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MHC class I-presented peptides and the DRiP hypothesis

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Abstract

MHC class I molecules present peptides derived from intracellular proteins, enabling immune surveillance by CD8+ T cells and the elimination of virally infected and cancerous cells. It has been argued that the dominant source of MHC class I-presented peptides is through proteasomal degradation of newly synthesized defective proteins, termed defective ribosomal products (DRiPs). Here, we critically examine the DRiP hypothesis and discuss recent studies indicating that antigenic peptides are generated from the entire proteome and not just from failures in protein synthesis or folding.

Keywords

DRiP; proteasome; antigen presentation; MHC class I

Proteolysis and MHC class I antigen presentation

All cellular proteins are continually being degraded and replaced by new synthesis. The bulk of cellular proteins, both short-lived and long-lived components, are degraded in the cytosol and nucleus by the ubiquitin proteasome system [1–6], and the remainder of proteins by lysosomal proteases after endocytosis of membrane components or by engulfment of cytosolic proteins and organelles by autophagy [7]. The proteasome digests primarily ubiquitinated proteins to oligopeptides ranging from 2–20 residues in length [8]. The great majority of these fragments are hydrolyzed very rapidly by cytosolic peptidases to amino acids [10]. However, a small fraction of the longer peptides escape this fate because they are transported by TAP into the lumen of the endoplasmic reticulum [9–11] where they may be trimmed by aminopeptidases, especially ERAP1 [12–15] (Fig 1). Then peptides of appropriate length and with key residues become bound by MHC class I molecules, and the complex is then transported to the cell surface (Fig 1). This process allows the $CD8^{+}$T cells

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to detect and eliminate cells expressing foreign genes (e.g. from a viral infection) or ones that are mutated (e.g. in a cancer).

Although there is now extensive evidence and a consensus about the proteolytic systems that generate the MHC class I-presented peptides [8,16,17], the nature of the proteins that are the source of the presented peptides is less clear. In the DRiPs model [18], which has gained prominence and favor in the field, MHC I-presented peptides are predominantly generated from newly synthesized proteins that are somehow defective in sequence or folding and rapidly degraded. In this model, well-folded, more stable proteins, which constitute the bulk of the proteome, are the source of relatively few, if any, MHC I-presented epitopes. This proposal contrasts with an older and presently disfavored model in which MHC I-presented peptides are generated from the turnover of all cellular proteins, whether defective or error-free. In this latter view, the majority of presented peptides derive from all of a cell's polypeptide constituents, including normal proteins. In this review, we discuss the strengths and weaknesses of the evidence for both models and on this basis, advance the view that the MHC class I pathway monitors all cellular proteins.

The origins of presented peptides

With the introduction of proteasome inhibitors [1,19–21], it became clear that the ubiquitin proteasome system was the predominant source of MHC class I-presented peptides. Although a fraction of MHC class I bound peptides are also derived from membrane or secreted proteins during processing of signal peptides [22]. It was originally assumed that the majority of presented peptides were generated by proteasomes during the normal turnover of the whole proteome (Fig 2). However, this idea was challenged because many viral antigens are degraded slowly, if at all [18]. It was therefore reasoned that it would take a long time for newly infected cells to generate, from such stable viral proteins, sufficient amounts of antigenic peptides to be recognized by $CD8^+$T cells, and thus, it seemed impossible for the immune system to detect infected cells sufficiently early to eliminate infections. However, $CD8^+$T cells can recognize presented peptides derived from stable viral proteins soon after cells are infected. Therefore, it was postulated [18] that there must be a mechanism to allow a portion of otherwise stable proteins to be rapidly degraded and presented.

A fundamental role of the ubiquitin-proteasome system (UPS) is to rapidly eliminate misfolded proteins arising from mutations, errors in transcription or translation, excess components of multimeric complexes, failures in folding, and postsynthetic damage [4,23–25]. On this basis, it was postulated that the rapid presentation of such viral antigens could arise if appreciable errors were made during their synthesis and during synthesis of cellular proteins generally [18] (Fig 2). These defective molecules could arise by mutations or misincorporation of amino acids, premature termination of translation, and/or protein misfolding [4,23–25]. Because such proteins are rapidly degraded by the UPS, peptides would be presented from newly synthesized polypeptides, regardless of the stability of the mature protein. These error-containing proteins were named Defective Ribosomal Products (DRiPs). Since the initial proposal, the definition of what constitutes DRiPs has evolved, but their key features are that they are newly synthesized, have never achieved their mature,

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functional conformations and are much more rapidly degraded than the bulk of cell proteins, i.e. with half-lives of <10 minutes [26]. Some investigators have also viewed DRiPs as damaged mature proteins that may result, e.g. from exposure to "free radicals" [27,28]; however this usage seems inappropriate since the biosynthesis and maturation of such proteins has been successfully completed.

While a DRiP mechanism might allow rapid sampling of many of a cell's newly synthesized proteins, this sampling would not be uniform for all antigens. The actual quantity of defective molecules is still largely uncertain and likely to vary widely between proteins depending on their structures [Box 1]. Furthermore, while misfolded proteins are generally degraded rapidly, many mutant or truncated proteins are not [29] and would not generate DRiPs. Therefore, the proportion of DRiPs produced and their potential contribution to antigen presentation should vary from protein-to-protein. In addition, the rather surprising prompt generation of epitopes from stable viral proteins, which the DRiP hypothesis aims to explain, might arise simply because a fraction of all normal proteins are in fact generated immediately after synthesis following first order rate kinetics [Box 2].

DRiP proponents have argued that the production of a small number of peptides would be insufficient to allow recognition of antigen-presenting cells, because they would be outcompeted by the large number of peptides generated from cellular proteins [30]. However, there is no evidence that such competition actually limits antigen presentation under physiological conditions. On the contrary, there is strong evidence that the generation of peptides by proteasomes is rate-limiting [31]. Unfortunately, the actual efficiency of epitope generation by proteasomes, transport into the ER, and processing have been examined quantitatively in only a very few instances and may vary widely between antigens [32]. Moreover, very few (1–100) peptides-MHC I complexes are needed to stimulate $CD8^+$T effector cells [33]. So, the small numbers of peptides generated from "stable" viral proteins early after infection could be sufficient for immune responses. In addition, the efficiency of presentation must depend on how many precursor peptides are generated by proteasomes; i.e. by how many protein molecules are being degraded at any instant. At steady state, this rate is proportional to the protein's abundance and inversely related to the protein's stability. In general, most abundant cell proteins tend to be long-lived (i.e. with half-lives of more than six hours). However, because of their greater abundance, more of these stable molecules may be degraded per minute than of short-lived proteins, which tend to be rather rare regulatory proteins.

Clearly, the degradation of misfolded polypeptides (i.e. DRiPs) or excess free subunits of complexes or viruses, which tend to be loosely folded and unstable in the absence of other subunits, will generate presented peptides, as is evident from experiments expressing mutant or truncated proteins [19,25,29,34]. However, the fundamental questions are: 1) how quantitatively important is this source of presented peptides in overall antigen presentation; and 2) whether there are specialized mechanisms to preferentially present peptides from the "DRiPome", as postulated [26,35] (Fig 2). These issues need to be addressed experimentally, and the limited data on these points are discussed below.

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Evidence for the existence of DRiPs and their abundance

Do DRiPs, as hypothesized, actually exist? Although the error rates in transcription and translation appear quite low, a number of early biochemical studies on mammalian cells, and even bacteria concluded that a large fraction (6–35%) of newly synthesized proteins are short-lived and degraded to amino acids in 30–60 minutes [23,36,37]. While these numbers may be taken as evidence for DRiPs, such a rapidly turning-over pool will also include signal peptides released and degraded during synthesis of membrane and extracellular proteins, as well as mitochondrial components. Also, this fraction will include many short-lived regulatory proteins, whose rapid elimination is critical for the regulation of transcription, metabolism, and growth. For example, the protein HIF-1α normally is degraded with a half-life of 10 minutes [38], and many key proteins have half-lives of less than 20–30 minutes (e.g. ornithine decarboxylase, p53, Myc, etc.) [3,24], and thus, would resemble DRiPs in such experiments.

The fraction of newly synthesized proteins that are short-lived was reexamined by briefly incubating cells with a radioactive amino acid, and then quantifying the increase in labeled protein in cells when the degradation of rapidly degraded proteins was blocked with proteasome inhibitors [37]. Proteasome inhibition caused about a 30% increase in labeled proteins, which was interpreted to come from DRiPs that were protected from degradation [37]. This result was taken as evidence that DRiPs are very abundant. However, a subsequent study [39] argued that much of this increase in labeled proteins was a n artifact of the amino acid starvation (generally used by investigators to enhance ^{35}S-methionine incorporation) and also from inhibition of proteasomal function prior to the pulse label, both of which alter the intracellular specific activity of the radioactive precursor pool. This is because amino acid starvation stimulates proteasomes to degrade unlabeled proteins and therefore in the control cells but not ones treated with proteasome inhibitors, unlabeled amino acids dilute the radioactive precursor. In other words, newly synthesized proteins in proteasome-treated cells are being labeled at higher specific activity.

Correcting for this artifact, the actual amounts of rapidly-degraded proteins were much lower, and as mentioned above, many of these components are short-lived regulatory proteins. Therefore, the actual failure rate in the expression and folding of proteins remains controversial and may be relatively low. In fact, one of the limitations in evaluating the contribution of DRiPs to antigen presentation is that data quantifying the amount of DRiPs generated during the synthesis exists for only one *bona fide* antigen [40]. Another factor that can influence the fate of abnormal proteins are cellular quality control mechanisms, some of which are associated with ribosomes [41] that can rescue some misfolded proteins (multiple molecular chaperones) and/or target them for degradation (ubiquitin ligases) [Box 3]. Nevertheless, whether there are many DRiPs generated or only a few, the key question is how much do they contribute to antigen presentation.

The contribution of newly synthesized proteins to antigen presentation

Most evidence suggesting that DRiPs are the dominant source of MHC class I-presented peptides comes from experiments that observed a rapid reduction in the generation of new

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peptide-MHC complexes when de novo protein synthesis was inhibited, even though the cells still contained a substantial pool of previously synthesized mature antigen [26,37,40,42–45]. If mature proteins were a significant source of the presented peptides, then presentation would have been expected to continue after synthesis ceased, but, if DRiPs were the primary source, then they would have been rapidly depleted when synthesis stops. Thus these findings were interpreted to indicate that DRiPs were the predominant source of MHC class I presented peptides.

However, this argument is not as straightforward as it might initially appear. For example, it assumes that all mature proteins are accessible to the MHC class I pathway. However, if an antigen is degraded by autophagy or targeted to membranes and lysosomes, then it would escape proteasomal degradation and fail to be presented on MHC class I molecules. In addition, a number of factors cause a fraction of normal proteins to be degraded early after synthesis [Box 2].

If the blockage of antigen presentation upon inhibition of protein synthesis is in fact due to stopping the formation of DRiPs, it would indicate that peptides produced from DRiPs are somehow segregated from those produced from mature proteins [30], even though both are generated by proteasomes (Fig 2). Those peptides produced from DRiPs would be supplied to MHC class I molecules, while those produced from mature proteins would somehow be excluded from antigen presentation (Fig 2). This model would require the existence of some as yet undiscovered mechanisms for segregating and sampling different pools of peptides in the cytosol. This idea has even led to speculation about the existence of specialized ribosomes ("immunoribosomes") [46] and novel subcellular compartments [35], for which there is as of yet no evidence but would be quite important if true (Fig 2). Also, this idea of segregated compartments does not fit with the well-established observation that peptides which are injected into the cytosol [1,19,47] or synthesized there from minigenes are presented very efficiently [17,48,49]. Such cytosolic peptides are in the same subcellular compartment as those generated when proteasomes digest mature proteins. On the other hand, it is possible that the presentation of mature epitopes introduced into the cytosol somehow uses different mechanisms from the peptides generated by proteasomes [50]. It therefore is very important to evaluate whether protein synthesis inhibition is truly blocking antigen presentation by eliminating the generation of DRiPs.

Most experiments aimed at establishing DRiPs as the source of antigenic peptides have utilized general inhibitors of protein synthesis such as cycloheximide to terminate antigen synthesis [37,42–44]. These agents also terminate the production of components of the antigen presentation pathway and have the potential to indirectly affect many cell processes that are regulated by short-lived proteins, whose concentrations fall rapidly upon inhibition of protein synthesis. In these experiments, it was argued that besides blocking antigen synthesis, these treatments did not affect other steps in antigen presentation, because under some conditions [26], new peptide-MHC complexes could appear on the cell surface after synthesis was terminated, (albeit in lower amounts than in control cells). More recent experiments, however, have directly assessed the effects of protein synthesis inhibitors and revealed that such agents do indeed block MHC class I presentation from preexisting cellular proteins [51,52], or from exogenously loaded ones [52–54]. Because these agents

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clearly block steps in the MHC class I pathway, beyond antigen synthesis, these kinds of experiments are problematic and cannot be used as evidence for a dominant role of newly synthesized proteins and thus, DRiPs.

Similar experiments have been reported in which tetracycline-regulated expression systems were used to selectively terminate antigen synthesis and thus to avoid the potential artifacts from blocking synthesis generally. In these experiments, terminating the synthesis of several EBV antigens (including EBV nuclear antigens), and the LCMV nucleoprotein, resulted in a partial or complete loss of antigen presentation, even when the cells contained pools of mature protein [40,55–57]. While these results appear consistent with a major contribution from newly synthesized proteins, there are some caveats. Specifically, these studies did not examine whether the particular antigens are still degraded by proteasomes and thus can be substrates for MHC class I presentation. In fact, EBV proteins have been reported to be degraded by a ubiquitin-independent proteasomal process [58,59] and EBV nuclear antigens by autophagy [60]. Therefore, the implications of these experiments for understanding the origin of the class I-presented peptides are uncertain. Thus, experiments in which synthesis of the antigen is inhibited selectively or globally do not yet make a compelling case for a dominant role of newly synthesized proteins or DRiPs in antigen presentation.

SILAC (non-radioactive isotope) Mass Spectrometry pulse-chase methodology has been used to examine the length of time after synthesis that labeled peptides can continue to be generated and presented [61] (Fig. 3A). At various intervals after the pulse, peptides were eluted from secreted MHC class I molecules and analyzed for the presence of label by Mass Spectrometry. Some peptides appeared in a biphasic pattern, and the early wave of presented epitopes was interpreted as coming from DRiPs. However, less than 15% of antigens displayed this biphasic pattern, and interestingly, most of these precursor proteins were components of multimeric complexes. Therefore, this early phase of presentation most likely reflects the breakdown of these proteins before they were fully assembled into the mature complexes [62]. The selective degradation of excess subunits of multi-molecular complexes or of partially formed complexes has been observed for many complexes, even though the components were not inherently defective, e.g. the CD3 complex [63]. In fact, such larger structures are generally more stable thermodynamically than individual subunits or partially assembled complexes. However, the actual times r equired to assemble such stable multimeric complexes and the efficiency of this process in cells are unknown.

Most importantly, for all 51 peptides examined, the labeled species continued to be incorporated into MHC class I complexes for 6–96 hours following synthesis. Although it was not pointed out in that study, this time far exceeds the time needed for generating and transporting peptide-MHC complexes. Therefore, these results actually demonstrate that antigenic peptides are generated from cellular proteins for many hours (or days) after synthesis. In other words, these presented peptides can all come from a protein source other than DRiPs (Fig 3A) (although the relative importance of the mature and newly synthesized species remains uncertain).

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The presentation of peptides from newly synthesized functional mature proteins

Several experiments have now established that fully functional proteins, which by definition are not DRiPs, can be a source of presented peptides. In recent studies, proper synthesis and folding of endogenous antigenic proteins was confirmed based on their achieving catalytic activity [64] (Fig 3B). These constructs were made with inteins, which, only when properly folded, perform a self-cleavage and internal ligation reaction. Epitopes were split between two ends of the splice sites so that they would be generated only when the proteins folded properly and became catalytically active. These epitopes, which therefore could not come from DRiPs, were subsequently digested by proteasomes and presented on MHC class I molecules. Most, importantly, they were presented as efficiently as the same epitopes from the same proteins, in which there were no inteins and therefore could come from DRiPs. Thus, class I peptides were generated primarily, perhaps exclusively, from the mature protein.

A related set of experiments further established that MHC class I peptides can be generated from proteins that were properly folded, as shown by their ability to form a ligand-binding conformation [52] (Fig 3C). These studies took advantage of a mutant form of FKBP that was stabilized when it bound its ligand [65]. This stabilization inhibited its degradation and the presentation of an FKBP epitope [44]. In recent studies, epitope presentation from this construct and a distinct one was inhibited upon ligand binding by 80–95% [52]. These large reductions in presentation upon substrate stabilization indicate that most of the presented peptides are coming from the functional proteins capable of ligand binding. On the other hand, a similar study with an EBNA1 protein came to the opposite conclusion (i.e. that ligand binding did not inhibit presentation) [55].

However, this study did not investigate how the EBV protein was degraded and as noted above, EBNA1 may be degraded by autophagy rather than by proteasomes. These various experiments demonstrate that fully mature functional proteins, including ones with long half-lives, can certainly be the dominant source of presented peptides. Whether this is true for the bulk of antigenic peptides remains to be seen.

The presentation of peptides from “old” proteins

It has been suggested that MHC class I molecules are somehow preferentially present peptides from newly synthesized proteins [18] (Fig 2). This idea, which is inherent in the DRiPs hypothesis, was reinforced by the probably misleading finding that inhibiting protein synthesis blocked antigen presentation [26,37,42,44]. Also, this idea is at variance with early findings on the presentation of mature antigenic proteins that were microinjected into cells and had half-lives ranging between several to many hours [66,67]. In one such experiment, the injected protein was affinity purified on a substrate column and thus was clearly folded and functional [66]. In each example, MHC class I bound peptides were presented from these long-lived functional proteins, although shorter-lived proteins were presented more rapidly after microinjection.

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Similarly, using the ligand stabilized FKBP system described above, it has been possible to build up a cohort of "old" functional proteins that had been synthesized in the cell and then determine if they could be presented, when the stabilizing ligand was removed, and the "old" molecules were degraded. This approach further extends the time of the degradation process from the time of synthesis. The initial experiments using this approach suggested that peptides from old proteins were presented on class I molecules about 35-fold less efficiently than from newly synthesized antigens [44]. However, subsequent experiments by this same group [45] and others [52] found that the old proteins were equally, if not more efficiently, presented than newly synthesized antigens. Therefore, these data do not support models where there is a special linkage between antigen synthesis and antigen presentation. Instead, it appears likely that antigen production simply reflects the flux of the protein through the proteasome, which in turn depends on the structure and abundance of the mature protein, and is inversely related to its degradation rate.

Concluding remarks

The proposal that MHC class I molecules preferentially present peptides that come from errors in protein biosynthesis or failures of successful folding (DRiPs), is an attractive idea, and one that has influenced general conceptions of how the immune system monitors for nonnative gene products. However, on close examination, the data that actually support this proposal are still quite limited, often indirect, and based on questionable assumptions. Certainly, recently synthesized proteins tend to be degraded preferentially because of signal peptide destruction, the difficulties in successfully folding large multidomain proteins and assembling multimeric complexes and of exponential decay kinetics of most proteins. Before the evolution of the immune system, protein degradation by the UPS served as an efficient mechanism to eliminate proteins failing to fold correctly, and this mechanism must still be an important source of MHC class I presented peptides. Additionally, recent data indicate that for many antigens, a majority of the MHC class I-presented peptides probably originates from functional mature molecules, and not defective, newly synthesized proteins. The immune system thus appears to monitor the peptides that are generated through proteasomal breakdown of both short-lived and stable cellular proteins. However, further research is necessary to quantify the relative importance of the newly synthesized and mature proteins in generating the bulk of MHC class I-presented peptides and whether DRiP-like mechanisms may function in certain cases, e.g. oxidative stress [27,68,69] to enhance the efficiency of this critical process.

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Highlights

- Class I presented peptides may derive from normal functional protein and DRiPs
- The favored model posits that most peptides were derived from DRiPs
- Definitive evidence supporting the dominance of DRiPs is still lacking
- New evidence suggests that mature proteins are an efficient source of peptides.
- Recent data questions the primacy of the DRiPs mechanism in immune surveillance

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Text Box 1: The proportion of DRiPs produced should vary from protein-to-protein

The success rate for folding of most proteins is not known [57]. The folding of small (single domain) polypeptides is typically very rapid and efficient (i.e. the failure rate is quite low). In contrast, for some proteins, especially large molecules, achieving proper folding involves sampling many possible conformations and achieving the final active conformation requires the involvement of multiple molecular chaperones [57,70]. Consequently, the efficiency of folding is low for some multi-domain proteins, multi-typic membrane proteins, proteins requiring post-synthetic modifications, and large multisubunit complexes. For example, most CFTR molecules (the polytypic membrane chloride channel) normally never fold properly and are rapidly degraded by the UPS [71]. It is unclear to what extent such failures and proteolysis occur during initial folding on the ribosome or subsequently, when the released polypeptide tries to assume its active quaternary structure, binds cofactors and other subunits, or assembles into large complexes. Because cell proteins differ widely in structure, their proper folding and assembly into stable complexes must involve very different kinetic and thermodynamic challenges [57,70]. Consequently, the frequency of biosynthetic failures or DRiPs is likely to differ widely between for diverse proteins.

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Text Box 2. Protein degradation early after synthesis

The degradation of cell proteins follows exponential decay kinetics (first-order), so that, twice as many molecules are degraded during the first half-life as in the second half-life, etc. [23]. Thus, independent of DRiPs, even well formed long-lived proteins are more likely to be degraded and function in antigen presentation soon after synthesis. Moreover, in yeast, and presumably higher eukaryotes, there is a window of time early after their synthesis, when normally long-lived proteins are much more susceptible to damage that triggers degradation than at later times when the proteins are successfully incorporated into multimeric complexes [62,70]. In addition, the early detection of viral infections may be due to the degradation and presentation from cellular antigen pools before the proteins (e.g. nucleocapsids components) are incorporated into the mature viral particles, which probably resist proteasomal degradation. After viral infection, measurements of protein stability are complex and potentially misleading, since the viral proteins are almost certainly found in multiple forms with distinct half-lives – i.e. viral constituents and their precursors. Thus, attributing presentation from newly synthesized proteins to DRiPs may, for many proteins, be an unnecessary hypothesis.

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Text Box 3: Quality control and protein degradation

Cells contain multiple systems for ensuring quality control of proteins present in, the cytosol, nucleus, endoplasmic reticulum, and mitochondria [4,7]. In addition to many molecular chaperones that promote folding of newly synthesized polypeptides and the refolding of damaged or spontaneously denatured mature proteins [70], there are multiple ubiquitination enzymes that recognize and selectively ubiquitinate proteins misfolded in the cytosol and the endoplasmic reticulum (ERAD pathway) [3,72–76]. In the best-characterized example, the ubiquitin ligase, CHIP, targets for destruction cytosolic proteins that have the chaperones Hsp70 or Hsp90 bound to them and thus, reflect failures in protein folding or refolding [72]. Several ubiquitin ligases are bound to the ribosome, where folding begins, and misfolding is initially monitored [75,77]. One-ribosome-associated ubiquitin ligase selectively eliminates abnormal gene products that lack stop-codons and contain C-terminal extensions [75]. Most of these quality control mechanisms have been described in yeast [73–75] and their mammalian counterparts are largely uncharacterized, but clearly, they evolved long before the class I antigen presentation pathway. To what extent these systems contribute to the elimination of the hypothesized DRiPs and antigen presentation has not been studied.

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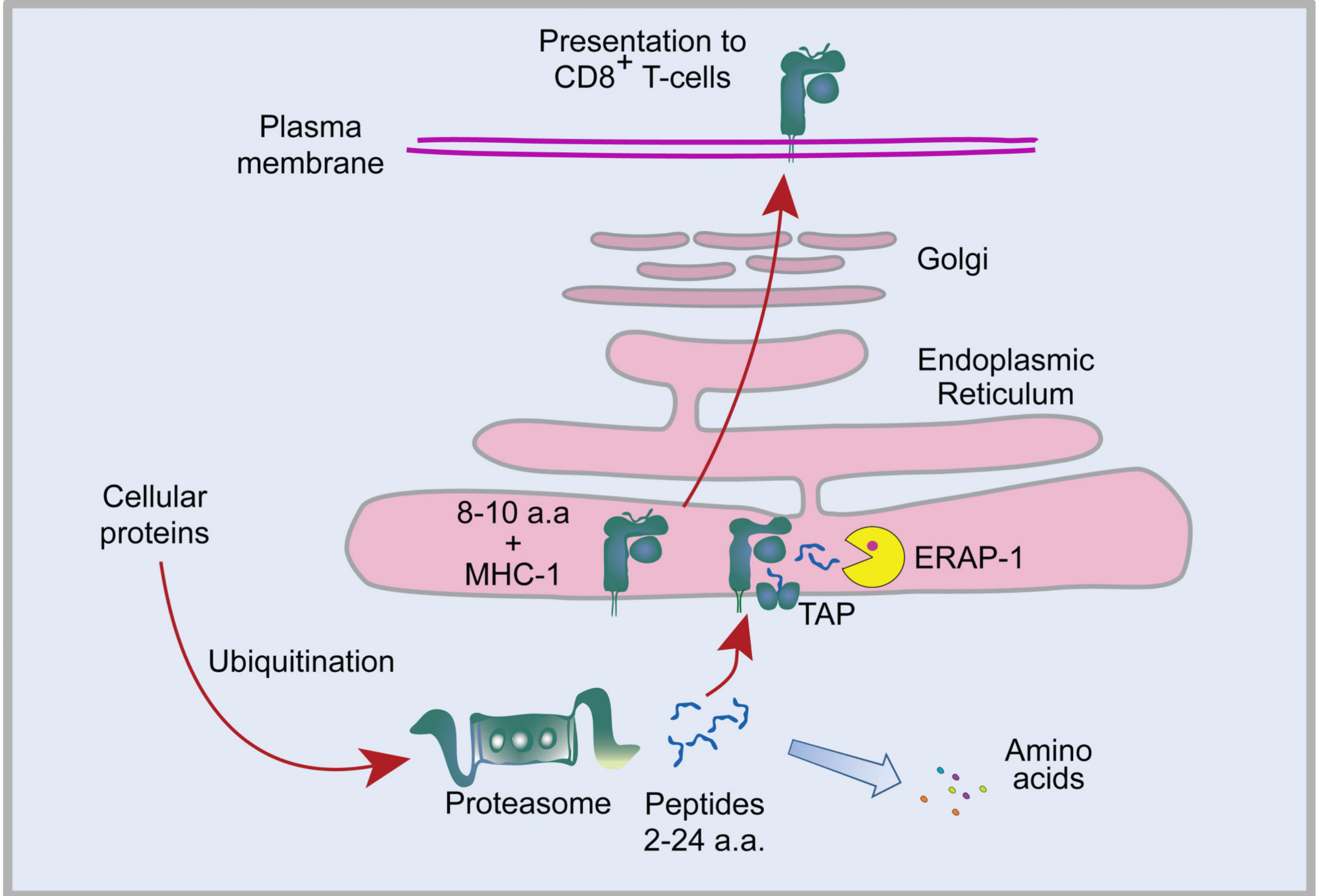

Figure 1. MHC class I antigen presentation pathway

Cellular proteins are degraded into oligopeptides by the ubiquitin-proteasome pathway. Most of these peptides are further degraded by peptidases into amino acids, but a small fraction are transferred into the lumen of the ER by the TAP-transporter, where they can be trimmed further into 8–10 amino acid fragments by the endoplasmic reticulum aminopeptidase-1 (ERAP-1). These peptides can then be bound by newly synthesized MHC class I and transported to the plasma membrane for display to CD8+ T cells.

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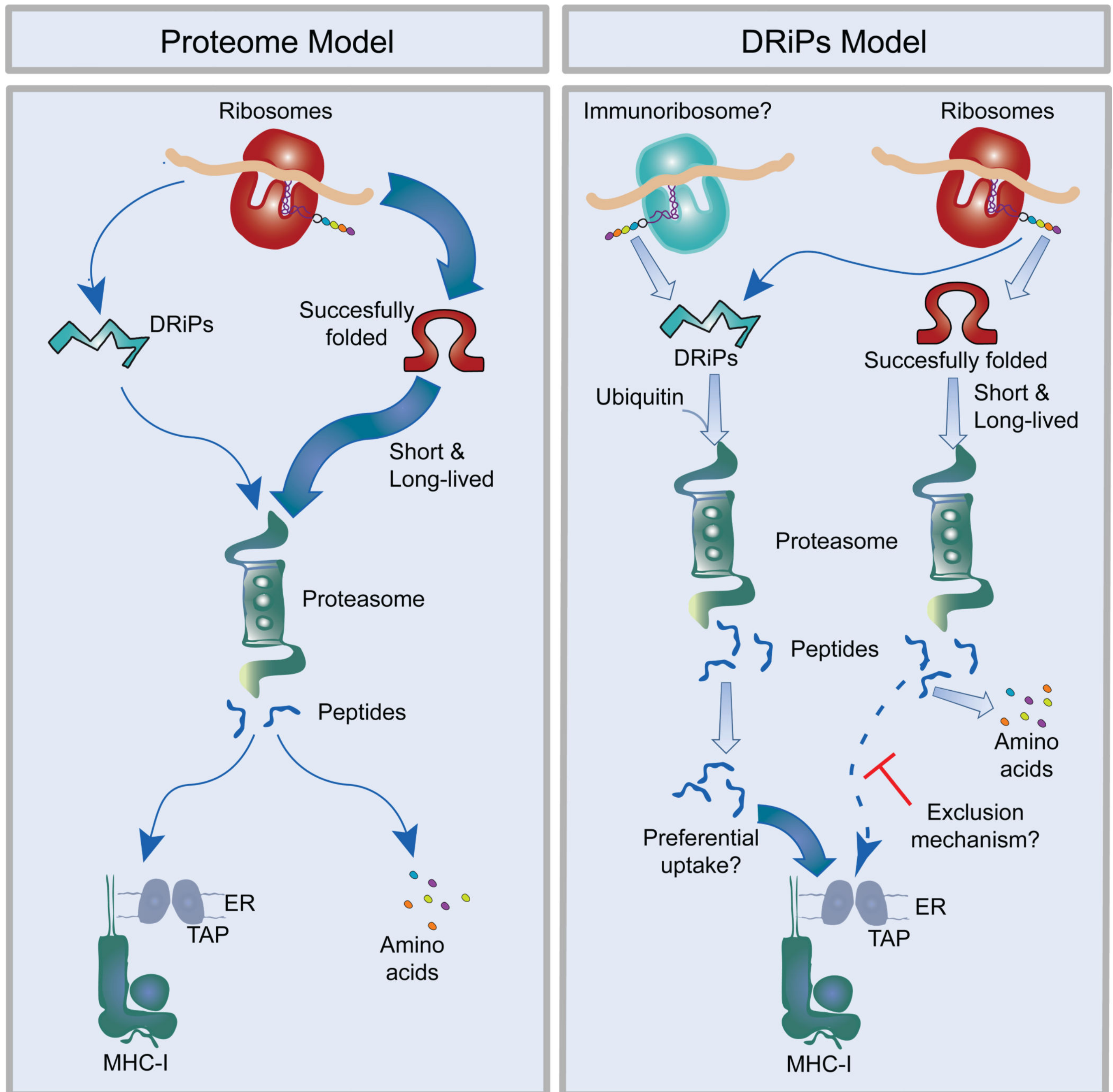

Figure 2. Models for the source of MHC class I peptides

Proteome Model-Proteins are synthesized by ribosomes and subsequently fold into their mature conformation. The degradation of folded or unfolded proteins begins immediately after synthesis, following first order rate kinetics. Most of these cellular proteins are degraded by the ubiquitin-proteasome pathway into oligopeptides. A fraction of these peptides escape further hydrolysis into amino acids when they are transported into the ER. This process provides the MHC class I antigen presentation pathway with a sampling of

peptides from all cellular proteins, including mature folded proteins and defective ones (the proteome).

DRiPs Model- Ribosomes, or the hypothetical error-prone immunoribosomes, synthesizes a cohort of functional and defective proteins. The defective proteins (DRiPs) are ubiquitinated and rapidly degraded by the proteasome. By an unknown mechanism (possibly preferential uptake into the ER and/or exclusion of peptides derived from mature proteins) the DRiP-derived peptides may be preferentially loaded on MHC class I molecules. Consequently, newly synthesized defective proteins constitute the dominant source of presented peptides, either at early time points (e.g. post viral infection) and/or at all time points [35].

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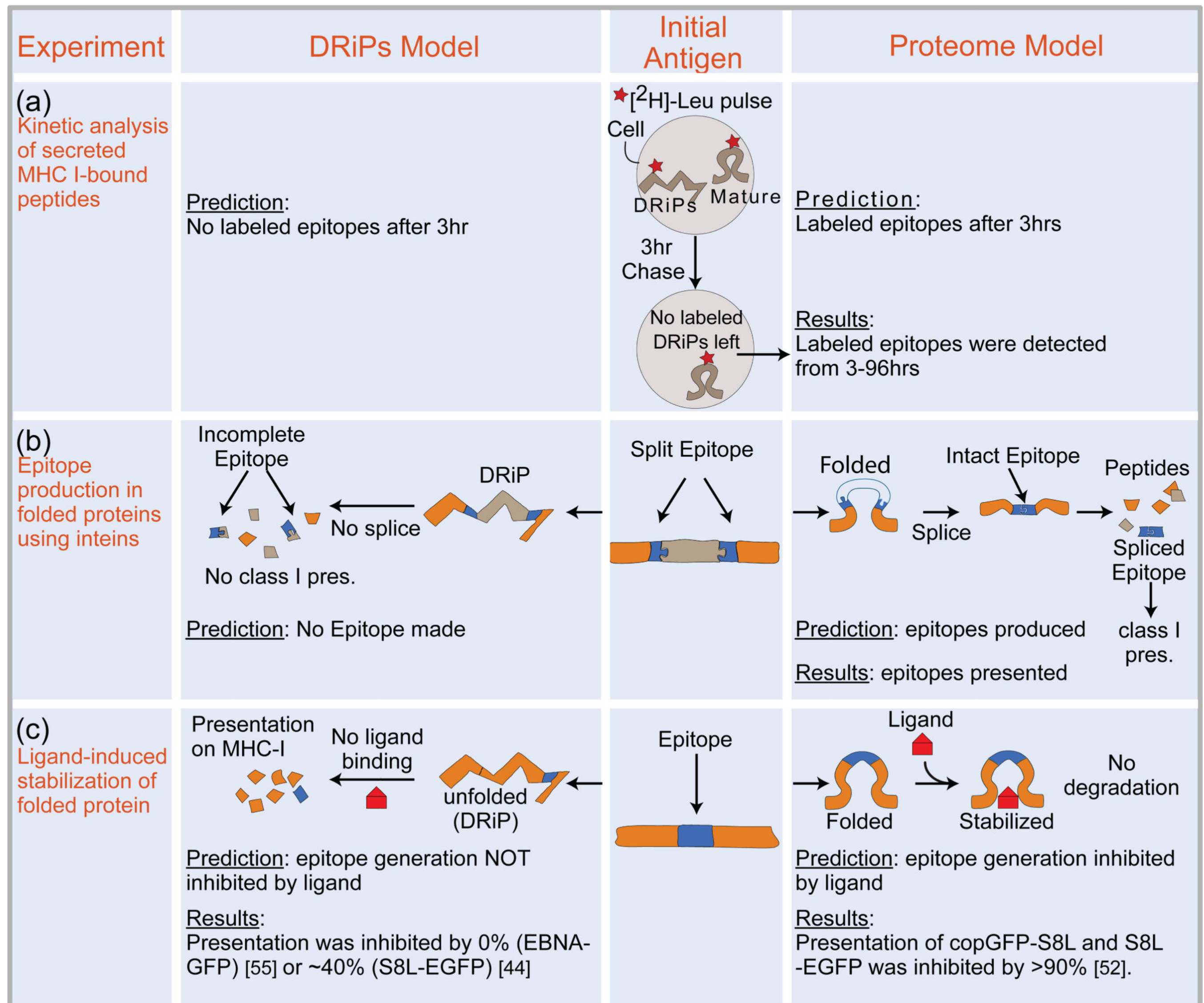

Figure 3. Experimental evidence supporting the DRiPs Model and the Proteome Model

(a) Cells were labeled for SILAC using heavy leucine (red star) for 7 days. Cells were then transferred to light leucine and secreted MHC:peptide complexes were collected every 3hrs for up to 96 hours. Peptides bound to the soluble MHC class I complexes were eluted and characterized by Mass Spectrometry. Since the labeled DRiPs would be very rapidly degraded, the DRiPs model predicts that peptides containing the heavy isotope would only be detected at early time points; in contrast the proteome model predicts that labeled peptides would continue to be generated over time. The actual data showed that peptides containing heavy leucine continued to be detected for 6 hours (with a half life of the label 3hrs), consistent with many presented peptides coming from the proteome [61] (b) Proteins were expressed with T cell epitopes (blue) split by an intervening sequence (inteins) so that the intact epitope would only be generated post-translationally from mature protein by intein catalysis and protein ligation. Since the epitope would not exist in an unfolded protein, the DRiP model predicted that no presented epitopes would be generated. The actual data

showed the epitopes were presented with the same efficiency as ones that did not require splicing demonstrating that mature proteins were the dominant source of the presented peptides [64] (c) Antigens were expressed as protein fusions to a destabilizing domain causing the protein to rapidly degrade (left panel). Addition of a ligand (red) specific for the folded form of the protein prevented its turnover (right panel). In two studies, presentation of SIINFEKL fused to EGFP (S8L-EGFP) and the EBV protein EBNA1 fused to EGFP (EBNA-EGFP) were inhibited by 40% and 0%, respectively, suggesting that a majority of presented peptides came from unfolded protein species [44,55]. However, a later study using one of the same antigens (S8L-EGFP) and a new antigen (copepod GFP fused to SIINFEKL, copGFP-S8L), showed near complete inhibition (80–95%) of presentation in the presence of stabilizing ligand, indicating that the presented peptides came from mature protein [52].

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