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Translating DRiPs: progress in understanding viral and cellular sources of MHC class I peptide ligands

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Abstract

It has been 15 years since we proposed the defective ribosomal product (DRiP) hypothesis to explain the rapid presentation of viral peptides by MHC class I molecules on the surface of infected cells. Here, we review the evidence for the contribution of DRiPs to antigen processing, pointing to the uncertainties regarding the physical nature of DRiPs, and emphasizing recent findings suggesting that peptide generation is a specialized process involving compartmentalized translation.

Keywords

Antigen processing; MHC class I; Proteasome; Translation; Virus

That we find out the cause of this effect. Or rather say, the cause of this defect. For this effect, defective comes by cause.

Polonius, regarding Hamlet's insanity

Hamlet, Prince of Denmark,

William Shakespeare

The race goes to the swift

Doherty and Zinkernagel's demonstration of the dual viral-antigen and MHC-restricted nature of cytotoxic T cell recognition of virus-infected cells [1] triggered a mad scramble to understand the molecular nature of the recognition event. Unanue [2] and Grey [3] provided the initial conceptual breakthrough, in demonstrating that MHC class II molecules bind to short peptide sequences and present them to CD4 T cells. This work was quickly extended by Townsend [4] to MHC class I molecules and viral peptides. Because Townsend [4] arrived at this answer by demonstrating CTL recognition of cells expressing truncated metabolically unstable forms of a viral protein (a phenomenon reported initially by Tevethia [5]), it was immediately clear that peptides were derived from protease products of biosynthesized proteins.

The problem of presenting peptides from metabolically stable viral proteins with sufficient alacrity was given little thought, but this poses a major problem for immunosurveillance. A single cell has $\sim 3 \times 10^9$ copies of proteins, yet only expresses $\sim 1 \times 10^5$ class I molecules [6]. Were it necessary to wait for the degradation of a viral protein with a half-life measured in days, antigen presentation would initiate only well after viral replication was completed.

Standard CTL assays through the 1980 with lytic viruses like influenza A virus (IAV) or vaccinia virus (VV) were performed over a period of 10–16 h postinfection, giving little reason to consider the kinetics of peptide generation. By the late 1980, however, the introduction of inhibitors like cycloheximide (CHX) [7] and brefeldin A [8] to antigen presentation studies led naturally to consideration of the kinetics of antigen presentation. In the guise of quantitating the requirement for TAP in endogenous viral-antigen presentation, we found that a sufficient vesicular stomatitis virus (VSV) nucleocapsid protein (N) is synthesized within the initial 45 min of infection to enable CTL killing of target cells [9].

Nucleocapsid protein, like most viral proteins, is extremely stable in infected cells, so how could enough N be made in 45 min to enable T cell recognition? Further, how could we explain why CHX treatment greatly retarded the export of nascent class I molecules from the ER, enabling antigen presentation of cytosolically-delivered exogenous viral proteins more than 24 h after addition of CHX to cells [10]? These findings prompted the DRiP hypothesis, which posited that peptides are generated from defective ribosomal products, i.e. defective forms of newly synthesized proteins that are degraded rapidly due to inability to properly fold, assemble, or traffic [11].

DRiP: it's not just semantics

After 15 years, what is the status of the DRiP hypothesis? Antigen presentation kinetics inspired the DRiP hypothesis, and kinetics provides the most compelling evidence for relevance of DRiPs to antigen presentation. This evidence, which continues to deepen [12–14] has been reviewed recently [15–17]. In short, studies in both viral and host antigen systems indicate that the generation peptides relevant for antigen processing is closely tied kinetically to increases and decreases in protein synthesis, strongly implying that many/most peptides derive from pool degraded with a half-life two orders of magnitude less than the average half-life of proteins, namely, 10 min versus 24 h (1,440 min).

It is worth digressing on the nature of the evidence. A key strategy in gauging the contribution of DRiPs to peptide generation is to examine the effect of abrogating protein synthesis with translation inhibiting drugs, typically CHX, which blocks polypeptide elongation. Based on the immediacy of the shut of peptide generation, the half-life of the peptide precursor pool can be calculated. A potential limitation, however, to interpreting the action of CHX on peptide generation is that it effects protein degradation, or other features of class I assembly or transport unrelated to its effects on precursor pool size.

A number of recent studies support the conclusion that CHX treatment does not inhibit the antigen processing and presentation machinery. We reported that presentation of the influenza virus peptide PA_{224–233} synthesized as a VV minigene product or Ub-liberated peptide continues for hours in the presence of CHX due the unusual ability of PA_{224–233} to generate a sizable intracellular pool [18]. This extended our prior demonstration [19] that CHX has no significant effect on degradation of RDPs or slowly degraded proteins measured by pulse chase experiments, and that peptide generation is prolonged after CHX treatment if a pool of precursors (presumably DRiPs) is expanded by treating cells with a proteasome inhibitor. Similarly, Neefjes and colleagues showed that peptide generation continues unabated in the presence of CHX for at least 7 h if cells are exposed to γ -irradiation [19]. Taken together, these findings provide solid support for the validity of short term CHX-shutdown (on the order of hours) as a means of measuring the metabolic stability of peptidogenic substrates.

While the evidence supporting an important role for DRiPs in antigen processing mounts, there has been somewhat less progress in understanding the physical nature of DRiPs. As this is a key area for future studies, it is useful to discuss the meaning of “DRiP”, which

ultimately must have a physical definition, or more accurately, definitions, since DRiPs almost certainly comprise different categories of substrates. Casting a wide net, we originally defined DRiPs as any non-functional form of a gene product, including forms that would not qualify as proteins per se, which is why the P in DRiP is product and not protein. This includes unfolded or misfolded proteins of the proper length and sequence, proteins with sequence errors or errors in posttranslational modification, prematurely terminated proteins, and proteins translated from the wrong start codon. We later extended DRiPs to include wrong reading frames, and sequences that do not encode traditional proteins, as encoded by introns, upstream sequences etc., all of which are now known to be sources of antigenic peptides [17].

Based, however, on the identification of thousands of defined viral peptides by functional assays and thousands of cellular peptides by mass spectrometry, it is reasonably clear that nearly all abundant peptides derive from standard open reading frames. Eisenlohr [20] and colleagues recently revisited the DRiP hypothesis, insightfully raising the issue that many misfolded proteins are degraded with kinetics that are approximately 10-fold slower than DRiPs relevant to antigen processing (i.e. on the order of hours). They propose that peptides are generated from otherwise normal full length gene products that are stochastically ignored by the folding machinery and targeted for immediate destruction by 20S proteasomes (more on this below). As it is essentially impossible experimentally to know whether such substrates are not defective in some essential manner, there seems to be little purpose in not terming them DRiPs. It might be worth pointing to the original DRiP model, which proposed that a subset of DRiPs “may be directly derived from ribosomes by association with “anti-chaperones” that target the polypeptide to proteases” [11], which seems to jibe closely with the thinking of Eisenlohr.

Physically defining DRiPs

In 2000 we unwittingly rediscovered the original finding of Denys Wheatley that a large fraction (nearly 40% in Wheatley's dexterous hands) of nascent total proteins are degraded with a half-life of 10 min after 1 min labeling with [³H]-Leu [21, 22]. The true fraction of such rapidly degraded polypeptides (RDPs) is subject to some uncertainty, as we pointed out in our original review of DRiPs [23], and questioned experimentally by Vabulas and Hartl [24] (but see Yewdell and Nicchitta [17] for rebuttal). The size of the RDP fraction, which is of great interest and importance to cell function and evolution, is, however, a red herring in terms of the contribution of DRiPs to antigen processing. This is from Yewdell and Nicchitta [17]:

It is crucial to stress that the RDP fraction is only tangentially related to the fraction of MHC class I peptide ligands that derive from DRiPs. In other words, RDPs could constitute 1% (or less) of newly synthesized proteins, yet still provide all of the peptides presented by class I molecules.

Readers might feel that we are being a tad slippery with our words here. In our defense, nature itself is slippery, since there is not a simple digital distinction between DRiPs and native proteins. An entire class of proteins, termed intrinsically disordered proteins (IDPs- we swear, not our acronym!) are naturally disordered, and fold into stable structures only conditionally, typically when bound to other proteins [25, 26]. It is thought that more than 30% of eukaryotic proteins contain at least one disordered domain of more than 50 residues [26].

That not all RDPs contribute equally to antigen processing is suggested by our findings that RDPs can be divided into two classes based on their solubility following their rescue from proteasome degradation [27]. The major fraction (~75%) of RDPs is soluble in mild

detergent, modulated by HSC70 levels, and degraded by the classical ubiquitin (Ub)-26S proteasome pathway. The remainder is insensitive to HSC70 levels, become insoluble within an hour of blocking proteasome activity, and are degraded independently of ubiquitylation by proteasomes lacking 19S regulators. The latter appear to represent the bulk of DRiPs from standard proteins, while the former appear to be the principal source of peptides with exogenous degradation signals that interfere with folding or directly target the proteins to the Ub-proteasome pathway. What are these insoluble products? We found one clue in this study. A COOH-fragment of GFP, probably generated by auto-catalytic cleavage associated with fluorophore maturation [28], exhibited all the characteristics of the bulk insoluble fraction [27].

Such fragments might be a common source of DRiPs. We provided evidence that common downstream initiation events generates peptidogenic DRiPs when antigens are expressed by standard Semliki forest virus vectors [16]. Downstream initiation in this case is due to SFV modification of translations factors, and is dependent on eIF2a phosphorylation. Gu et al. [29] used shRNAs directed against a target antigenic protein to cleave its mRNA. When SIINFEKL was inserted upstream of the interfering target, K^b-SIINFEKL complex expression was enhanced relative to cells expressing control shRNA, despite decrease expression of full length protein. Cardinaud et al. [14] investigated the long known ability of the Gly Ala repeat (GAR) of the gamma herpes virus EBNA-1 protein to interfere with immunosurveillance of the EBNA-1 gene [30]. It was originally believed that the GAR completely abrogated antigen processing of EBNA-1 by blocking proteasome mediated peptide generation in a cis acting manner [31]. Subsequently, it was shown that EBNA-1 is immunogenic in humans and antigenic in cell lines [32–35], and critically, that EBNA-1 diminishes antigen presentation by interfering with translation [36]. Cardinaud et al. found that the GAR interferes with translation by inducing premature termination in or just upstream of the GAR, an effect that is likely due to the secondary structure of mRNA encoding the repeat, since altering codon usage of this segment abrogates its activity [37]. Most importantly, Cardinaud et al. found that regardless of the presence of the GAR, truncated polypeptides were generated in substantial amounts, and tied their degradation to peptide generation (surprisingly in cells, as opposed to in vitro translation, the amount of truncated protein was greatly increased by removal of the GAR).

A key challenge for future studies is to determine the overall contribution of downstream initiation and premature termination to DRiP generation. If one is the predominant mechanism for generating DRiPs, there should be respectively, COOH-terminal or NH₂-terminal bias in class I peptide ligands defined structurally and class I antigens defined functionally. Given the ever-increasing number of identified class I ligands, there clear opportunities for bioinformatics, particularly combined with quantitative mass spectrometry [38], to provide important clues as the biochemical nature of DRiPs.

Mass action: it's not for everyone

The law of mass action holds that the concentration of reactants is proportional to the reaction rate and at equilibrium to the concentration of product. Given a finite supply of class I molecules, as peptides approach saturating concentrations, they should compete for binding, assuming they are in a common compartment. To test this, we took advantage of the ability of VV to increase viral gene expression in proportion to the multiplicity of infection (10-fold increase in multiplicity leading to a 3-fold increase in gene expression) [39]. We found that it was possible to saturate class I presentation at a relatively low MOI using viruses expressing class I binding peptides as minigene products or Ub-liberated products. This enabled us to perform competition experiments by co-infecting cells expressing different defined peptides. As predicted from the law of mass action, the high

affinity K^b binding peptide VSV N₅₂₋₅₉ (“RGY”) competed for presentation of K^b-SIINFEKL complexes as determined flow cytometrically using the 25-D1.16 mAb to directly quantitate cell surface K^b-SIINFEKL complexes. Competition occurred at the level of class I (and not TAP, for example) since RGY did not compete with presentation of D^b complexed with a rVV-expressed D^b-binding peptide. But, we made a curious observation when SIINFEKL was expressed in a protein context requiring proteasomal liberation. Despite the expression of a saturating amount of competing RGY, we were unable to reduce K^b-SIINFEKL complex expression. We interpret this to mean that peptides introduced into the cytosol by direct ribosomal synthesis or liberation from nascent protein by Ub-hydrolases are at a competitive disadvantage for loading class I molecules in the ER relative to peptides liberated from DRiPs by proteasomes.

We can think of two explanations for this finding. First, the NH₂-terminal extensions present on naturally processed peptides provide some sort of “handle” that enables much more efficient loading onto class I molecules. Second, cells are compartmentalized in some manner to prevent cytosolic peptides from competing with naturally processed peptides. Taking this a step further, the fact that DRiPs are the principal source of peptides despite the near equal amount of degradation of retirees [22, 23, 40], strongly suggests heterogeneity in the access of proteasome substrates to this putative compartment.

This idea that classes of proteasome substrates differ widely in their efficiency of accessing the class I pathway is central to the current thinking in our laboratory about antigen processing. As discussed above, slowly degraded DRiPs generated by misfolding are a less efficient source of peptides than rapidly degraded DRiPs. Even among rapidly degraded DRiPs there can be distinctions. NP targeted for rapid and complete destruction by the N-end pathway appears to be a far less efficient source of peptide than natural NP DRiPs, since increasing the immediate degradation to 100% only increase peptide generation 3-fold [41]. Since we cannot detect any difference in NP expression in the presence of proteasome inhibitors (which block peptide generation), this suggests that true DRiPs are many fold more efficient than the artificially generated DRiPs.

We recently extended these findings using a system developed by the Wandless lab to rapidly and reversibly control the folding status of reporter proteins using the drug Shield-1 [42]. Creating a Shield-1-controlled SIINFEKL-tagged GFP-fusion protein, we found that K^b-SIINFEKL complexes are generated much more efficiently from Shield-1 insensitive DRiPs than from either Shield-1-rescued nascent or “aged” proteins [43]. In all cases, antigen presentation was completely blocked by proteasome inhibitors.

This provides yet another (Orwellian) example that not all proteasome substrates are treated equally: peptides liberated from “true” DRiPs have more efficient access to the pathway than peptides liberated from retirees or “mildly” misfolded nascent proteins. The key idea is that DRiP processing may be compartmentalized to enhance efficiency.

Compartments within the cell

What type of compartment might house high efficiency antigen processing and presentation machinery? As the fundamental unit of life, cells are highly organized structures that contain organelles and sub-organelle domains evolved to increase the efficiency of metabolic processes. Not all domains form structures that can be visualized by current microscopic techniques. Restricted domains are proposed to explain H₂O₂-mediated signaling in the face of cytosolic peroxiredoxin levels that should immediately destroy nascent H₂O₂ [44]. Protein translation itself seems to be compartmentalized to ensure that aminoacylated-tRNAs are in close proximity to the translation machinery and do not have to passively diffuse to ribosomes after their generation by tRNA aminoacyl synthetases [45, 46].

One physically defined compartment is related, at least tangentially, to DRiP processing. Dendritic cells segregate and store newly synthesized and polyubiquitylated proteins in dendritic cell aggresome-like induced structures (DALIS) during the process of maturation [47, 48], demonstrating that molecular mechanisms exist to ensure compartmentalization of at least a subset of DRiPs. Stress induces similar structures in other cell types, providing clear precedent for cellular compartmentalization of DRiPs [49].

There is also evidence for compartmentalized processing of cross-presented antigens. Guermónprez et al. [50] made the initial observation that class I complexes were selectively generated in the compartment containing phagocytosed antigen. Since peptide generation was both TAP- and proteasome-dependent, they proposed that peptides were generated locally and transported into their source compartment. Nearly identical observations were made by Burgdorf et al. [51] studying antigen endocytosed via the mannose receptor.

Though Occam's razor can be a weapon of mass destruction when applied to biological systems, it is tempting to speculate a connection between the organization of the cross-presentation- and DRiP-presentation compartments. Both face the common problem of avoiding competition from peptides generated by turnover of normal cellular proteins, and both appear to be able to generate peptides at high efficiency relative to the efficiency of generating peptides from retirees and proteins targeted for more rapid destruction [19, 41, 52].

ERAD or PrERAD?

Since the electron microscope was first employed to scrutinize cells, the presence large numbers of ribosomes on the endoplasmic reticulum (classically referred to as rough ER) has repeatedly demonstrated that a substantial fraction of cellular protein translation is associated with this organelle. The recent work of Nicchitta and colleagues puts a new twist on this finding, as it appears that a substantial fraction of proteins synthesized on ER-associated ribosomes are not imported into the ER by Blobel's signal based mechanism [53], but rather are destined for the cytosol/nucleus [54–56].

Still, a surprisingly large fraction of proteins, more than 25% of individual gene products [57] and total synthesized protein [58] are targeted to the ER. ER proteins are a rich source of class I peptides. This is particularly well established for viral proteins. Indeed, at the dawn of the-peptide-as-epitopes era (i.e. pre-Unanue), the first peptides identified as CTL antigens, derived from viral membrane proteins [59, 60].

Although there are exceptions [61], presentation of most ER-targeted proteins is TAP- and proteasome-dependent, implicating cytosolic processing in peptide generation. We recently performed careful kinetic studies on the presentation of the SIINFEKL peptide embedded in the neuraminidase (NA) stalk of recombinant IAVs [13]. Although SIINFEKL insertion has subtle effects on IAV pathogenesis in mice [62], it has no significant effect on viral growth in cultured cells, and NA biogenesis is not detectably affected. Antigen presentation and NA accumulation at the surface of infected cells occurred with nearly identical kinetics and required ongoing synthesis of NA protein. Remarkably, only 12 min separated abrogation of K^b-SIINFEKL presentation when we treated cells with proteasome inhibitor versus CHX. Assuming that this would account for ~3 half-lives of the relevant substrate (=87.5% of the available antigen), these data imply a ~4 min half-life of the NA-DRiP pool. Notably, we previously found similar kinetics for ovalbumin, that natural source of SIINFEKL, which is also an ER-targeted secreted protein.

For typical ER-targeted proteins like NA and Ova, that are not known to be re-imported into the cytosol after achieving native state, but rather are either secreted/released from cells or

degraded in endo/lysosomal compartments, it is hardly surprising, on moment's reflection, that peptides derive from DRiPs. We can divide such DRiPs into two bins: those that never entered the ER, and those that were re-exported from the ER into the cytosol by ER-associated degradation (ERAD). ERAD is actually a misnomer, since it was coined by Klausner and colleagues to describe the proteolysis of misassembled TCRs (DRiPs!), which they believed occurred in the ER itself [63]. It was first shown by Brodsky and McCracken in yeast that ERAD actually occurs in the cytosol [64]. Independently of these findings, our group and Engelhard's group showed that peptides from ER-targeted proteins almost were certainly generated by ERAD since they were TAP-dependent and were affected by N-linked glycosylation, a process that exclusively (a dangerous word!) occurs in the ER [65, 66]. Engelhard later showed that in their tyrosinase antigen system, substrates that enhance folding in the ER reduce antigen presentation, demonstrating that at least some of the sources of peptide are not irretrievably defective [67].

A key issue in relating these findings to our concept that DRiPs are frequently not salvageable is quantitation and kinetics. T cell activation-based assays are poorly suited to generate these data, and the application of flow cytometry based quantitation of peptide class I complexes is needed to gauge the real degree of differences between our findings with SIINFEKL based antigens and other systems.

We would be remiss if we left the reader with the impression that ERAD is the major route of presentation of ER-targeted antigens, since its relative importance in antigen processing is uncertain. Groettrup and colleagues provided solid evidence that for prostate stem cells antigen, the major source of antigen is a pool of DRiPs that were not properly targeted to the ER and were degraded by proteasomes (a process we term preERAD or PrERAD) [68]. To the extent that available ERAD inhibitors (drugs and siRNA) effectively inhibit overall ERAD (they probably do not), evidence would suggest that non-targeting is the major route of presentation, since blocking ERAD has little effect on peptide generation from endogenous sources of peptide antigens, though ERAD has been implicated in antigen cross-presentation [69, 70].

Given the vagaries, the only solid conclusion regarding the nature of DRiPs for ER-targeted proteins is that the major pathways have been lightly sketched out and much remains to be learned.

Translation at the center of it all

Given the critical role of translation in the class I pathway, the clear potential for compartmentalization, and over-representation of minor sources of antigen processed with high efficiency, it is worth considering all sources of nascent peptides, with particular emphasis on the oddballs. In a recent report we used RNA polymerase II inhibitors to “trap” influenza late mRNAs in the nucleus [71] and observed on-going antigen presentation of IAV NA peptides (albeit at lower levels than untreated cells) despite the almost complete lack of NA in treated cells [72]. We proposed that NA DRiPs synthesized in the nuclei of drug treated cells are a source of peptides. But can proteins be translated in the nucleus?

Nuclear translation was initially reported in 1957 [73], re-discovered a decade ago [74–76], but is widely considered by translation mavens to be an artifact [77]. As more and more of the components of translation are detected in the nucleus, such as charged-tRNAs [78], amino acyl tRNA synthetases [79, 80], and translation initiation factors [76, 81–84] the case for nuclear translation mounts. What might be translated in the nucleus? Perennial suspects are pioneer translation products from nonsense mediated decay (NMD) of mRNAs [85]. The NMD pathway is a quality control mechanism that degrades mutant or mis-spliced mRNAs by detecting premature termination codons in a process that entails translation of nascent

messages (the pioneer round). We previously suggested NMD as a source of peptides for extremely low abundance antigens, whose presentation is difficult to square with the law of mass action [17]. Interest in the potential contribution of NMD to antigen processing is heightened by the exciting recent findings linking NMD to tumor rejection [86].

Met-misacylation? probably not

In collaboration with Tao Pan, we recently reported that up to 14% of Met is attached to the “wrong”, i.e. non-cognate tRNA by aminoacyl synthetases (probably Met tRNA synthetase when cells are subjected to stressful conditions, including viral infections [87]). We were testing the hypothesis that tRNA-misacylation is a source of DRiPs, with the idea that misincorporation of amino acids leads to misfolding. Indeed, we also provided multiple lines of evidence that non-cognate Met is incorporated into proteins (our strongest evidence is the similar off rate of Met from cognate and non-cognate tRNA and the complete blockade of both when CHX is added to block protein synthesis).

While it is certainly plausible that Met-misacylation contributes to peptidogenic DRiPs, our available evidence suggests this is not a major effect of Met replacement. First, misacylation is induced by a number of conditions that seem to be unrelated to any need to enhance peptide generation, including exposure to LPS, oxidants, and even overcrowding of cells. Second, as far as we can determine, Met-misacylation is induced by intracellular ROS generation by NADPH oxidases, a common signalling event in the cellular response to infectious, chemical, and physical stress. Third, as Met is known as a “bodyguard” residue that protects proteins against ROS mediated damage [88, 89], and Met misacylates specific tRNAs, we have proposed that Met-misacylation functions (ironically) as an anti-DRiP, i.e. to reduce degradation of nascent proteins, and not to enhance it.

While do not favor the idea that Met-misacylation is a major contributor to DRiPs, we are keeping an open mind on the issue, and encourage others, particularly mass spectrometrists, to do so as well. When encountering class I associated peptides with masses that do not match the predicted proteome, it would be a useful exercise to research using an algorithm that replaces the mass of the 19 other common amino acids with Met.

The ribosome-proteasome link

The close kinetic relationship between translation and peptide generation along with our surprising (non-) competition results [39], suggests a potential linkage between ribosomes and proteasomes (and other cytosolic proteases implicated in antigen processing). Detailed analysis of the yeast proteasome interaction network revealed several proteins involved in translation [90]. eIF3 [91] and EGD [92] have been found to interact with both ribosomes and proteasomes by biochemical analysis. An additional translation factor, eIF1A is thought to interact with the Rpt1 subunit of the proteasome to facilitate co-translational degradation of mis-folded proteins [93].

Analysis of mammalian cells is more limited, but two translation factors, eIF2 and eIF1 α , are reported to associate with proteasomes isolated from cells [94]. It is of obvious interest to examine the interaction of ribosomes and proteasomes via microscopy, particularly in living cells with fluorescent versions of the protagonists.

What's next?

Fifteen years and counting, there is solid evidence that DRiPs account for the majority of viral peptides. There is compelling, but less extensive evidence for a major contribution of DRiPs to cellular peptides. With the accretion of Ab/TCR-based probes with TCR-like

specificity it will be important to extend flow cytometry based kinetic studies comparing antigen expression and generation of class I peptide complexes using genetically unmodified versions of antigen. It is particularly important to compare the properties of antigen presentation between cell lines and real differentiated cells, ideally analyzed in vivo, but initially ex vivo using dissociated tissues. For example, it should be possible to measure levels of a nominal class I peptide complex on epithelial cells at various times after initiating infections.

The most significant weakness in the DRiP hypothesis is the poor biochemical definition of DRiPs. Though it is reasonably clear that a subset of DRiPs are generated via premature termination [14] and downstream initiation [16], the relative contribution of these processes to DRiPs is uncertain, not to mention other myriad sources, including NMD and other transcription error sources. Eisenlohr et al.'s [20, 95] point is well taken that misfolded proteins are typically degraded too slowly to account for the rapid kinetics of peptidogenic DRiPs, but half-lives are based on the assumption of uniform exponential decay (being modeled on the law of mass action), and it is possible that a subset of misfolded proteins are degraded with much more rapid kinetics than the majority population. Indeed, we found that although the amino acid canavanine induced slowly degraded antigen, it still enhanced presentation from a cohort that was present with kinetics identical to non-canavanyl DRiPs in CHX shut down experiments [19]. As the number of defined IDPs increases and their average degradation kinetics emerges, this will provide a handle on gauging the contribution of this potentially important subset of proteins to the RDP pool and to class I peptide ligands.

It is highly unlikely that we have identified all of the important gene products that participate in class I peptide ligand generation. With a little luck, a few surprises are still in store for the field, and will likely come from high throughput screens for compounds/siRNAs that modulate antigen presentation. Our recent results suggest a role for deubiquitylases in peptide generation [43], which is consistent with our prior findings that ubiquitylation is not required for generating peptide class I complexes [27, 96].

Given the role of MHC class I molecules in multiple “alternative” processes (which may actually be more important evolutionarily than anti-pathogen immunity) that now include tumor immunosurveillance, NK recognition, mate selection, and neuronal organization, it seems increasingly likely that the repertoire of presented peptides that regulate these processes is not simply left to chance. Despite the doubts of some of our colleagues dismayed by our slow progress, we will continue to investigate the immunoribosome hypothesis that cells segregate a subset of mRNAs for high efficiency conversion to antigenic peptides.

Finally, the forest: why come to grip with DRiPs? A better understanding of the cellular and molecular biology of DRiP antigen presentation will lead to novel insights into myriad aspects of cellular function, and is required for practical applications, including rational vaccine design, enhancing immune eradication of pathogens and tumors, and blocking autoimmunity.

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