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Nowhere to hide. Unconventional translation yields cryptic peptides for immune surveillance

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Summary

Effective immune surveillance by CD8+ cytotoxic T cells of intracellular microbes and cancer depends upon the antigen presentation pathway. This pathway produces an optimal peptide repertoire for presentation by MHC class I molecules (pMHC I) on the cell surface. We have known for years that the pMHC I repertoire is a reflection of the intracellular protein pool. However, many studies have revealed that pMHC I present peptides not only from precursors encoded in open-reading frames of mRNA transcripts but also cryptic peptides encoded in apparently “untranslated” regions. These sources vastly increase the availability of peptides for presentation and immune evasion. Here, we review studies on the composition of the cryptic pMHC I repertoire, the immunological significance of these pMHC I, and the novel translational mechanisms that generate cryptic peptides from unusual sources.

Keywords

Antigen presentation; antigen processing; cytotoxic T cells; major histocompatibility complex; protein synthesis

Introduction

Adaptive immunity mediated by conventional CD8+ cytotoxic T cells depends upon expression of major histocompatibility class I molecules (MHC I) on the cell surface. The MHC I molecule present a peptide mixture derived from virtually all intracellular proteins. Together the thousands of different peptides presented by MHC I (pMHC I) make up a comprehensive reflection of diverse intracellular proteins (1). In pathogen infected or tumor cells, the pMHC I repertoire, in addition to self-peptides, also includes peptides derived from microbial sources or mutated proteins. These novel pMHC I serve as flags recognized by antigen receptors of CD8+ T cells which in turn eliminate the infected or cancer cells.

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The pMHC I are produced by the antigen processing and presentation pathway. For most pMHC I this pathway begins in the cytoplasm (2, 3). The antigenic precursor polypeptides are cleaved in the cytoplasm primarily by the multicatalytic proteasome into peptide fragments (4, 5). The peptide fragments are transported from the cytoplasm to the endoplasmic reticulum (ER) by TAP, a channel dedicated to transport of antigenic peptides (6, 7). Within the ER, the peptides are brought into the peptide-loading complex that includes the empty MHC I molecules. With help from housekeeping chaperones and ER aminopeptidases, the peptides are trimmed to appropriate length and loaded onto the MHC I molecules (8, 9). The MHC I molecules serve as chaperones to transfer the peptides to the cell surface where they can engage the appropriate CD8+ T cells. Importantly, the mechanisms for peptide generation, MHC I loading and recognition by CD8+ T cells do not distinguish among the potential polypeptide sources. Thus, peptides for loading MHC I can arise from both conventional and novel mechanisms.

Among the potential sources for processed peptides, new protein synthesis has emerged as a major contributor to the pMHC I repertoire on the cell surface (10, 11). Notably, Yewdell and colleagues have argued in favor of a mechanism that uses DRiPs, or defective ribosomal products as precursors for generating peptides for loading MHC I (12, 13). Nevertheless, protein turnover also contributes to the pMHC I repertoire (14, 15). Interestingly, accumulating evidence has revealed that novel protein synthesis mechanisms also yield antigenic peptides for presentation by MHC class I (2, 16–19). We focus here on these novel cryptic translational mechanisms that give rise to pMHC I for immunity. We begin with some historical background on the discovery of cryptic peptides. We then describe the role of cryptic peptides in immunity and our current understanding of the molecular mechanisms that produce cryptic peptides for presentation.

Discovery of cryptic peptides in the pMHC I repertoire

Over 25 years ago, Boon and colleagues first described an odd observation that T cell stimulating antigenic pMHC I were generated in cells that had been transfected with genomic DNA fragments (20). They called these peptides “peptons”. It was puzzling how the cells produced the peptides because the transfected DNA fragments lacked obvious transcriptional and translational control elements such as promoters or translation initiation codons. Other examples of unexpected pMHC I were also discovered in the SV40 and influenza viruses (21, 22). Although not immediately appreciated at the time, each of these unexpected examples of pMHC I was a likely result of cryptic translation mechanisms as definitively established by our studies on the presentation of peptides encoded in alternate translational reading frames (23–26).

Subsequently, the number of known antigenic peptides derived from unconventional sources has substantially increased (reviewed in (16–18). Many of these peptides are encoded in regions of mRNAs considered “untranslated”. For example, 5′ and 3′ UTRs of mRNA or even introns raising questions on the very definition of an “open-reading frame” (ORF), and peptides that are encoded in alternate translational reading frames (ARF) that are produced from initiation at non-canonical initiation codons from both endogenous and viral mRNAs (18). Because cryptic pMHC I are generated from both endogenous as well as viral mRNAs

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indicates that immune surveillance mechanisms have evolved to take advantage of highly conserved aspects of protein translation.

From the known examples of antigenic precursors, it is evident that eukaryotic ribosomes translate the principal ORF of mRNAs not only for making proteins that serve their normal biological functions, but also translate a variety of other mRNA regions to produce peptides for presentation by MHC I. These unusual translation products are referred to as cryptic peptides and form a substantial and significant part of the overall pMHC I repertoire (19). When Perreault and colleagues examined the MHC I associated peptides with mass spectrometry, they discovered ~10% of these peptides were actually encoded by transcripts normally considered to be untranslated. Peptides were found in anti-sense transcripts and in 5′ and 3′ UTRs as well as alternative translational reading frames. This elegant study makes it clear that the proportion of cryptic pMHC I in the overall pMHC I repertoire has been highly underestimated and as discussed below, so is its impact on immunity.

Immunological significance of cryptic translation

Cryptic peptides were originally detected using T cell activation as an assay, thus proving the corollary that cryptic peptides were capable of eliciting T cell responses. Thus it was very likely that cryptic pMHC I would also play a role in immunity to pathogens. The two examples of cryptic epitopes referred to above are derived from the influenza and SV40 viruses that elicited T cell responses albeit during experimental manipulations (21, 22). More recently, T cells specific for non-conventional epitopes have been identified as encoded in ARFs of the murine AIDS viral mRNAs underscoring the importance of cryptic pMHC I in immune surveillance (27, 28). Protective cytotoxic CD8+ T lymphocytes (CTLs) response was elicited by a peptide encoded in the ARF of the LP-BM5 *gag* gene. Additionally, infection of rhesus macaques with the simian immunodeficiency virus (SIV)mac239 elicited a strong CTL response towards an epitope that was translated from another alternate translational reading frame relative to the *env* ORF (29). Cardinaud *et al.* used transgenic HLA-B*07 mice to model anti-HIV responses and discovered HLA-B*07 restricted CTLs specific for a peptide encoded in the ARF of the *gag* gene. The same epitope was also found among the CTLs individuals infected with HIV (30). Subsequently, Maness *et al.* (31) identified that a large fraction of the CD8+ T cell responses in virus infected monkeys were specific for cryptic epitopes that were encoded in viral ARFs.

The development of increasingly sophisticated algorithms to predict antigenic peptides presented by specific MHC I molecules has allowed identification of potential cryptic peptides encoded by viral genomes and to determine whether cryptic peptides pay a role in immune surveillance and in the evolution of HIV virus sequences in infected humans (32–35). Sequence variations were analyzed in the *nef*, *pol* and *gag* genes from a large number of patients to identify specific sequence variations associated with the MHC molecules expressed by the individual. Such variants would be expected to result from evolutionary selection or immune evasion under pressure from a particularly effective CTL response. The results revealed that in addition to the variants in the ORFs, more than 60 variations had occurred in ARFs of the three genes. In individuals that expressed the HLA-A*03 allele, a specific serine residue was found to be under represented compared in the integrase gene to

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viral sequences in individuals that did not express the HLA-A*03 allele. In subsequent studies, the optimal RTSKASLER peptide was identified with serine at position 6 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). Interestingly this peptide sequence was translated from an alternate reading frame without a requirement for the conventional AUG initiation codon. Rather, a CUG codon that encodes for the leucine amino acid was found as a potential translation initiation codon. Because mechanisms that allow virus to evade immune surveillance by cytotoxic T cells depends upon MHC I molecules, Bansal and colleagues searched for potential changes in all six translational reading frames of the HIV genome that correlated with the MHC molecules expressed by the infected hosts. This analysis revealed HLA-associated polymorphisms in the alternate translational reading frames of HIV-1 *pol*, gag and *nef* including those in antisense mRNAs. They found peptides encoded in ARFs of both sense- and antisense transcripts and these epitopes were often mutated during the first year of infection. The MHC-associated changes in these epitopes strongly suggests a role for CTLs specific for these cryptic peptides in early phase of infection. Mutations within these epitopes is likely to provide a mechanism for immune evasion by the HIV virus.

In a different study examining the HLA-alleles specific responses in HIV-patients, Bansal and colleagues discovered that presentation of cryptic peptides correlated with favorable clinical outcomes (36). By bioinformatic prediction of HLA-binding cryptic peptides, they identified peptides that could be presented by HLA-B*27 like protective alleles verses peptides that could be presented by HLA-B*5301 like non-protective alleles. Among the HIV-infected patients tested with these predicted peptides, they found higher responses to cryptic peptides in protective versus non-protective groups. Most of the cryptic epitopes identified in this study arose from anti-sense transcripts from the HIV genome which appears to have a larger potential for encoding cryptic epitopes. Why this interesting correlation exists and the translational mechanisms used for generating cryptic peptides remains unknown.

In another elegant study, Eisenlohr and colleagues discovered presentation of cryptic pMHC I in the antibiotic gentamycin treated cells (37). Interestingly antibiotic treatments that can affect translational fidelity have been proposed as a treatment of diseases that involve premature stop codons. The antibiotic treatment causes ribosomes to read-through the stop codon and thus allows the otherwise truncated protein to be produced as the native full-length form. The antibiotic treatment, however, also allows read-through of normal stop codons to generate cryptic peptides encoded in the 3′ untranslated regions. Thus therapies based upon treatments that affect translational accuracy can have unintended consequences such as autoimmunity.

These examples of cryptic epitopes in anti-viral response suggests the possibility that vaccines could be improved by including non-conventional epitopes, such as those found in the ARFs of HIV-1 transcripts (35). Indeed, in rhesus macaques the immune responses elicited by ARF epitopes were far stronger when DNA vaccines were compared to normal infection (31). On the other hand, the presentation of immunogenic ARF peptides can have undesirable consequences as shown in antibiotic treated cells discussed above (37) and in a

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human gene therapy trial reported by Samulski and colleagues (38). Unexpectedly, the DNA construct that was used to restore expression of coagulation factor IX in patients also encoded antigenic cryptic peptides in alternate translation reading frame. These epitopes elicited a CTL response that eliminated the genetically altered cells as well as their therapeutic efficacy. Thus, to exploit the advantages of immunity elicited by cryptic peptide and yet evade the undesirable outcomes, it is important to understand the underlying molecular mechanisms that produce the precursors of these antigenic cryptic epitopes.

Unanticipated cellular mechanisms produce cryptic peptides for presentation by MHC I

Protein biosynthesis is a wasteful process with a large proportion of newly translated polypeptides targeted for rapid degraded (39). Non-conventional or cryptic epitopes are derived from this pool of newly translated precursors with no obvious cellular role, yet are processed for presentation by MHC class I molecules. These polypeptide intermediates are described as DRiPs (Defective Ribosomal Products) and are poised for rapid processing in the MHC I presentation pathway (12, 13). DRIPs serve as an efficient strategy to alert the immune system that particular cells are infected or transformed. Rapid MHC class I presentation from a virally-infected cell is essential for timely activation of the immune system. Since many viral proteins, such as the Herpes viral proteins EBNA1 and LANA1 (40–43) are extremely stable with long half-lives (t½ >24h), generation of rapidly processed viral polypeptide precursors is an efficient way to mark infected cells for immune recognition. Elegant studies from the Yewdell laboratory have shown that newly synthesized proteins are preferential source of antigenic pMHC I (44). As an example, Cardinaud *et al.* demonstrated that during EBNA1 biosynthesis a proportion of ribosomes prematurely terminate and generate truncated antigenic intermediates that are efficiently presented in contrast to antigens derived from the long-lived full-length EBNA1 protein (45). Other examples of unanticipated MHC I peptides have been observed from translation within non-coding regions of the mRNA, translation from alternate reading frames, and from initiation at non-AUG start codons (16, 18, 46). The plasticity of translation is observed from the variety of nonconventional mechanisms which could serve to generate CD8+ epitopes during infection and transformation.

Translation of “untranslated” regions of the mRNA

A peptide encoded in the “non-translated” 5′ UTR was found to be presented by the HLA-B*2705 and to be recognized by CD8+ T cells suggesting that cryptic translation products could be novel sources for immunotherapy of cancer (47). This antigenic peptide is presented by HLA molecules due to overexpression of VEGF in cancer cells, which underscores the notion that tumor-specific antigens could potentially hide within untranslated regions of the mRNA. Translation of “untranslated” regions of mRNA indicates that ribosomes are capable of recognizing and initiating translation at codons different from the initiation codon of the normal ORF. These findings are not in agreement with the currently accepted model of translation that limits translation initiation at only the first AUG codon of the ORF in an appropriate Kozak context (48, 49).

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During conventional translation initiation, the initiator methionine-tRNA (Met-$tRNA_i^{Met}$) is loaded onto the small 40S ribosomes before the ribosome binds to the mRNA. The 40S ribosome complex bound to the Met-$tRNA_i^{Met}$ and other initiation factors recognizes the 5′-end of the mRNA and scans from the 5′ to 3′ direction to identify the authentic start codon (50). The initiation step of translation is highly regulated and requires more than 13 initiator factors as well as other auxiliary factors (51). It plays a prominent role in a variety of cellular pathways during different physiological condition (52, 53). Whether ribosomes recognize and translate from untranslated regions and other non-coding regions of the mRNA is presently an area of active research. Historically, the notion that regions outside of CDSs could be translated was limited by the perception of an ORF from standard gene annotations which currently do not distinguish ORFs outside of the standard 'normal' CDS (54, 55). Given that ribosomes are anticipated to encounter a variety of AUG and non-AUG start codons as they scan from the 5′ end of the mRNA, the use of uORFs and other small coding regions may serve to generate a relevant pool of epitopes during normal and disease states. The recent development of ribosome profiling by Ingolia and his colleagues (56, 57), now commonly used by many laboratories, has changed the traditional view of non-coding regions of the mRNA. Many ribosome profiling datasets indicate that thousands of translation events can potentially occur in regions outside the standard CDS. As such, untranslated regions and other non-coding RNAs should be considered sources of cryptic antigenic peptides during epitope discovery searches in exome data. Polypeptides translated from short uORFs, which is often less than 100 amino acids (58), would yield precursors that require minimal processing in the cytosol prior to TAP translocation into the endoplasmic reticulum (ER) where peptides are loaded onto MHC I molecules. Notably, translation of non-coding regions of the genome, such as long non-coding RNA (lncRNA), reveals an array of short polypeptides with bioactive properties that could also be available to the antigen presentation pathway (59).

We recently identified a novel translational mechanism that uses non-AUG codons in the nucleotide sequence upstream of the canonical AUG start codon in mRNAs translated in stressed cells (60). In cells undergoing ER stress, the unfolded protein response (UPR) (61), causes inactivation of eIF2, the key factor that allows loading of the 40S ribosomes with the canonical Met-$tRNA_i^{Met}$. Loss of eIF2 activity inhibits canonical translation initiation at AUG codons with Met-$tRNA_i^{Met}$. Nevertheless, certain proteins such as BiP continue to the produced and play key roles in the stress response. We took advantage of the efficiency with which cells generate pMHC I and CD8+ T cells that detect the pMHC I to assess whether a non-AUG translation mechanism was used to maintain BiP levels during stress and reduced eIF2 activity. Indeed, we discovered that translation was initiated at UUG and CUG codons in the 5′ UTR, two non-AUG codons via a distinct mechanism dependent on the non-standard initiation factor eIF2A. Thus, numerous translation events using upstream initiation codons under stress conditions could serve as a unique source of cryptic pMHC I for immunity.

Translation from non-canonical reading frames

Many of the characterized non-conventional MHC class I epitopes arise from translation of non-canonical translational reading frames or ARFs on both viral mRNA and cellular

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mRNAs associated with cancer and autoimmunity (18). It is now clear that ribosomes can begin translation not only at the primary ORF initiation codon of the CDS, but can also translate mRNAs at positions shifted by +1 or +2 nucleotides from the standard reading frame on both endogenous and viral messages. Indeed, as is observed for a variety of viruses discussed above, translation of anti-sense transcripts would generate polypeptides in all six reading frames of a given DNA which could yield a complex mixture of antigenic precursors. In addition, many ARFs encode polypeptides less than 50 amino acids in length (30, 62–64) and would require modest cytosolic processing prior to entry into the ER and loading onto MHC class I molecules. From a kinetic perspective, the reduced requirement for proteolytic processing would favor presentation of many of these ARF epitopes compared to peptides originating from long-lived protein precursors.

A variety of non-standard translational mechanisms likely account for the generation of ARF epitopes such as initiation codon read-through, frame-shifting, and re-initiation. In a seminal study, Bullock and Eisenlohr showed that ribosomes scanned past the standard ORF start codon in an ideal Kozak context showing a preference for translation of the antigenic precursor from a downstream start codon.(65). Since this discovery, there are multiple examples of cryptic epitopes generated from regions of the mRNA downstream from the annotated ORF initiation codon on both cellular and viral mRNAs.

Ribosome frame-shifting happens during translation around regions of secondary structure or a ‘slippery’ sequence thought to encode for rare tRNAs. After initiation at the standard ORF start codon, the ribosome slips forward (+1 frame-shift) or backwards (−1 frame-shift) and resumes translation in the ARF. This event, which is known to occur frequently in viruses and can occur on cellular mRNAs as well, can yield N-terminal extensions derived from the primary ORF polypeptide directly fused to the new polypeptide generated from ARF translation. ARF translation from a +1 frame shift in the IL-10 mRNA sequence is the mechanism responsible for generation of a cryptic epitope derived from translation of the normal ORF and from translation of the +1 ARF (66). In another example, an epitope was generated from ribosomal frame-shifting within the thymidine kinase mRNA which was sufficient to elicit an effective cytolyitc T cell response (67). These examples highlight frame-shifting as an additional mechanism for generating cryptic peptides for immunity.

Re-initiation of translation is another strategy that cells could employ to generate ARF epitopes during cellular stress (68). Cells harbor the distinct cellular kinases PKR, PERK, GCN2 and HRI which are activated by divergent stimuli and lead to phosphorylation of Ser^{51} on the α subunit of eIF2, thereby limiting its availability to mediate conventional translation initiation at the canonical AUG codon. Interestingly, mammalian cells require stress-induced phosphorylation of eIF2α and ribosomal re-initiation for translation of *ATF4*, the transcriptional regulator activating transcription factor (69). The molecular mechanism for how translational re-initiation operates *in vivo* remains elusive but is being characterized *in vitro* studies (70).

The ribosome profiling technique simultaneously measures the position of ribosomes on thousands of mRNAs and captures a snap-shot of translation from distinct regions of the mRNAs (56, 57). The remarkable single nucleotide resolution of this unbiased approach also

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reveals the translational reading frame being used by the ribosomes including translation of "non-coding" regions of the mRNA. Further improvements have allowed prediction of translational initiation at canonical AUG and non-AUG start codons at any regions of the mRNA (71) Interestingly, in starved yeast cells when eIF2α phosphorylation levels are upregulated, ribosome initiation at non-AUG start codons is dramatically increased. Likewise, thousands of non-conventional translation events utilizing non-AUG codons were identified in mammalian cells (57) as well as in viral genomes (72, 73).

Together these studies suggest that translation of unconventional regions of mRNAs in mammalian cells utilizing non-AUG start codons may also persist during cellular stress conditions. For example, particular viral infections which activate PKR to enhance eIF2α-phosphorylation (74) could be associated with persistent translation at non-AUG-initiated upstream ORFs (uORFs), but also at other regions of the mRNA which could be potent sources of immunologically relevant MHC class I epitopes.

Translation initiation at non-AUG codons

In addition to initiation at canonical AUG start codons, ribosomes can also recognize a variety of non-AUG initiation codons (e.g. CUG, UUG or ACG, etc.). Initiation at non-AUG start codons in a variety of reading frames on both cellular and viral mRNAs is another mechanism that cells employ to generate non-conventional antigenic peptides (24). Previously, we established that ribosomes can initiate at a CUG start codon present either in the primary ORF or located within a "non-translated" 3′-UTR. With the utilization of a CUG start codon, we showed that ribosomes participated in an unprecedented alternative translational mechanism by using leucine to initiate translation (24, 25). To determine if cryptic pMHC I could be generated from non-AUG start codons in normal cells, Schwab *et al.* showed that a CUG-initiated peptide is translated from the 3′ UTR of a conventional antigenic peptide ORF *in vivo* (25). Using T cell assays, Schwab et al. showed that the a CUG/leucyl-initiated peptide which comprised approximately 1 copy/cell nevertheless induced tolerance in transgenic mice and elicited a potent $CD8^+$ T cell responses in mice not expressing the a CUG/leucyl-initiated peptide. These findings established that even low levels of CUG/leucine initiation can produce antigenic precursors for loading MHC I molecules with immunological significance. It is possible that the distinctly regulated non-AUG initiation event can offer the cell an alternate means to generate precursors for antigen presentation when initiation with Met-$tRNA_i^{Met}$ and/or associated initiation factors are limiting in stressed cells. Note that numerous proteins also appear to be translated using this unconventional mechanism such as human trypsinogen (75), the tumor suppressor PTEN (76) as well as potential polypeptide precursors identified in ribosome profiling studies (77).

Other examples of translation using non-AUG initiation codons have been characterized. The translation mediated by an IRES, the internal ribosome entry site of the capsid protein encoding transcript of the Cricket Paralysis (CrPV) and the *Plautia stali* intestine viruses begins without using Met-$tRNA_i^{Met}$ (78, 79). Indeed, translation is initiated at these viral non-AUG start codons using alanine for the GCU or glutamine for the CAA codons, respectively, in mammalian cells indicating that the ribosomes are amenable to a variety of non-conventional initiation mechanisms (80). However, in contrast to translation of Cricket

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Paralysis or the *Plautia stali* intestine viruses, CUG/leucine initiation is not directed by a specific sequence or IRES element (25, 26), yet it is enhanced by the Kozak context (48) analogous to the optimal sequence context for the canonical AUG initiation codons. However, only upstream, in-frame CUG codons, but not AUG codons, could trap the ribosomes that initiates translation at the CUG codon with a leucine residue (26). These findings suggest that a different non-canonical translation mechanism directs ribosomes to decode CUG start codons with leucine.

As in stressed cells discussed above, for some viral infections, the antiviral response is mediated by translational down regulation induced by phosphorylation of eIF2α which limits initiator Met-$tRNA_i^{Met}$ recruitment to the ribosome (reviewed in (81, 82). Interestingly, the use of sodium arsenite which activates the HRI kinase leading to phosphorylation of eIF2α to mimic the antiviral response showed that while translation at the canonical AUG start codon was inhibited, cryptic translation initiated at the CUG codon was not inhibited and was even enhanced (26). Interestingly, ribosome profiling studies using showed that translation of 5′ leaders occurred frequently in eIF2 repressed cells although whether the cells used the CUG/leucine initiation mechanism was not directly tested (83). Together, these studies suggest that distinct ribosome translation events continue initiation at CUG start codon with leucine, independent of the Met-$tRNA_i^{Met}$ which would contribute a consistent source of epitopes for identification of potential targets for appropriate $CD8^+$ T cells.

To begin to uncover the molecular explanation for initiation at CUG start codons with leucine, we directly analyzed ribosome initiation complexes assembled at the CUG initiation codon. Starck *et al.* used "toe printing" or the primer extension inhibition analysis to directly measure ribosome recognition of CUG start codons in a cell-free extracts. This approach showed that ribosomes recognized cryptic CUG start codons with approximately 10% the efficiency by which the AUG start codon was recognized in antigenic precursor mRNAs (84). In addition, initiation at cryptic CUG start codons was dependent on recognition of the m^7GpppN cap structure by eIF4E, which supports the conclusion that unconventional CUG initiation differs from standard initiation at the earliest steps of translation and does not result from post-translational phenomena. These data began to provide a molecular counterpart for the studies from Schwab *et al.* which indicated that CUG/leucine-initiation was an eIF2-independent event (26) arising from functional differences intrinsic to the ribosome translation initiation complex. This is consistent with the independent hypothesis of Yewdell and Nicchitta that cells generate a robust supply of antigenic precursors from dedicated "immunoribosomes" (13).

Starck et al. identified a variety of small molecules that differentially affected CUG versus AUG initiation, with activity within or near the active site of initiator Met-$tRNA_i^{Met}$ binding during initiation (84). This data suggested that there are molecular features of the CUG ribosome initiation complex, such as a unique initiator tRNA. Indeed, analysis of ribosomal complexes assembled on CUG initiation codons showed the unique presence of an elongator $tRNA^{Leu}$ (85). Moreover, translation initiation at the CUG codon required a distinct initiation factor called eIF2A, rather than the canonical eIF2 initiation factor required for AUG initiation. In more recent work, eIF2A levels were found to be enhanced during

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conditions that limit initiator Met-$tRNA_i^{Met}$ availability (60) suggesting that a distinct translation initiation pathway may constitutively operate to circumvent the sensitivity of eIF2-mediated initiation especially during stress to ensure a supply of pMHC I precursors.

Conclusions and future perspectives

More than 25 years have elapsed since MHC class I molecules were found to present cryptic peptides that elicited $CD8^+$ T cell responses. We currently recognize that cryptic peptides are normally generated from nonconventional translation of endogenous as well as viral transcripts. Numerous studies have established that cryptic peptides are immunologically relevant and play a protective role in immunity to viral infections and cancer or a less desirable role in eliciting autoimmunity. While DRIPs generate a significant fraction of antigenic peptides, cryptic peptides are included in this pool and arise from a variety of novel translational mechanisms. These include ribosomes that initiate translation at conventional AUG as well as non-AUG codons in various alternate translational reading frames as well as in 5′ and 3′ "untranslated" regions. We anticipate that a better understanding of these unusual mechanisms and the cellular signaling pathways that regulate their activity will reveal the complex mechanisms underlying effective immune surveillance.

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