OPINION

Rethinking peptide supply to MHC class I molecules

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Abstract | The notion that peptides bound to MHC class I molecules are derived mainly from newly synthesized proteins that are defective, and are therefore targeted for immediate degradation, has gained wide acceptance. This model, still entirely hypothetical, has strong intuitive appeal and is consistent with some experimental results, but it is strained by other findings, as well as by established and emerging concepts in protein quality control. While not discounting defectiveness as a driving force for the processing of some proteins, we propose that MHC-class-I-restricted epitopes are derived mainly from nascent proteins that are accessed by the degradation machinery prior to any assessment of fitness, and we outline one way in which this could be accomplished.

CD8$^+$ T cells prevent the spread of intracellular pathogens through the recognition of pathogen-derived peptides (epitopes), generally 8–10 amino acids in length, that are produced by intracellular proteolysis and are displayed at the surface of the infected cell in combination with MHC class I molecules[1,2]. In addition, CD8$^+$ T cells, with their combination of sensitivity, specificity and lethality, may be the best hope for immune-based cancer therapy[3]. A great deal has been learned about how peptides that bind to MHC class I molecules are generated, but many important issues remain unresolved. One of these is the driving force for peptide production. In this Opinion article, we explore this topic, focusing on the more established direct presentation pathway in which antigen production, 'processing' and presentation occur within the same cell. This appears to be mechanistically distinct from the less well understood cross-presentation pathway in which antigen in an undefined form is transferred to another cell for processing and presentation[4,5]. We focus mainly on the processing of cytosolic proteins, as these have been used in most relevant studies.

The DRiP model of peptide supply

Most proteins, including those that contain MHC-class-I-restricted epitopes, are turned over very slowly with half-lives of many hours if not days. As pointed out by Yewdell *et al.* in 1996, this rate is inconsistent with *in vitro* assays showing that cells become recognizable by CD8$^+$ T cells soon after they are infected[6]. Indeed, accounting for the time needed for viral proteins to be expressed within the cell, for antigen to be processed and for peptide–MHC class I complexes to be transported from the endoplasmic reticulum (the site of peptide loading) to the cell surface, peptide production must commence very shortly after protein synthesis. Rapid peptide display would appear to be crucial, as some viruses can complete their replication cycles in a matter of hours and killing the cell after that period would serve little purpose. Accordingly, Yewdell *et al.* proposed that immediate peptide supply is driven not by senescence of mature proteins but by errors in protein production that result in some copies of any protein being defective from the outset (FIG. 1). These misfits, which they termed defective ribosomal products (DRiPs), are flagged by the quality-control machinery and are rapidly degraded[6], a scheme that was supported by several earlier findings.

First, early studies of mutant proteins that cause disease (haemoglobin variants being a primary example) suggested a fate of immediate degradation for mutant proteins[7–10]. These results were complemented by other studies showing that protein species formed in the presence of compounds that induce truncation or severe misfolding also seemed to disappear soon after synthesis[11–13]. Second, under conditions of brief metabolic labelling, Wheatley and colleagues observed that a substantial fraction of newly synthesized proteins is rapidly turned over — at an astonishing 40% per hour[14]. Two decades later, using an inhibitor of the proteasome (see Glossary), Schubert *et al.* came up with a similar figure (~30% per hour)[15]. So, it appeared that a large number of errors during protein production were creating defective proteins that shared the same fate as the mutant haemoglobins. This calculation showing that ~30% of newly synthesized proteins are immediately degraded has recently been challenged on the basis of the apparent effects of protein-synthesis inhibition[16] and amino-acid starvation[17] on proteasomal activity, and both challenges have been rebutted[18]. Wherever the truth lies, a high error rate of protein production is not crucial to validate the DRiP model. CD8$^+$ T cells are extremely sensitive[19] and even the peptides derived from the 'few percent' immediate turnover rate that was calculated by Vabulas and Hartl[17] would be more than sufficient for robust activation of CD8$^+$ T cells. Finally, in 1988, Townsend *et al.* reported that increasing the degradation rate of an antigen results in a substantially higher level of epitope production[20]. Destabilization was achieved by following the N-end rule, which states that certain amino acids at the amino terminus of a protein provide a signal for immediate degradation[21] (BOX 1). In contrast to this overt degradation signal, the accessibility of other degrons is regulated, which provides a means of rapidly eliminating a specific activity.

This indirect support for the DRiP model was subsequently bolstered by experiments that tested a key prediction of the model: interruption of protein synthesis should

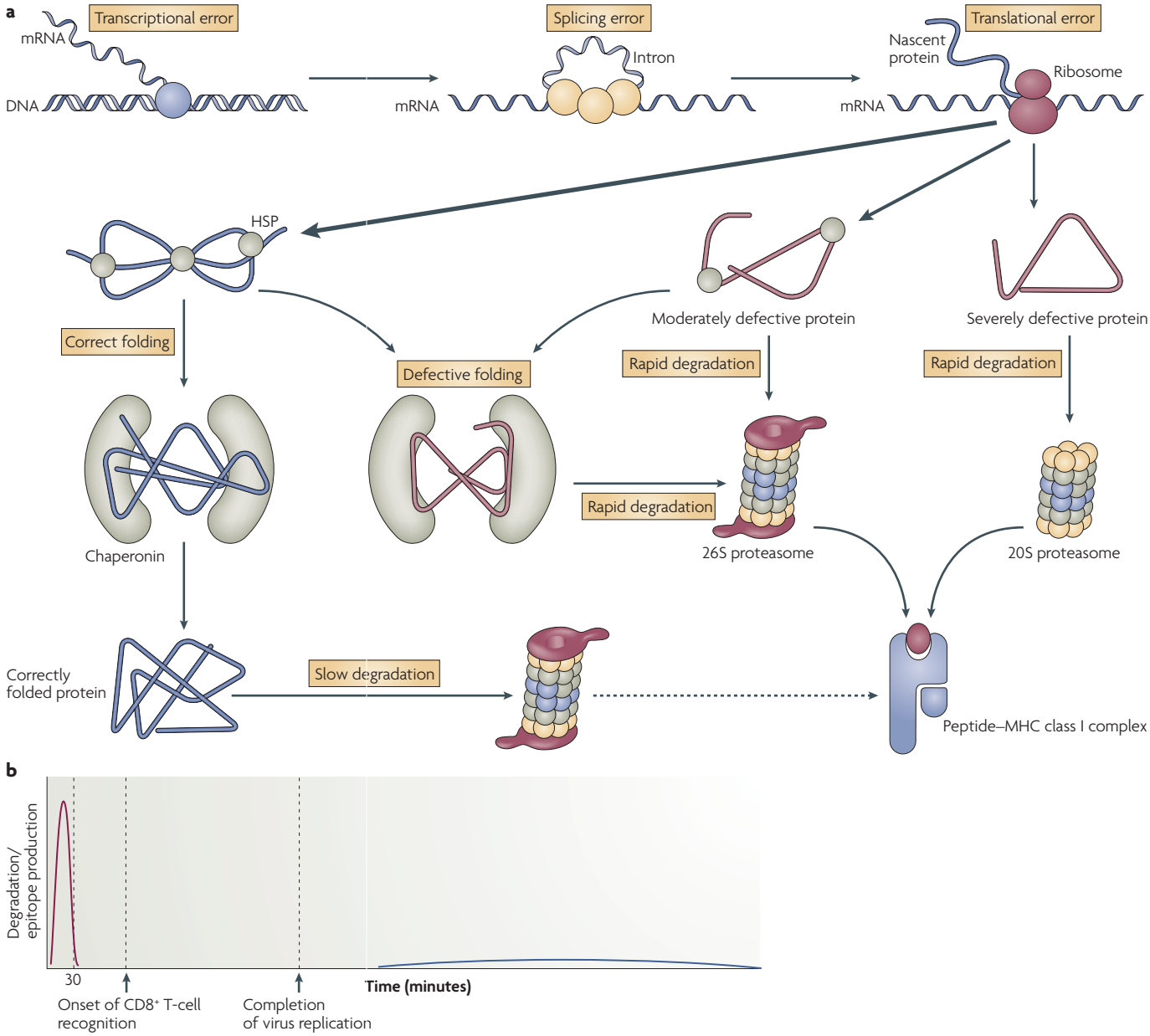

Figure 1 | **The DRiP (defective ribosomal product) model of peptide supply. a** | Accumulated errors during protein production (transcription, splicing, translation and folding) can lead to defective proteins that are instantly recognized by the quality-control machinery of the cell and targeted for rapid degradation, with some products ultimately becoming MHC-class-I-bound epitopes. Arrows of different thicknesses indicate unequal distribution, but they are not to scale as relative proportions are still unclear. The same applies to transcriptional, splicing and translational errors, the rates of which are much lower than implied. In this updated version of the model, misfolded proteins are subdivided into severely misfolded proteins that cannot be engaged by the folding machinery and are consequently degraded by the 20S proteasome, and moderately misfolded proteins that are engaged by the quality-control machinery but are also rapidly degraded by the 26S proteasome. **b** | Epitope recognition cannot be based on the turnover of mature proteins alone because this is generally much slower than the observed time that it takes for infected cells to be recognizable by antigen-specific $CD8^+$ T cells and the replication time of many viruses. Indeed, experiments with protein synthesis inhibitors indicate that epitopes are mainly, if not exclusively, produced from proteins that are degraded within 30 minutes of their production.

cause a rapid and substantial decrease in peptide supply, as mature protein turnover is too slow to make a meaningful contribution to the peptide pool. The initial experiments assessed epitope production through maturation of MHC class I molecules[15,22] or, more directly, by transport of peptide to the endoplasmic reticulum through the transporter of antigenic peptide (TAP) heterodimer[23]. By these measures, all detectable peptide production ceases after 30 minutes of exposure to an inhibitor of protein synthesis. Recent work with an inducible antigen expression system led to the same conclusion; direct processing is focused mainly, if not exclusively, on recently synthesized proteins[5].

Why the DRiP model seems problematic

A window of 30 minutes for peptide production implies a very short half-life of 10 minutes or less for the substrates from which the peptides are derived. More recent analyses have raised the estimation to a half-life of ~15 minutes for some proteins[24], but this is still quite rapid. For reference, the

degradation of N-end rule substrates can commence even before translation has been completed[25] (probably because the degron is N-terminally positioned and disordered) and this half-life, which would seem to approach a theoretical limit, has been measured at ~10 minutes[24]. That defective proteins would be disposed of just as quickly is not in line with current concepts of protein production and quality control.

Intracellular protein concentrations have been estimated at 300–400 mg per ml[26]. Despite the innate capacity for many proteins to carry out self-directed folding[27], the chances for inappropriate and incapacitating associations within the cell are exceedingly high. So, in real life, nascent proteins are intercepted by complex cellular machinery that directs folding in an insulated environment[28–32]. This machinery also provides quality control; those proteins that do not assume proper conformation are turned over to the degradation machinery for recycling and also to prevent the accumulation of misfolded, aggregated proteins, which can be lethal[33,34]. Initial stages of protein folding, which consist of shielding and compacting hydrophobic domains, are generally carried out by members of the heat-shock protein (HSP) family. The binding specificities of the HSPs are not well understood but the available evidence shows them to be broad and overlapping, which is consistent with their need to handle a tremendously heterogeneous population of primary sequences[30,35]. Partially compacted proteins are then transferred to the chaperonins, which shepherd the substrate through the final stages of condensation.

Rapid interception of nascent polypeptides is clearly important, as indicated by the positioning of specialized HSPs at the exit channel of the ribosome. In *Escherichia coli*, this function seems to be fulfilled by a single protein known as trigger factor (Tig)[36], whereas in yeast and higher eukaryotes, it is carried out by two different complexes: the heterodimeric nascent polypeptide-associated complex (NAC) and the HSP70-associated ribosome-associated complex (RAC)[37–39].

Several observations support the idea that quality-control decisions are not made hastily. First, many misfolded proteins can be rescued by prolonged interaction with HSPs[31,40], an opportunity that would be missed by an immediate decision to degrade. Second, a protein need not be perfect to be judged acceptable. Many mutant proteins do not display optimal activity but pass quality-control checkpoints despite the possibility of a reduced lifespan[41–44]. This flexibility makes some intuitive sense, as, in many cases, partial function is far better than no function. We have observed this flexibility at first hand. Several years ago, as part of our attempt to understand the enigmatic presentation behaviour of an epitope in influenza-virus nucleoprotein, we mutated the protein in ways that were expected to alter folding, anticipating that this would induce rapid degradation and enhance epitope production[45]. Although folding was indeed altered, the half-life of the nucleoprotein mutants was not substantially changed and peptide supply was not significantly different. Only when we applied the N-end rule was peptide supply markedly increased. We are not alone in this observation. Gileadi *et al.* carried out comparable manipulations of influenza-virus matrix proteins and similarly observed marginal changes in peptide supply[46]. By contrast, alteration of the sequence of HIV Gag did result in a rapidly degraded protein (half-life of 20 minutes) and in increased peptide supply. However, many variations were apparently tested before an effective one was identified that resulted in rapid degradation of the protein. This involved shuffling one-quarter length segments of the protein[47], a manipulation that would seem beyond the capacity of the cell. So, it is not so easy to produce a protein that the cell finds wholly and immediately unacceptable. Finally, there is an ever growing list of proteins that are described as 'natively' or 'intrinsically' unfolded[48]. Such proteins seem to exist in a completely or partially unfolded state until they are able to interact with a binding partner. Rapid quality-control decisions would seem to preclude the existence of such proteins.

So, are any naturally or experimentally defective proteins degraded with a half-life of 10–15 minutes? Inspection of the recent literature for mutant proteins that are misfolded (TABLE 1) suggests that, at best, such cases are rare. Some mutants of the cystic-fibrosis transmembrane receptor (CFTR) are degraded within an hour[49–51], but, as is shown in TABLE 1, this seems to be exceptional. Earlier studies, such as those with mutant haemoglobins, did show very short half-lives (10 minutes, for example), but this is attributable to experimental technique; only detergent-soluble cellular fractions were analysed. It was eventually appreciated that the abnormal haemoglobin subunits become detergent insoluble before their more protracted destruction[52], and this is now known to be the case for most if not all other misfolded proteins that are destined for destruction. First interpreted as mere aggregation, this transition now seems to reflect entry into a sophisticated subcellular domain that contains all of the components that are necessary to reconcile cases of misfolding: HSPs and chaperonins, which can attempt to refold the protein while simultaneously making the quality-control assessment, and proteasomes, should the substrate ultimately fail. When proteasome inhibitors are provided or when proteins that are predisposed to misfolding are overexpressed, this domain becomes overloaded, leading to formation of the 'aggresome', a perinuclear structure consisting of, at least in part, the aforementioned components[53]. It is important to note that increased degradation is not always the fate of misfolded proteins. They can also enter aggregates that resolve very slowly or not at all, becoming candidates for ubiquitin-mediated autophagy and not proteasomal degradation[54].

Box 1 | **The N-end rule**

The gene that encodes ubiquitin, the 76-amino-acid protein that tags proteins for destruction by the 26S proteasome, encodes four back-to-back copies of ubiquitin that are then separated into single copies by ubiquitin hydrolase. During their dissection of the ubiquitylation pathway using different fusion proteins, Varshavsky and colleagues discovered that the amino acid that immediately follows a ubiquitin moiety, which becomes the amino terminus of the protein once ubiquitin is removed, strongly influences the stability of the protein[80]. This association was formalized as the N-end rule[21]. As ubiquitin hydrolase is only specific for the sequence within the carboxyl terminus of ubiquitin, it is a straightforward procedure to control the half-life of a protein by substituting the amino acid immediately following the ubiquitin moiety.

Seizing on the N-end rule, Townsend *et al.* made ubiquitin–influenza-virus-nucleoprotein fusion constructs in which the N-terminal amino acid was engineered to be a methionine (that is, stabilizing the protein) or an arginine (that is, destabilizing the protein)[20]. They observed that proteins generated by the arginine-containing construct were much more rapidly degraded and that the number of presented peptides from the arginine-containing nucleoprotein was substantially higher than the number of presented peptides derived from the methionine-containing nucleoprotein. The N-end rule has been subsequently exploited by other laboratories using other antigens with a similar outcome in almost all cases.

Table 1 | **A sample of recently described naturally mutant proteins and their kinetic fates**

Protein	Cellular location	Disease	Mutation	Effect on degradation	Refs
Protein S	Secreted	Protein S deficiency	Tyr595Cys	2 hour lag before the mutant protein is degraded in the 6 following hours	82
PMA1	Plasma membrane	Experimental mutation	Ala165Gly and Val197Ile (temperature-sensitive mutant)	Reaches the cell surface and is degraded in vacuoles	83
SMAD4	Cytosol and nucleus	Experimental mutation	Premature termination	Lifespan of ~4 hours	84
EB1	Cytosol	Experimental mutation	Truncation (amino-terminal 100 amino acids)	Stably aggregated in the aggresome	85
Transthyretin	Secreted	Central nervous system amyloidosis	Asp18Gly	74% of transthyretin is degraded after 6 hours	86
Superoxide dismutase	Cytosol	Familial amyotrophic lateral sclerosis	various	Half-life ≈ 5 to >24 hours	87
Antithrombin	Secreted	Antithrombin I deficiency	ΔMet103	Half-life ≈ 6 hours	88
CFTR	Plasma membrane	Cystic fibrosis	Leu346Pro	Most copies are degraded within 1 hour	51
Kidney anion exchanger	Plasma membrane	Distal renal tubular acidosis	Ser773Pro	Half-life ≈ 5–6 hours	89
DJ-1	Cytosol and nucleus	Familial Parkinson's disease	Leu166Pro	Half-life ≈ 1 hour	90

CFTR, cystic fibrosis transmembrane conductance regulator; EB1, end-binding protein 1; PMA1, plasma membrane H^+ ATPase 1; SMAD4, mothers against decapentaplegic homologue 4.

Because a DRiP has not yet been identified[22], N-end-rule substrates have served as surrogate DRiPs in some studies of rapid peptide supply[24,55]. Given that the degradation rates for most defective proteins fall outside the observed peptide supply rates[15,23], the strategy seems problematic. If defectiveness and quality-control decisions drive acute peptide supply, something beyond the conventional framework is required for the model to hold. Several recent and innovative refinements to the DRiP model address this quandary.

Refinement of the DRiP model

Once a protein does fail quality control, according to the standard model, it is targeted to the 26S (capped) proteasome following ubiquitylation. There are now numerous exceptions to this scenario, beginning with the substitution of ubiquitin with other targeting molecules such as antizyme[56,57]. An even more radical departure from this convention is the targeted delivery of substrates to the 20S (non-capped) proteasome[58,59]. This scheme is not so surprising considering that the archaebacterial proteasome functions without extensive cap structures and more than half of the proteasome population in at least some higher eukaryotic cells is in the 20S form[60]. More striking is the recent description of self-targeted (non-tagged) protein delivery to the 20S proteasome[61,62]. The basis for this self-targeting seems to be the HSP-like properties of the 20S proteasome, which has been reported to prevent the aggregation of heat-denatured proteins[63]. However, 20S core particles seem incapable of refolding proteins, which suggests that this interaction only prefaces degradation[64].

These evolving concepts in quality control and degradation, together with a series of recent studies have motivated several updates to the original DRiP model. First is the proposition that there are different degrees of defectiveness[55]. Using a combination of established and new reagents, Qian *et al.* concluded that N-end-rule substrate degradation is ubiquitin dependent for only ~75% of the molecules, with the other 25% being degraded by the 20S proteasome in a process that is unaffected by HSC70, an important constitutive HSP that is distinct from HSP70 and that interacts with nascent proteins[30]. It was, therefore, proposed that moderately defective proteins, represented by the 75% cohort, are degraded by the 26S proteasome in a ubiquitin-dependent manner. Members of the remaining 25% were proposed to be so severely defective that interaction with the folding machinery is compromised, which would result in targeting to the 20S proteasome. Of particular note, acute epitope production from a stable protein, which is arguably a more representative processing substrate, was observed to be entirely ubiquitin independent. We have made similar observations by overexpressing a dominant-interfering ubiquitin mutant; presentation of a stable cytosolic protein is ubiquitin independent, whereas presentation of an N-end rule substrate is substantially ubiquitin dependent (L.H., unpublished observations). In the context of the updated DRiP model, ubiqitin-independent presentation of stable 'wild-type' antigens combined with a window of 30 minutes for processing suggests that moderately misfolded (26S degraded) species are relatively rare. However, another possibility is that such species are common but not efficiently processed.

The notion that misfolded species are common but not processed has been supported by studies that compared the efficiency with which an epitope is generated from an N-end-rule substrate or from a substrate with a slower rate of turnover (half-life of 4 hours), the former being superior[65]. This, in turn, has inspired the concept of an 'immunoribosome' that directs nascent proteins to the 20S proteasome and TAP, perhaps by direct linkage[65]. Furthermore, it has been proposed that this ribosome population may preferentially bind nascent messenger RNA (mRNA) that has not yet undergone nonsense-mediated decay (a translation-coupled process by which mRNAs that contain premature termination codons are eliminated) making them more prone to producing defective (prematurely truncated) proteins than conventional ribosomes[18].

A proposed alternative

The intuitive appeal of the DRiP model is obvious, and it is highly probable that some epitopes are naturally produced, at least partially, as a result of defectiveness. We are nevertheless motivated to propose an

alternative for the following reasons. First, we are sceptical that a protein could be so severely defective as to confound the folding machinery, considering the generic- and linear-binding specificities of HSPs[30,35,66], the myriad normal sequences that are successfully intercepted, the difficulty we and others have experienced in engineering a rapidly degraded defective protein, and the existence of natively unfolded proteins. Furthermore, the available information on mutant proteins suggests that defectiveness follows a continuum that contrasts the sharp 30 minute demarcation defined by the experiments with protein synthesis inhibitors. Second, given the current view of protein folding, it is difficult to envisage the equivalent degradation, at least kinetically, of a protein that undergoes a quality-control decision and an N-end-rule substrate. Again, very few, if any, natural or synthetic proteins are degraded this quickly on the basis of defectiveness rather than on possession of a degron. It follows that epitopes would be derived from a relatively small set of proteins and such a bias has not been observed. Rather, the collective experience is that most proteins of sufficient size contain at least one MHC-class-I-restricted epitope. Fourth, we felt challenged by Occam's Razor (that is, the simplest solution is generally the right one) to identify a scheme that might not depend on so many novel concepts (severe defectiveness, immunoribosomes and DRiP production coupled to nonsense-mediated mRNA decay). Finally, as a DRiP has not yet been identified[22] nor produced, despite the existence of the model for over a decade[18] and some effort in this direction, alternatives seem worth considering.

We begin with the proposition that selection for immediate degradation is stochastic with no consideration for the potential to achieve an acceptable conformation. A model that we have devised to explain how this might be possible is outlined in FIG. 2. In a way, it also invokes the notion of a specialized ribosome. As already stated, the general release of unfolded nascent proteins to the open environment of the cell would be catastrophic and ribosome-associated HSPs (NAC and RAC) have a key role in preventing this from happening. In *E. coli*, in which this system has been most thoroughly investigated, association with trigger factor is known to be transient[36,37]. This is likely because trigger factor dissociates from the exit channel along with the domain it has just compacted[36], and this leaves the ribosome temporarily unoccupied. At steady state, it has been estimated that 10% of ribosomes are in the unoccupied state[67]. As trigger factor can substitute for RAC in yeast[68], we speculate that this fundamental theme persists in eukaryotic cells with the consequence that some nascent proteins will emerge from the ribosome unattended, a potentially catastrophic condition. How could such species be negated? The 20S proteasome, with its generic HSP-like α rings, and already implicated in acute epitope generation by two lines of investigation (see above), would appear to be ideally suited to act as a safety net, absorbing and degrading these 'escapees' and thereby preventing them from wreaking havoc. This may be one reason for the high cellular levels of the 20S proteasome. This scenario would also explain why levels of HSC70, so far not implicated in ribosome-associated folding[39], were not observed to influence immediate, 20S-mediated degradation[55].

"We begin with the proposition that selection for immediate degradation is stochastic with no consideration for the potential to achieve an acceptable conformation."

In our scheme, if anything were to be labelled defective, it would be the folding machinery. However, a certain amount of stochastic turnover of nascent protein may not be accidental. In an attempt to rationalize a perceived nascent protein turnover rate of 40% per hour[14], Wheatley proposed that such a system of basal proteolysis "ensures a flux of proteins through the cytoplasm upon which the cell draws for use for its maintenance or growth. At the same time, it optimizes the adaptability and responsiveness of the cell to its environment."[69] Although the degree of turnover may be lower than originally estimated by Wheatley *et al.* and subsequently by Schubert *et al.*[15,17,18], some amount of constitutive turnover might still be crucial for rapid responsiveness. Under more extreme conditions, such as nutrient deprivation, a more aggressive turnover programme would be required[17]. An intriguing notion is that, in this scheme, the 20S proteasome is more than just a safety net and instead precludes HSP association in some way, thereby actively establishing the level of basal proteolysis.

For any antigen, this basal level of peptide presentation from immediately degraded substrate will be supplemented by processing of the cohort of newly synthesized proteins that is successfully intercepted by the folding machinery. The rate and peak levels of epitope produced by this second pathway will depend on inherent stability (FIG. 2B). If the protein carries an overt degron (for example, an N-end-rule substrate), then there is substantial, essentially immediate supplementation by ubiquitin-dependent, 26S-proteasome-mediated degradation, which can commence even before translation is complete[25]. This, we suggest, explains the 75% to 25% split deduced by Qian *et al.*[55]. If the protein carries a cryptic degron, then a burst of epitope will be produced at the time the degron is revealed (for example, phosphorylation that triggers cyclin degradation). In addition to targeted degradation, there is an entire range of defectiveness, from profound to absent; the more defective the protein, the sooner and more intense the presentation of the peptide, which is due to more rapid rejection by the quality-control machinery. Shuffled Gag (with a half-life of 20 minutes) is one example[47], for which basal supply of the mutant protein is amply supplemented. There is also

Glossary

Chaperones
Proteins that assist in protein folding. Both heat-shock proteins and chaperonins are chaperones.

Chaperonins
A set of proteins, all characterized by a double-stacked ring structure, that actively assist in the later stages of protein folding.

Degrons
Signals within proteins that target them for rapid degradation. Degrons can be overt, as in the case of the N-end rule, or covert, as in the case of cyclins. For example, cyclin B must be rapidly destroyed following mitosis, and this is achieved by kinase-regulated access to a 'destruction box' sequence in cyclin B that stimulates polyubiquitylation and subsequent degradation by the proteasome[81].

Heat-shock proteins
A diverse set of proteins, many of which have key roles in the early stages of protein folding, unfolding and refolding. They are so named because their expression increases following stresses, such as excessive heat, that cause widespread protein denaturation and the need for rapid repair and replacement.

Proteasome
A giant multicatalytic protease resident to the cytosol and the nucleus. The 20S core, which contains three distinct catalytic subunits, can be appended at either end by a 19S cap or an 11S cap. The binding of two 19S caps to the 20S core forms the 26S proteasome, which degrades polyubiquitylated proteins. In addition to having a crucial role in protein turnover, the proteasome is thought to carry out the first catalytic step in the MHC-class-I-restricted processing of most, if not all, antigens.

a modified variant of influenza-virus nucleoprotein into which a modified segment of the JAK1 (Janus kinase 1) kinase motif was embedded (half-life of 70 minutes) that resulted in increased presentation[70].

By contrast, proteins synthesized in the presence of canavanine, an arginine analogue that causes misfolding (half-life of 4 hours)[65], and the influenza-virus nucleoprotein mutants generated in our laboratory (with a half-life of several hours), none being associated with enhanced presentation, are closer to the other end of the spectrum. For these and even longer-lived wild-type proteins, it may be that the only source of T-cell-stimulating

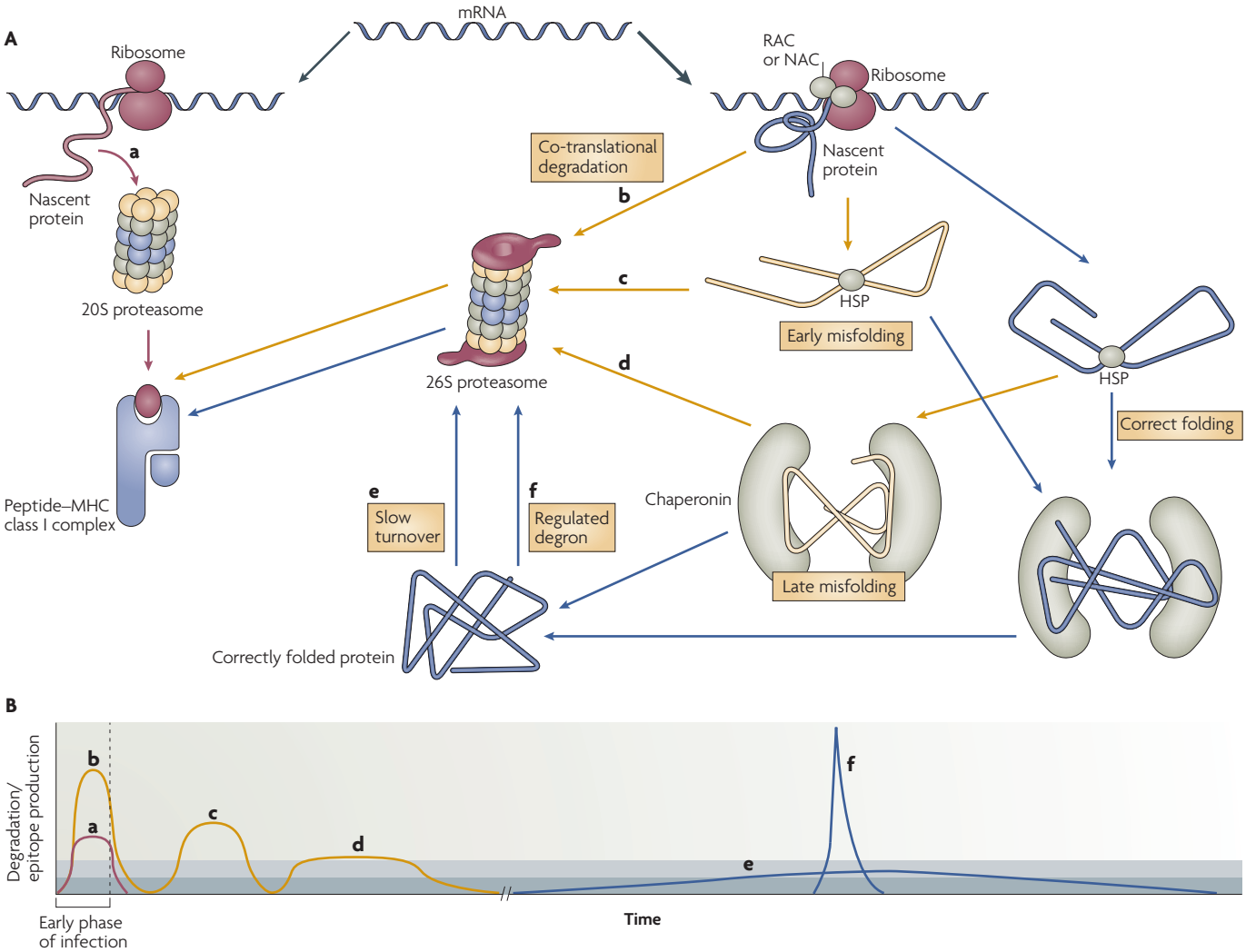

Figure 2 | **An alternative to the DRiP (defective ribosomal products) model. A** | Most messenger RNA (mRNA) molecules are translated by ribosomes with associated heat-shock proteins (HSPs; such as HSP70-associated ribosome-associated complex (RAC) and nascent polypeptide-associated complex (NAC)), and this results in partial compaction of the nascent polypeptide, and its delivery to downstream chaperones. We propose that a small fraction is translated by unengaged ribosomes and that these unfolded protein species are immediately intercepted and degraded by the 20S proteasome before the undue consequences of aggregation can take effect (**a**). This results in an immediate burst of epitope production (part **B**, red line) soon after translation has started. In the case of HSP-associated translation, nascent proteins are subsequently engaged by downstream HSPs and chaperonins until the mature folded state is achieved, or the protein is deemed defective and targeted for degradation. If a protein carries an overt degron, such as an arginine at the amino-terminus, then degradation by the 26S proteasome commences immediately (**b**). If the protein carries a covert degron, then it is rapidly degraded at a specific time after having achieved the fully mature state (**f**). In either case, targeted degradation results in kinetics that are similar to those associated with 20S-proteasome-mediated processing. Proteins are identified as defective at different steps towards maturation (**c** and **d**). The earlier the detection of defectiveness, the more focused the period of degradation and the higher the peak epitope levels (part **B**). Note that experiments with inhibitors of protein synthesis (defining a 30 minute window of epitope production) suggest that these kinds of protein species (**c** and **d**) are relatively rare under normal conditions or less efficiently processed. Mature, wild-type proteins are turned over slowly (**e**), resulting in protracted, low-level epitope production. **B** | As an example, the kinetics of antigen processing of a viral protein produced during the early phase of infection are shown. In all cases, a fraction is rapidly degraded by the 20S proteasome, which is due to unattended translation (red line). The manner in which this initial burst of catalysis is supplemented by 26S-proteasome-mediated destruction depends on the stability of the cohort that is successfully intercepted by the folding machinery. The horizontal shaded bands represent hypothetical thresholds for biochemical detection (monoclonal-antibody staining and TAP mobility) of epitope (light grey) versus a hypothetical threshold for T-cell activation (dark grey). So epitope production that is undetectable by the *in vitro* methods may still be of significance *in vivo*. All lines are speculative and, therefore, not to scale.

epitope is derived from the nascent phase, with turnover of mature protein being too protracted to sustain threshold levels of epitope at the cell surface. It must be kept in mind, however, that the 30 minute processing window was defined with assays that are not as sensitive as T-cell activation and so it remains possible that the slow turnover of more stable proteins may be meaningful to the immune system (FIG. 2B).

Two corollaries of this model seem worth pointing out. First, because proteins are randomly selected for degradation, there is no bias towards epitopes derived from proteins that tend to misfold, thereby ensuring the broad array of targets that is crucial for optimal immune responses. Second, during an acute viral infection, the folding machinery will probably be overwhelmed by the high rate of protein synthesis, which would result in more 20S-proteasome-mediated degradation and lead to an increased level of presentation. In addition, the 20S proteasome might be a more efficient antigen-processing machine than the 26S proteasome (due, for example, to a more direct connection with TAP), and this would provide an alternative to the immunoribosome[65] as an explanation for the more efficient presentation of nascent protein.

A potential sticking point for both models is the processing of non-cytosolic proteins, mainly untethered and membrane-bound glycoproteins, which are well represented in the range of peptides bound to MHC class I molecules[71]. According to convention, proteins with signal sequences are efficiently translocated to the endoplasmic reticulum[72,73] and those that fail quality control are ejected back into the cytosol for ubiquitin-dependent degradation by the 26S proteasome[74]. By this scheme, there would be no opportunity for instant degradation, as the protein materializes in a proteasome-free compartment. However, the recent report of Oyadomari *et al.* provides a possible solution[75]. Proteins that are inefficiently translocated owing to limited engagement by chaperonins in the lumen of the endoplasmic reticulum are intercepted on the cytosolic side by the chaperonin 58 kDa interferon-induced, protein-kinase-interacting protein (p58IPK), which leads to proteasomal targeting. During times of high glycoprotein production, as would occur during an acute viral infection, luminal chaperonins are overtaxed and the degradation pathway operates at high levels — a scheme that is similar to the one we have proposed for cytosolic proteins.

Concluding remarks

The DRiP model promotes defectiveness as the driving force for direct peptide presentation, but the rapidity with which peptides are produced excludes most defective proteins. Corollaries have proposed several mechanisms for generating and/or selecting a subset of defective proteins that is rapidly and efficiently channelled into the MHC class I processing pathway. We propose an alternative model in which a subset of nascent polypeptides is stochastically delivered to the 20S proteasome owing to neglect by the folding machinery. We stress that the two models are not mutually exclusive but we resist the idea that defectiveness drives much of the processing within the first 30 minutes of production. Our model is unconventional but there are many cases in which the study of antigen presentation has led to surprising discoveries in fundamental cell biology, proteasome-mediated splicing of eukaryotic proteins being one recent example[76,77]. The distinction between the DRiP model and stochastic selection, whatever the underlying mechanism, is not trivial. For instance, it affects options for escape of immune surveillance. Of note, reduced protein expression[78] and direct interference with proteasome function[79], perhaps the only viable options for reducing peptide supply in the context of a stochastic model, have both been described. Evolution towards minimal production of defective copies — admittedly, a difficult process to study — has not. From a vaccine development point of view, our model predicts that engineering strategies to increase $CD8^+$ T-cell responses to specific epitopes or proteins may be marginally effective if anything short of adding a degron is attempted. Time will tell where the truth lies but it seems clear that a deeper understanding of protein production and turnover will be needed to resolve the question of peptide supply.

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Competing interests statement

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