Non-conventional sources of peptides presented by MHC class I

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Received: 17 February 2011 / Revised: 17 February 2011 / Accepted: 18 February 2011 / Published online: 10 March 2011
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Abstract Effectiveness of immune surveillance of intracellular viruses and bacteria depends upon a functioning antigen presentation pathway that allows infected cells to reveal the presence of an intracellular pathogen. The antigen presentation pathway uses virtually all endogenous polypeptides as a source to produce antigenic peptides that are eventually chaperoned to the cell surface by MHC class I molecules. Intriguingly, MHC I molecules present peptides encoded not only in the primary open reading frames but also those encoded in alternate reading frames. Here, we review recent studies on the generation of cryptic pMHC I. We focus on the immunological significance of cryptic pMHC I, and the novel translational mechanisms that allow production of these antigenic peptides from unconventional sources.

Keywords Antigen presentation · Antigen processing · Cytotoxic T cells · Major histocompatibility complex · Protein synthesis

Introduction

Nearly all vertebrate cells express molecules of the major histocompatibility complex class I (MHC) on their surface. Each MHC I molecule contains a peptide derived from one of various endogenous proteins. Thus, the peptide repertoire presented by MHC I reflects the intracellular protein milieu and, in infected or transformed cells, includes additional proteins such as those derived from intracellular pathogens. Because the pMHC I serve as unique ligands for the CD8$^+$ cytotoxic T cell receptors, the generation of pMHC I by the antigen presentation pathway allows the CD8$^+$ T cells to detect and eventually eliminate infected or transformed cells.

The antigen presentation pathway begins in the cytoplasm (Fig. 1) [1, 2]. Most polypeptide precursors are fragmented by the proteasome into proteolytic intermediates in the cytoplasm. The intermediates are then transported into the endoplasmic reticulum (ER) by the TAP heterodimer. Once in the ER, the MHC I molecules in the peptide-loading complex (PLC) are loaded with appropriate peptides with the help of ER aminopeptidases and housekeeping chaperones. After being loaded with the appropriate peptide cargo, MHC I carry the bound peptides to the cell surface for presentation to CD8$^+$ T cells. Because neither the MHC I molecules nor the CD8$^+$ T cells discriminate among sources of precursor polypeptides, immune surveillance can use pMHC I that arise from normal as well as non-conventional translational mechanisms.

New protein synthesis supplies a significant portion of peptides presented on the cell surface as pMHC I [3, 4]. Indeed, it has been argued that a ribosome-based mechanism may have evolved to use a fraction of newly translated polypeptides exclusively as substrates for antigen processing and presentation [5]. In addition, evidence has accumulated showing that non-conventional translational mechanisms also serve as a unique source of peptides for presentation by MHC class I [1, 6, 7]. Here, we review recent immunological and biochemical studies of non-conventional sources of MHC I ligands that arise during unexpected ribosome decoding events on endogenous as well as viral mRNAs.
Immunological significance of cryptic translation

In the last few years, the identification of non-conventional epitopes arising from ARF translation on viral mRNAs has highlighted the functional significance of cryptic epitopes during immune surveillance (Table 1). In addition to murine AIDS which was found to elicit protective cytotoxic CD8+ T lymphocyte responses (CTLs) towards an ARF peptide encoded in the LP-BM5 gag gene [9, 10], simian immunodeficiency virus (SIV) mac239-infected rhesus macaques mounted a strong CTL response towards an epitope that was translated from the +2 reading frame relative to the env ORF [11]. Further, using HLA-B*07 transgenic mice, Cardinaud et al. [12] showed that CTLs target an HLA-B*07-restricted ARF epitope translated from the +2 ORF within the gag gene and that this ARF epitope was recognized by CTLs in HIV-infected individuals. More recently, Maness et al. [13] have determined that nearly one-quarter of the anti-SIV CD8+ T cell responses in SIV-infected rhesus macaques are directed towards cryptic epitopes generated from ARF translation.

To assess the global impact of these cryptic epitopes for both immune surveillance and viral evolution, several groups have used bioinformatic prediction methods to identify potential non-conventional epitopes in HIV-infected individuals [14–16]. Berger et al. analyzed HIV gag, pol, and nef sequences from a cohort of 765 individuals to identify HLA allele-associated viral polymorphisms. A total of 64 HLA-associated viral polymorphisms translated in the +2 or +3 reading frames were identified. One particular polymorphism showed that individuals expressing the HLA-A*03 allele were significantly less likely to have serine at position 241 in the +2 reading frame of integrase compared with individuals lacking this HLA type. Indeed, epitope mapping showed the optimal sequence to be RR9 (RTSKAśler) with serine at position 6 (underlined) which potently inhibited viral replication in vitro. As predicted from the identified polymorphism within this sequence, the most common escape variant RPR9 (RTSKAPLER) contained proline at position 6 (underlined). Not only was this sequence translated in an alternate reading frame, but no upstream in-frame AUG start codons were present. Instead, a codon normally encoding leucine was identified as a likely start site. Bansal et al. also determined HLA-associated polymorphisms in the six possible translational reading frames of HIV-1 gag, pol, and nef which include antisense transcripts. They found both sense- and antisense-encoded ARF epitopes were immunogenic during primary and chronic infections and that these ARF epitopes were often mutated during the first year of infection. This observation is consistent with an important role for ARF epitopes in immune responses that inhibit viral replication and can thus select for escape variants.

Naturally processed cryptic peptide ligands for T cells

Since the original observations of Boon and colleagues describing presentation of antigenic peptides referred to as “peptons” that seemed to arise from unexpected sources [8], the list of cryptic peptides recognized by T cells has grown considerably (reviewed in [6, 7]). The examples listed in Table 1 include peptides that arise from untranslated regions (UTRs or introns) of the mRNA—challenging the very definition of an open reading frame (ORF)—as well as peptides encoded in alternate translational reading frames (ARF) and from non-AUG start codon initiation on both endogenous and viral mRNAs. That cryptic pMHC I arise from endogenous as well as virally-encoded mRNAs suggests that immune surveillance has evolved to exploit highly conserved aspects of protein translation.

The examples listed in Table 1 suggest that while mammalian ribosomes must carefully select the correct start codon of the ORF for synthesis of functional proteins they also promiscuously translate other regions of the mRNA irrespective of the ORF location for MHC class I presentation. On one hand, it may be tempting to dismiss these translational events simply as mistakes without any physiological relevance. On the other hand, the fact that cryptic peptides effectively compete with the hundreds of thousands of other peptides presented by MHC class I on the cell surface and elicit T cell responses argues that cryptic peptides are immunologically relevant for immune surveillance.

Fig. 1 Schematic diagram of the MHC class I antigen processing pathway that culminates in display of peptide bound MHC I on the cell surface. Most antigenic peptides are derived from polypeptides synthesized in the cytoplasm by translation of open reading frames (ORF). However, many other peptides arise as defective ribosomal polypeptides (DRiPs) by translation of ORF or cryptic reading frames (RF). The polypeptides undergo proteolysis in the same compartment and are then transported into the endoplasmic reticulum (ER). In the ER, many peptides are further trimmed by aminopeptidases and assembled with the resident MHC molecules. The MHC I molecules then chaperone the peptides to the cell surface where they serve as potential ligands for the killer CD8+ T cell repertoire.
**Table 1** Examples of natural sources of cryptic pMHC I

| Cell type | Peptide source | References |
|-----------|----------------|------------|
| **Endogenous sources: non-coding mRNA** | | |
| BALB/c radiation-induced leukemia | 5' untranslated region of c-akt oncogene | [60] |
| Human melanoma | Region of the MUM-1 gene spanning intron–exon junction | [61] |
| Human melanoma | Intron of the N-acetyl glucosaminyltransferase V gene | [62] |
| Human melanoma, normal cultured melanocyte cell line | Intron of gp100 gene | [63] |
| Human melanoma | Intron of TRP-2 gene | [64] |
| Human renal cell carcinoma | 5'-UTR region of VEGF, likely from initiation at CUG, a non-AUG start codon | [25] |
| **Endogenous sources: alternate reading frame** | | |
| Human melanoma, normal cultured melanocyte cell line | Alternate ORF of gp75 | [65] |
| Human squamous cell carcinoma, lung adenocarcinoma | Alternative ORF of SARC-1 | [66] |
| Human melanoma, breast cancer cells | Alternate ORF of NY-ESO-1 | [67] |
| Human melanoma | Alternate ORF of CAMEL | [68] |
| Renal cell carcinoma | Alternate ORF of the intestinal carboxyl esterase gene, initiated at ACG | [69] |
| Human renal cell carcinoma, normal kidney and liver cells | Alternate ORF of the M-CSF gene | [70] |
| Human melanoma cell lines | Alternate RF of BING-4 | [31] |
| Human synovial fluid from a patient with Reiter’s syndrome | Internal coding sequence with +1 frameshift (ORF2) of IL-10 | [35] |
| Mouse melanoma cell lines | Alternate RF of human HER-2, human PAP, and mouse TERT | [32] |
| Human renal cell carcinoma | Alternate RF of the C19orf48 gene | [33] |
| Mouse liver cells transduced with AAV2 | Alternate RF of coagulation factor 1X (F9) | [18] |
| Human leukemia cells | Alternate RF of the abl gene | [71] |
| **Viral sources: alternate reading frame** | | |
| Murine cell line (L929) infected with vaccinia | Alternate ORF of mutant influenza NP. The authors found that out-of-frame epitopes were expressed even when the AUG for the primary ORF was in an excellent context for translation initiation. The level of expression of out-of-frame epitopes increased as the context of the primary AUG worsened | [72] |
| Cells from mouse infected with LP-BM5 MAIDS retroviral complex | Alternate ORF of MAIDS gag gene. Mice infected with MAIDS generated CTL against this epitope | [9] |
| Human cell line (T1-B7 and Jurkat) infected with HIV human PBMC | Alternative RF of HIV gag, pol and env genes | [12] |
| Human cell line (HEK293) | DNA vaccine with alternate RF of pol gene from HBV, Alternate RF of OVA | [73] |
| SIV-infected rhesus macaques | Alternate RF of SIV env gene | [11] |
| PBMC, monocyte-derived dendritic cells, and B cells from rhesus macaques | DNA vaccine/vector encoding a portion of the env ORF likely through non-AUG initiation | [17] |
| Human CD4+ T cells from chronically-infected individuals | Alternate RF of HIV-1 integrase gene likely through a non-AUG start codon | [15] |
| SIV-infected rhesus macaques | Alternate RF of SIV pol, tat/vpr, env, and env/Rev genes; some likely through non-AUG initiation | [74] |
| **Endogenous sources: non-AUG initiation** | | |
| Human B cell acute lymphoblastic leukemia, Epstein-Barr virus-transformed B cells | HB-1, initiated at CUG codon | [75] |
A different escape variant was recently identified by Cardinaud et al. who characterized a mutation within the HIV-1 gag ARF (QPRSDTHVF, Q9VF) (personal communication). They found that patients eliciting CTL responses against Q9VF did not contain provirus encoding this epitope, but rather Q9VF/N (QPRSNTHVF), a parental epitope with a D to N mutation. Apparently, this point mutation inhibits presentation of Q9VF/N by a proteasome degradation mechanism. This is an interesting example of how ARF peptides providing a cellular defense against infection allow viruses to evolve, within a short period of time, to subvert effectiveness of immune surveillance by CD8+ cytotoxic T cells.

These examples underscore the possibility that vaccine design could be enhanced by the inclusion of non-conventional epitopes, including ARF epitopes described from HIV-1. Indeed, Maness et al. [17] show that ARF epitopes in DNA vaccines elicit a much stronger response in rhesus macaques than normal infection. However, presentation of immunogenic peptides from ARFs can also have deleterious effects as shown by Samulski’s group, where a DNA cassette being used for human gene therapy trials generated unexpected ARF polypeptides [18]. The targeting of these pMHC I by cytotoxic T cells caused a loss of genetically modified cells and failure of their therapeutic potential. Certainly, it is desirable to understand the fundamental mechanisms which direct non-conventional translation on both endogenous and viral message in order to harness the utility and prevent unwanted effects of non-conventional epitopes.

### Mechanisms for producing cryptic peptides for presentation

Non-conventional epitopes are derived from polypeptide precursors that serve no obvious function in the cell but are nevertheless presented on MHC class I molecules. By definition, these precursors could be described as DRiPs (defective ribosomal products), destined for automatic entry into the MHC class I presentation pathway [19]. If such precursors were readily available for antigen presentation, they could serve to efficiently report the status of the cell (infected or transformed) to the immune system. This would be particularly beneficial during viral infection since many viral proteins have evolved to be extremely stable [half life \( t_{1/2} \approx 24 \text{ h} \)] and recalcitrant to proteasomal degradation [e.g., EBNA1 from Epstein-Barr virus (EBV) and LANA1 from Kaposi’s sarcoma-associated herpesvirus (KHSV)] [20–23]. The different translational mechanisms for producing cryptic polypeptide precursors could circumvent the delay in presentation of stable viral proteins through protein turnover. Indeed, Cardinaud et al. show that truncated antigenic precursors are generated when ribosomes terminate translation of EBNA1 prematurely [24]. Other mechanisms include translation from non-coding regions and alternate reading frames of the mRNA as well as from non-AUG start codons within any region of the mRNA. The flexibility to use a variety of translation mechanisms might relieve the bottlenecks of conventional translation such as when translation initiation factors are limiting or even missing (Fig. 2).

### Translation of “non-coding” mRNA

The recent discovery of an HLA-B*2705 bound peptide translated from the 5'-UTR of VEGF, likely through initiation at a CUG start codon, suggests cryptic translation products are novel targets for tumor therapy [25]. This peptide is abundantly presented by HLA-B*2705 molecules in tumor tissues as a result of VEGF mRNA overexpression, and draws attention to the existence of tumor antigens within non-coding regions of the mRNA.

| Cell type                        | Peptide source                                                                 | References |
|----------------------------------|-------------------------------------------------------------------------------|------------|
| Human renal cell carcinoma       | 5'-UTR region of VEGF, likely from initiation at CUG, a non-AUG start codon   | [25]       |
| Renal cell carcinoma             | Alternate ORF of the intestinal carboxyl esterase gene, initiated at ACG       | [69]       |
| **Viral sources: non-AUG initiation** | DNA vaccine/vector encoding a portion of the env ORF likely from non-AUG initiation | [17]       |
| PBMC, monocyte-derived dendritic cells, and B cells from rhesus macaques | Alternate RF of HIV-1 integrase gene likely from non-AUG start codon           | [15]       |
| Human CD4+ T cells from chronically-infected individuals | Alternate RF of SIV pol, tat/vpr, env, and env/Rev genes; some likely from non-AUG initiation | [74]       |
| SIV-infected rhesus macaques     |                                                                                |            |

Table 1 continued
Translation of “untranslated” regions indicates that ribosomes are capable of initiating translation upstream or downstream of the normal ORF start codon (including introns from unspliced mRNA). These findings challenge the model of translation where ribosomes scan for and initiate only at the first AUG start codon of an ORF in a proper Kozak context [26].

In the conventional model for translation, ribosomes preloaded with initiator methionine-tRNA (Met-tRNA\textsubscript{Met}), the only tRNA thought to initiate translation, bind to the 5’-end of the mRNA and scan linearly in the 3’ direction for the start codon. Initiation of protein synthesis is a tightly regulated step requiring at least ten initiator factors that comprise more than 26 polypeptides [27] and is the target of many cellular pathways during normal and stress conditions [28]. How frequently non-coding regions of the mRNA are translated is presently unknown. The analysis is made especially difficult by our reliance on current gene annotations, including the specified 5’- and 3’-UTR sequences. Indeed, construction and sequencing of human 5’-UTRs show that many annotated genes are missing on average 45 bases of the 5’-UTR and nearly 30% contain upstream AUG starts (uAUG), many with a suitable Kozak context [29, 30]. Given that ribosomes enter the mRNA at the 5’ end, use of uAUGs or non-uAUGs could be an important source of cryptic epitopes. Accordingly, UTRs should be considered during epitope discovery, especially when short uORFs yield precursors that require minimal processing prior to entry into the antigen presentation pathway.

**Translation of alternate reading frames**

More than half the documented non-conventional MHC class I epitopes arise from ARF translation in a variety of human diseases such as influenza virus infections, cancer, and autoimmunity (Table 1). In addition to initiation at the primary ORF start codon, ribosomes can also translate the mRNA in the two other reading frames on both endogenous and viral messages. Furthermore, many ARF epitopes arise from precursors that are <50 amino acids [12, 31–33], which should require minimal degradation in the cytosol prior to entry into the ER and loading onto MHC class I molecules. A reduced requirement for proteolytic processing places many of these ARF epitopes at a kinetic advantage compared to the hundreds of thousands of peptides that are thought to compete for eventual loading onto MHC class I molecules either before or after arriving into the ER.

Several distinct translational mechanisms could account for the generation of ARF epitopes: (1) initiation codon read-through, (2) frame-shifting, and (3) re-initiation. Bullock and Eisenlohr [34] showed that, despite a primary ORF start codon in an ideal Kozak context, ribosomes often bypassed this codon and initiated translation further downstream to generate antigenic precursors. Since this seminal discovery, many examples have been described for both endogenous and viral mRNAs where the primary ORF start codon is bypassed for downstream initiation of cryptic epitopes (Table 1).

Frame-shifting is a phenomena whereby ribosomes may initiate at the primary ORF start codon but ‘slip’ either forward (+1 frame-shift) or backwards (−1 frame-shift) and continue translation in an alternate reading frame. This event produces an N-terminal primary ORF polypeptide fused to a polypeptide decoded from an alternate reading frame. Indeed, Saulquin et al. [35] showed that +1 frame-shifting in the IL-10 mRNA sequence generated a cryptic epitope derived partly from ORF1 and partly from ORF2. Likewise, another epitope within the thymidine kinase gene was generated only when ribosomal frame-shifting occurred and was effective in eliciting protective CD8\textsuperscript{+} T cells in vivo [36]. While frame-shifting has been best characterized in lower organisms [37], human mitochondrial (mt) ribosomes undergo frame-shifting at AGA and AGG codons due to lack of mt-tRNAs that recognize these codons [38]. These so-called ‘hungry’ codons signal the ribosome to frame-shift in the −1 direction which highlights the possibility that ribosomes on cellular messages may also be subject to frame-shifting during a reduced supply of tRNA due to either fluctuations in natural abundance or reduction in steady-state levels of aminocylated-tRNAs.

Re-initiation is an additional mechanism that could give rise to ARF epitopes. This mechanism has been best characterized from translation of Saccharomyces cerevisiae transcriptional activator GCN4 whereby amino acid starvation-induced phosphorylation of the initiation factor eIF2...
causes ribosomes to scan past inhibitory uORFs and re-initiate at the primary GCN4 ORF [39]. Initiation factor eIF2, recruits initiator Met-tRNA\textsubscript{Met} to the small ribosomal subunit in a GTP-dependent manner, prior to entry at the 5' end of mRNAs [40]. Different stresses activate cellular kinases (PKR, PERK, HRI, and GCN2) which phosphorylate Ser51 on the α subunit of eIF2 and limit its availability for initiation at AUG start codons. Interestingly, in mammalian cells, stress-induced phosphorylation of eIF2α causes ribosomes to bypass translation of uORFs and re-initiate at the transcriptional regulator activating transcription factor, ATF4-coding region [41].

Recently, Ingolia et al. [42] developed an elegant technique to globally monitor the position of ribosomes as thousands of mRNAs are translated in budding yeast under both rich and starvation conditions. Since initiation is a rate-limiting step of translation, this approach allows a direct determination of the translational reading frame utilized by the ribosomes anywhere along an mRNA sequence. Interestingly, during starvation conditions when eIF2α phosphorylation is enhanced, ribosome initiation at non-AUG start codons in uORFs dramatically increased. This suggests that translation of non-conventional regions of mRNAs in mammalian cells may also be upregulated during cellular stress. Interestingly, some viral infections activate PKR-mediated phosphorylation of eIF2α [43], which may encourage re-initiation not only at uORFs but other regions of the mRNA generating additional sources of non-conventional epitopes.

Non-AUG translation initiation

Initiation at non-AUG start codons on cellular and viral messages (CUG, ACG, etc.) is another mechanism to generate non-conventional antigenic peptides whether in the primary or alternate ORF (Table 1) [44]. In the scanning model of translation, the small ribosomal subunit containing initiator Met-tRNA\textsubscript{Met} along with eIF4E and a whole host of initiation factors scans linearly until the first AUG in a good Kozak context (CC(A/G)CC\textsubscript{AUGG}) is encountered [26, 45] This mechanism predisposes initiation to commence only with methionine. Even non-AUG start codons, such as CUG, are believed to be decoded only with Met-tRNA\textsubscript{Met} through ‘wobble’ interactions between the codon and anticodon of the tRNA. Interestingly, regulation of the innate immune response through type-I IFN has recently been shown to be linked to the phosphorylation state of eIF4E [46]. The precise connections between translational control and innate immunity are outside the scope of this review but highlight the emerging intersection between translational control and immune regulation [47].

Recently, we showed that the CUG start codon either in the primary ORF or within a 3'-untranslated region (3'-UTR) is a substrate for an unusual alternative translational mechanism using leucine as the initiating amino acid [48, 49]. To study this mechanism in vivo, Schwab et al. [49] generated mice with a transgene encoding a cryptic peptide initiated by CUG in the 3'-UTR of a conventional antigenic peptide ORF. T cell assays were used to detect the CUG/leucyl-initiated antigen and, despite its low abundance, the cryptic pMHC I was fully capable of inducing tolerance in transgenic mice as well as eliciting CD8\textsuperscript{+} T cell responses in wild-type mice. This suggests that even low levels of CUG/leucyl initiation, regardless of the reading frame, directs the generation of precursors which can efficiently compete for loading onto MHC I molecules. Further, the existence of a non-competing initiation event with leucine offers the cell a distinct advantage for antigen presentation when methionine and/or associated initiation factors are limiting. To what extent leucine initiation contributes to the pool of cryptic pMHC I awaits discovery of the set of proteins translated using the unconventional mechanism of CUG/leucine initiation, such as human trypsinogen [50].

The only other example of initiation without methionine is during IRES-mediated translation of the downstream capsid protein coding sequence of the cricket paralysis (CrPV) or the Plautia stali intestine viruses [51, 52]. Initiation at these non-AUG start codons using alanine (GCU) or glutamine (CAA) residues in mammalian cells suggests that highly conserved features of the ribosome are amenable to a range of non-canonical initiation events [53]. In contrast to translation on these viral messages, CUG/leucine initiation is not directed by a specific sequence or IRES element [49]. Furthermore, initiation at a CUG start codon, similar to AUG, was enhanced by the Kozak context [26]. This observation indicates that leucine insertion occurred during initiation as opposed to a post-translational modification. In contrast, CUG/leucine initiation was inhibited by insertion of upstream, out-of-frame CUG codons but not AUG start codons [54], suggesting that a distinct ribosome initiation mechanism specifically scans for and decodes CUG start codons with the alternative amino acid leucine.

The cellular response to viral infection is often manifested by shut-down of host-translation (reviewed in [55]). This antiviral translational response can induce phosphorylation of eIF2α and limit initiator Met-tRNA\textsubscript{Met} recruitment to the ribosome [40], which ultimately favors translation of viral mRNAs. Interestingly, chemical mimicry of the antiviral response using sodium arsenite to phosphorylate eIF2α showed that while conventional translation at AUG was inhibited cryptic translation from the CUG start codon was resistant [54]. This suggests that during viral infection a switch from AUG/methionine to
CUG/leucine would continue to supply a source of epitopes for immune surveillance by the CD8\(^+\) T cell repertoire.

Evidence that cryptic pMHC I is generated by a distinct initiation mechanism was recently investigated by analyzing ribosome initiation complexes at CUG start codons directly. Starck et al. used a technique called primer extension inhibition analysis or ‘toeprinting’ to monitor initiation complex assembly at CUG start codons in a cell-free extract. This approach showed that ribosome initiation complexes do assemble at cryptic CUG with up to 1/5th the efficiency of canonical initiation at AUG [56]. Further, these cryptic CUG initiation complexes were fully dependent on recognition of the mRNA m\(^7\)GpppN cap structure by eIF4E and the Kozak context, which further indicates that the cryptic translation product arises from a novel initiation mechanism in contrast to post-translational alterations of the epitope.

Using a series of protein synthesis inhibitors, Starck et al. further defined the requirements for cryptic translation at CUG. For example, both methionine-sulfamide and edeine, which inhibit initiator Met-tRNA\(^\text{Met}\)-eIF2 activity [57, 58], compromised ribosome assembly at AUG while CUG complexes were resistant. These data extend the measurements from Schwab et al. [54] which show eIF2-independent translation of cryptic pMHC and indicate that the functional differences are intrinsic to the ribosome initiation complex. Independently, Yewdell and Nicchitta [59] have argued in favor of an ‘immunoribosome’ dedicated to the efficient supply of polypeptides for presentation by MHC I.

The series of inhibitors which distinguish canonical initiation from cryptic CUG initiation all act within the P site of the ribosome, the site where initiator Met-tRNA\(^\text{Met}\)-eIF2 assembles for addition of the first amino acid [56]. This suggests that structural features of the ribosome initiation complex such as a unique initiator tRNA and/or ribosomal RNA directs CUG initiation. While the precise molecular mechanism awaits further characterization, we propose that CUG/leucine initiation increases the complexity of the proteome especially under certain cellular stress conditions that inhibit canonical initiation with Met-tRNA\(^\text{Met}\). These cryptic initiation events would continue to supply an important source of peptides for antigen presentation during cellular stress when conventional translation mechanisms are subverted.

Conclusions and future perspectives

More than 20 years have elapsed since CD8\(^+\) T cells were discovered to recognize cryptic peptides presented by MHC class I molecules. Although initially considered a curiosity, we now know that cryptic peptides can arise from polypeptides translated from many endogenous and viral mRNAs. These peptides are immunologically significant and can play a protective role in viral infections. Like the large fraction of antigenic peptides presented by MHC class I molecules that are derived from DRiPs, cryptic peptides arise from a variety of mechanisms also tied to protein translation. Recent studies have provided tantalizing hints that ribosomes that carry out the synthesis of antigenic precursors for cryptic peptides are distinct from those responsible for conventional translation. We expect characterization of these novel ribosomes as well as the pathways that regulate their activity will reveal further insights into the as yet mysterious sources of antigenic peptides that make immune surveillance possible.

Acknowledgements S.R.S. was supported by the Cancer Research Institute and the NRSA fellowship from the National Institutes of Health. We thank Sylvain Cardinaud for sharing findings before publication. Research in this laboratory is supported by the National Institutes of Health and the International AIDS Vaccine Initiative (IAVI).

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