newly synthesized proteins in HeLa cells are degraded by proteasomes, presumably because they represent DRiPs.

We noticed that increased Met starvation results in a greater DRiP-recovery, possibly owing to misincorporation of alternative amino acids in the presence of limiting amounts of [^{35}S]Met-charged tRNA. Furthermore, cells must be treated with proteasome inhibitors at least 10 minutes prior to labeling but not for more than 30-45 minutes. The lower limit reflects the time needed for the inhibitor to inactivate a sufficient fraction of proteasomes; the upper limit we are less sure about, but treatment of cells with proteasome inhibitors for this period

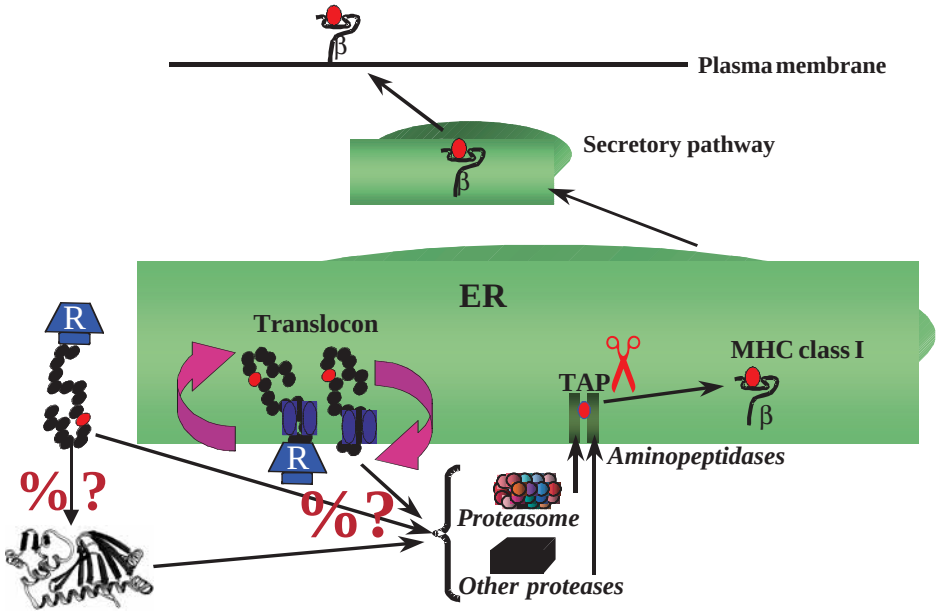

Fig. 1. Conventional MHC class I antigen processing pathway. Proteins (string of black balls, containing a red ball that symbolizes an antigenic peptide) synthesized by ribosomes (R) are either delivered to the cytosol or the endoplasmic reticulum (ER) through the translocon (blue ovals). A key number is the fraction of DRiPs, ribosomal products that fail to attain a stable, functional conformation. DRiPs originating in the cytosol or the ER are degraded predominantly by proteasomes, although other undefined cytosolic proteases (black box) also contribute. ER DRiPs are retranslocated to the cytosol via the translocon. Trimming of proteasome-generated peptides (red scissors), particularly of N-terminal extensions, can occur in the cytosol or in the ER following TAP-mediated (green bars) transport of peptides of 8-17 residues. Class I molecules that bind a peptide ligand above a threshold affinity are released from the ER and traverse the standard secretory pathway via the Golgi complex to reach the plasma membrane for perusal by T_{CD8+}.

has several effects, including a reduction in pools of free Ub available for conjugation, enhanced synthesis of molecular chaperones and, eventually, inhibition of protein synthesis. The last effect is probably responsible for the failure of previous investigators to discover the enhancing effects of proteasome inhibitors on the recovery of newly synthesized proteins.

Among the cell types we have investigated, DRiP synthesis is highest in HeLa cells, which is not surprising considering that HeLa cells no doubt have accumulated many mutations and gene imbalances in achieving 100% aneuploidy and a modal chromosome number of 82 (!) over the long course of their in vitro culture (>400 divisions since their establishment in 1952). DRiP synthesis is also substantial, however, in a hematopoietic-cell-derived dendritic cell line maintained in culture with cytokines, and even in activated lymph node cells processed immediately after their removal from a mouse. Under the most natural conditions – that is, in lymph node cells exposed for the minimal time to proteasome inhibitor treatment and labeled without Met pre-starvation – the DRiPs still represent 30% of newly synthesized proteins.

That DRiPs seem to make up 30-80% of newly synthesized proteins surprised us – considerably. It prompts three obvious questions. First, are they really DRiPs? Second, even if they are, is the high number an artifact of the methodology? Third, can cells decide so quickly to pull the degradation trigger on newly synthesized proteins?

Question 1: are they really DRiPs?

The strongest evidence for proteins rescued by proteasome inhibitors representing DRiPs is their insolubility: they selectively associate with insoluble material following freeze thawing (Schubert et al., 2000). DRiPs are also resistant to extraction with relatively mild detergents, such as TX-100 (unpublished observations). This insolubility is not a complete surprise, since unfolded proteins are generally insoluble owing to aggregation driven by solvent exposure of normally interior hydrophobic domains.

While compelling, this evidence is hardly conclusive, and additional supporting evidence is necessary. Demonstrating, for example, that a high percentage of the material is ubiquitinated would strongly support the DRiP hypothesis. The insoluble nature of the material makes quantitative analysis difficult. Thus, although proteasome inhibitors greatly increase the amount of newly synthesized protein reactive with Ub-specific antibodies, we could not determine the percentage of DRiPs that were ubiquitinated, since only a fraction could be solubilized. Use of a combination of detergents improves the solubilization of proteins, while maintaining their ability to interact with antibodies. In this way, we found that treating HIV-1-infected cells with proteasome inhibitors increases the amount of radiolabeled proteins recovered by antibodies specific for Gag by 30%. Some of the increase occurred in material migrating with a lower mobility than that of Gag, which probably represents polyubiquitinated Gag, since a fraction of it reacts with an antibody specific for polyubiquitinated proteins. The rest of the increase was in material migrating with the expected mobility of Gag. Frequently proteins targeted for proteasome destruction by ubiquitination are recovered in a non-ubiquitinated form following proteasome blockade, which suggests that ubiquitination often occurs well *after* a protein has been routed for destruction.

We are currently examining the DRiP fraction of other individual proteins. This will probably vary considerably, depending on the intrinsic complexity of the protein and the complexity of interactions with other proteins. At either end of the spectrum, small soluble proteins generally should have a low DRiP fraction, while large proteins that must interact with membranes and multiple partners should have a high DRiP

fraction. An interesting issue is whether proteins exported into the ER exhibit on average a higher or lower efficiency of folding and assembly.

Accurate measurement of the folding efficiency of individual proteins will not be an easy task, since detection methods inevitably favor folded forms of proteins. Were it possible to know the total amount of polypeptide synthesized, it would be a simple task to determine the folding efficiency by simply determining the amount of folded protein. Unfortunately, determining the amount of any given protein synthesized by live cells is exceedingly difficult, particularly given the possibility for alteration of the biochemical properties of the proteins by co-translational alterations. Although the problem is more tractable in in vitro translation approaches, they are doomed to the eternal purgatory of uncertain relevance to living cells.

Question 2: are the numbers real?

The first reality check regards the accuracy of the DRiP fraction calculated for cells in culture. Specifically, there are a number of potential artifacts associated with the radiolabelling method used that could falsely increase this. For example, there could be modified amino acids in the radioactive amino acid preparations used that interfere with protein folding and assembly. This seems unlikely given similar results with [^{35}S]methionine and [^{35}S]cystine, and this possibility can essentially be eliminated by using other amino acids (labeled with [^{3}H] or [^{14}C]), since various radiolabeled amino acids cannot all be contaminated in such a similar manner as to lead to the same artifactual effect on DRiPs.

A more serious problem is that blocking proteasomes itself reduces the fidelity of protein synthesis. Arguing against this possibility, however, is the absolute increase in radiolabel observed when cells are treated with proteasome inhibitors for a period sufficiently brief to avoid the inhibitory effects on protein synthesis. This could only be explained if, in addition to reducing the fidelity of protein synthesis, blocking proteasomes also increased the incorporation of [^{35}S]Met. Although this is theoretically a plausible indirect affect of blocking the recovery of unlabeled methionine from proteasome substrates and thereby reducing intracellular Met concentration, we have observed a high DRiP fraction in cells labeled with medium containing normal concentrations of methionine (unpublished results).

Whatever arguments can be mustered in favor of the metabolic labeling method, it is but a single method, and confirmation of the DRiP rate by alternative approaches is essential. It is particularly important to find conditions that enable determination of the percentage of newly synthesized proteins that are ubiquitinated *in the absence* of proteasome inhibitors. We did demonstrate that the amount of polyUb proteins detected by western blotting rapidly decreases after adding protein synthesis inhibitors to HeLa cells (Schubert et al., 2000). Although this is consistent with a high DRiP fraction, these data cannot be used for even a rough estimate of the real number.

The second reality check is the extent to which protein biosynthesis in cultured cells maintained in synthetic media mimics that in vivo. That this limitation applies to virtually all of modern molecular cell biology, which by necessity is performed *in polystyrene*, does not reduce its significance. There is no technical barrier to performing pulse-chase experiments in living animals. It will also be interesting to study *S. cerevisiae*, which offers a number of advantages including natural unicellular growth and the complete toolbox of defined mutations for studying the process.

Question 3: can degradation occur so rapidly?

DRiPs are degraded rapidly, so quickly as to be indistinguishable from co-translational degradation. Could cells really decide during translation that a protein should be destroyed? The answer here is yes. Two groups have elegantly demonstrated co-translational degradation in mammalian cells (Lin et al., 1998) and yeast (Turner and Varshavsky, 2000). Both groups agree that protein biogenesis in eukaryotic cells may represent a kinetic competition between folding and degradation. At first glance this might seem to be the cell biology version of a comedy sketch in which police riddle a prone, unarmed suspect with bullets, and then yell, 'Freeze!' Why must cells decide so quickly to terminate a nascent protein? Perhaps because, to a cell, the minutes required for translation (which proceeds at ~5 residues/second) represent a perfectly reasonable time for a protein to fold, or at least make good progress down the folding pathway. The potential of unfolded nascent proteins to cause mayhem may be so great that cells even choose to err on the side of safety and destroy a fraction of proteins that would eventually achieve native conformation if given enough time.

Linking DRiPs to antigen processing

If DRiPs are a major source of peptide ligands for MHC class I molecules, then blocking protein synthesis should rapidly decrease the peptide supply. This in turn should slow the export of class I molecules from the ER, since peptide binding is required for rapid export of class I molecules. Protein synthesis inhibitors do in fact slow the export of MHC class I molecules from the ER (Schubert et al., 2000).

Spectacular additional evidence for a link between DRiPs and antigen processing came from an unexpected quarter. In studying the mobility of GFP-tagged TAP in the ER membrane, Neefjes and colleagues found that its mobility was inversely proportional to the cytosolic peptide concentration (Reits et al., 2000). Thus, armed with a unique assay for peptide generation in the cytosol, they found that peptides are depleted 30 minutes after protein synthesis is blocked. Since a similar degree of depletion is achieved within 15 minutes of blocking proteasomes – both in uninfected and influenza-virus-infected-cells – the primary source of peptides is probably from proteins in the first 15 minutes of their synthesis.

Curiously, influenza infection is associated with an increase in peptide supply to a level that saturated TAP in the mobility assay. This suggests two alternative possibilities. First, DRiPs are produced from viral genes and cellular genes at a similar rate, but the rate of translation increases with viral infection. Second, viral gene expression is associated with a greater DRiP fraction. In this second case, there are numerous possible explanations. For example defects in viral genes or transcription could lead to translation of unintended proteins. Alternatively, viral proteins might have an intrinsic tendency to less efficient

folding or assembly relative to cellular proteins. Finally, global virus-induced alterations in the efficiency of protein synthesis may occur, perhaps reflecting an innate immune response intended to enhance the presentation of viral proteins.

Perspectives

Recent work indicates that protein biogenesis is far less efficient than commonly considered. We have labored to point out the limitations of this conclusion, hoping to stimulate further research into this important question. Whatever the true efficiency of protein biogenesis, it is clearly important to understand how cells decide the fates of individual nascent proteins, and how this process is altered during infectious diseases, in terms of both the effects of intracellular pathogens on the process and the effects of cytokines released by immune cells during the inflammatory process. No doubt, the process is also affected in other disease situations.

Although the evidence discussed here suggests that newly synthesized proteins are the major source of peptide ligands for MHC class I molecules, we would be remiss if we neglected to mention that other sources exist. Antigenic peptides can be derived from exogenous proteins if the proteins are delivered to the cytosol (Yewdell et al., 1988; Moore et al., 1988), as well as from post-translational proteasome degradation of proteins (Anton et al., 1999). The relative contributions of these potential sources of MHC class I peptide ligands remain to be carefully quantitated. Moreover, proteasome inhibitors often have little effect on the generation of a given antigenic peptide, or even the pool of peptide ligands for given MHC class I allomorph (Benham et al., 1998; Vinitsky et al., 1997; Anton et al., 1998; Luckey et al., 1998; Cerundolo et al., 1997). This implies that peptides are also generated in a proteasome-independent manner. Do these proteases also degrade DRiPs? If so, our estimates of the inefficiency of protein biogenesis may even be too low!

Following the technical advances of the past 25 years, cell biologists are poised to make great leaps in our understanding of basic cellular processes, including the synthesis and folding of nascent proteins. As the mists of ignorance dissipate, the processes co-opted by the immune system to enable immune surveillance should be revealed in all their glory.

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