Initiation Codon Scanthrough versus Termination Codon Readthrough Demonstrates Strong Potential for Major Histocompatibility Complex Class I-restricted Cryptic Epitope Expression

By Timothy N. J. Bullock, Anthony E. Patterson, Laura L. Franlin, Evangelia Notidis, and Laurence C. Eisenlohr

From the Department of Microbiology and Immunology, Kimmel Cancer Center, Jefferson Medical College, Philadelphia, Pennsylvania 19107

Summary

Accumulating evidence shows that the repertoire of major histocompatibility complex class I-restricted epitopes extends beyond conventional translation reading frames. Previously, we reported that scanthrough translation, where the initiating AUG of a primary open reading frame is bypassed, is most likely to account for the presentation of cryptic epitopes from alternative reading frames within the influenza A PR/8/34 nucleoprotein gene. Here, we confirm and extend these findings using an epitope cassette construct that features two well-defined CD8⁺ T cell (T<sub>CD8⁺</sub>) epitopes in alternative reading frames, each preceded by a single start codon. Expression of one epitope depends on scanning of the ribosome over the first AUG with translation initiation occurring at the second AUG. We find that scanthrough translation has great potency in our system, with its impact being modulated, as predicted, by the base composition surrounding the first initiation codon, the number of start codons preceding the point of alternate reading frame initiation, and the efficiency with which the epitope itself is generated. Additionally, we investigated the efficiency of eukaryotic translation termination codons, to assess codon readthrough as a mechanism for cryptic epitope expression from 3' untranslated regions. In contrast with initiation codons, eukaryotic stop codons appear to be highly efficient at preventing expression of epitopes encoded in 3' untranslated regions, suggesting that 3' untranslated regions are not a common source of cryptic epitope substrate. We conclude that scanthrough is a powerful mechanism for the expression of epitopes encoded in upstream alternative open reading frames that may contribute significantly to T<sub>CD8⁺</sub> responses and to tolerance induction.

CD8⁺ activation depends upon interaction between the clonotypic TCR and a MHC class I molecule complexed with an 8–10 amino acid peptide epitope (1–5). Conventional models of antigen processing and presentation generally presume these epitopes to be derived from the cytosolic proteolysis of full-length, fully translated proteins. However, it has been demonstrated that T<sub>CD8⁺</sub> are also capable of recognizing either the products of gene fragments or mini genes expressing the minimal sequence of an epitope (6–8). In addition, there is sufficient evidence in the literature to suggest that strict adherence to conventional translation is not required for antigen presentation. Examples include the presentation of epitopes encoded in untranslated regions (UTR) (9, 10) and in alternative reading frames (RF) (11–15). This implies that regions of genes that are traditionally thought to be inaccessible to translation could be a potentially significant source of substrate for antigen processing and presentation.

The generally accepted model for translation initiation of most messages is the ribosomal scanning hypothesis (16–18), where a ribosome scans from the 5' cap and begins translation from the first AUG in favorable context, as determined by surrounding nucleotide identity. This AUG defines the primary RF, or RF0. Extensive surveys of eukaryotic genes have shown that in most cases the first AUG encountered is in favorable context (19, 20) and biochemical studies have shown that a primary start codon is used by the great majority of scanning ribosomes (21). However, some ribosomes fail to initiate at the primary initiation codon, with factors such as proximity to the 5'-cap and lo-
cal secondary structures as well as initiation codon context having influence (22, 23). We have recently demonstrated that initiation codon readthrough (scannthrough) is probably responsible for the production of epitopes encoded in alternative R Fs from a mutated influenza A PR / 8/34 nucleoprotein (N P) gene (24). In the system studied, it appeared that scannthrough was capable of utilizing an internal AUG codon to rescue the presentation of three NP epitopes (N P30–57, N P147–155, N P366–376) that had been shifted out-of-frame by a base deletion in the second codon of the N P open reading frame (O R F). In addition to providing this alternative source of out-of-frame epitopes, scannthrough translation may simultaneously produce truncated, in-frame polypeptides that could be more efficiently processed than full-length protein by the class I processing machinery. This mechanism could also provide a plausible rationale for the presentation of exoyctic proteins that do not normally access the cytosolic protein-degradation apparatus, by allowing initiation of translation after the signal sequence encoding region, resulting in the production of a nontranslocated substrate.

As in the case of start codons, not all translation stop codons cause termination of translation with equal efficiency. The sequence surrounding the termination codon dictates whether some readthrough and continued translation can occur. In Saccharomyces cerevisiae, termination codon readthrough has been shown to occur at levels up to 3.35% (25). This mechanism would allow for translation from the 3' UTR, further expanding the available substrate for class I presentation.

In the current study, we have constructed a presentation cassette in order to assess the potential for ribosomal scanthrough in epitope expression under controlled conditions. This allowed us, first, to confirm our previous findings concerning initiation codon readthrough and, second, to assess directly the strength of initiation codon readthrough and, second, to access the cytosolic protein-degradation apparatus, by allowing initiation of translation after the signal sequence encoding region, resulting in the production of a nontranslocated substrate.

Materials and Methods

Chemicals

General chemical supplies were obtained from Sigma (St. Louis, M O). Molecular biology reagents were obtained from New England Biolabs (Beverly, M A), except where noted.

Animals

6 to 8-wk-old female inbred CBA (H-2k), BALB/c (H-2k), and C57BL/6 (H-2k) strain mice were obtained from Taconic (Albany, N Y) or Jackson Labs (Bar Harbor, M E) and maintained in Kimmel Cancer Center Animal Facilities (Philadelphia, PA).

Tissue Culture

L929 (H-2b), L-Kd (H-2k, H-2kd), L929 transfected with H-2Kd, reference 26) and M C57G (H-2k) cells were maintained under standard conditions. Specifically, they were grown in DMEM supplemented with 5% FCS (Sigma) and incubated at 37°C and 9% CO2.

Molecular Manipulations

All enzymes used for the manipulation of the NP gene were used according to the instructions of the manufacturer. PCR primers and oligonucleotide linkers were synthesized by the Kimmel Cancer Institute Nucleic Acid Facility (Philadelphia, PA). NP containing a silent Apa I site in the H-2Kd (bp 457–463) epitope has been described elsewhere (8). Silent PCR directed mutagenesis was used to incorporate an ATG ATG pair into the reconstituted epitope separated by 5 nucleotides (nt) and the initiation cassette consisting of an excellent context for ATG and an excellent context for ATG.

The two ATG codons were in alternate reading frames and separated by 13 nt, partially encoding a KpnI site for later manipulation. Oligonucleotide sequences (upper oligo only), TCG AGC GAT TAT GTC CGT AC. The null context, TCG ACG GTT TAT GTC CGT AC. Poor context, TCG ACG GAT TAT GTC CGT AC. Fair context, TCG ACG GAT TAT GTC CGT AC. These altered sites were then used in addition to cloning of the NP gene. Most manipulations were done using a Bluescript II SK (+) vector (Stratagene, LaJolla, CA) with NP inserted in the EcoRI site of the MCS.

Variable Initiation Cassettes. The region between the NP30–57 and NP147–155 epitopes was removed by AatI to Apal digestion of the NP gene in Bluescript II SK (+). The resulting vector was then ligated (T4 ligase; GIBCO BRL, Gaithersburg, MD) with an oligo pair encoding the reconstituted epitope separated by 5 nucleotides (nt) and the initiation cassette consisting of an excellent context for ATG and an excellent context for ATG. The two ATG codons were in alternate reading frames and separated by 13 nt, partially encoding a KpnI site for later manipulation. Oligonucleotide sequences (upper oligo only), TCG AGC GAT TAT GGC GGT ACC GGA TTA TGG CAA GCG ATT 1 startcontext of RF0 construct with KpnI and ApaI and replacing that fragment with a synthetic oligo pair that retained the KpnI site, digested with BstBI and treated with alkaline phosphatase. Into a supernatant.

After construction of the excellent–excellent cassette, we altered the context of the ATG147–155 initiation codon using synthetic oligonucleotides. The initial construct was digested with SalI and Kpnl to excise the ATG and translate a downstream epitope. These studies provide important insights into the translation mechanisms available for the production of substrate from unconventional coding regions.

Variable Context Stop Codons.

Availability of Cryptic Epitopes for MHC Class I–restricted Presentation
the BstBI site, self-annealing oligos encoding either a strong (CGT AT A AGC GCG CTT ATA) or weak (CGT ATG ACC GCG GTC ATA) stop signals were ligated. Correct ligation were analyzed by the loss of the BstBI site.

Virus

Control vaccinia virus (vac), N P -expressing vaccinia virus, and the NP

G5G mutant designed to ablate the NP50-57 epitope have been described elsewhere (24). Other recombinants described here were made as previously described (8). In brief, all altered genes were ligated between the SalI and NotI sites in modified pSC11 for expression from the P2.5 promoter. These plasmids were then introduced into the vaccinia genome via homologous recombination in CV-1 cells (American Type Culture Collection [ATCC], Rockville, MD; CCL 70) and plaque purified in 143B HuTK− (ATCC; CRL 8303) cells in the presence of BrdU (Boehringer Mannheim, Indianapolis, IN). In all cases, the integrity of the recombinant was determined by isolating vac DNA and following after PCR amplification of the mutant gene.

 CTL Assay

APCs were infected for 1 h at 37°C with vac recombinants at 10 PFU/cell at a concentration of 107 cells/ml in balanced salt solution containing 0.1% BSA. After 1 h, 10 ml of precoordinated (37°C, 9% CO2) DMEM + 5% FCS were added and the cells incubated a further 3 h with rotation. Cells were pelleted and resuspended at 50 μl/106 cells of IMDM with 7.5% FCS containing 100 μCi of Na32PO4 (Amersham Corp., Arlington Heights, IL) and incubated for 1 h at 37°C. APC were then washed three times with DPBS and resuspended in IMDM and combined with CTL populations in round bottomed plates at 106 cells/well. APCs and CTL were coincubated for 4 h at 37°C before 10 μl of supernatants were collected and counted in a γ detector (Pharmacia, Sweden). The data are presented as percent specific 32Cr release, defined as 100 × ([experimental cpm — spontaneous cpm]/[total cpm — spontaneous cpm]).

Generation of CTL

TCD8+ restricted to H-2d, H-2d, or H-2d were derived from N P -immunized CBA, BALB/c, or C57BL/6 mice, respectively, as described elsewhere (27). Mice were immunized by intraperitoneal injection of 107 PFU of NP-vac in the case of C57BL/6, a vac expressing the isolated NP50-57 epitope in the case of CBA, and a vac expressing the isolated NP147-155 epitope in the case of BALB/c. After at least 2 wk, spleens from appropriate mice were harvested and one-third of cells infected with PR8 for restimulation. Secondary cultures were incubated at 37°C, 9% CO2 for 6-7 d before harvesting for effector populations.

Results and Discussion

Confirmation of Initiation Codon Scanthrough as a Viable Mechanism for MHC Class I Epitope Production. Our analysis of a series of frameshift mutations of the NP gene led us to suggest that ribosomal scanthrough of an initiation codon can provide a significant amount of substrate for epitope production from unconventional coding regions (24). A recombinant vac encoding the NP gene with a deletion in the second codon was able to express three NP epitopes, NP50-57 (H-2Kk-restricted), NP147-155 (H-2Kk-restricted), and NP366-374 (H-2Dd-restricted), as assessed by sensitization of target cells in vitro. This mutant was also able to prime mice for anti-NP366-374 responses at levels similar to those achieved by priming with wild-type NP-expressing vac. We were able to identify an internal AUG codon that played a prominent role in overcoming the frameshift and permitting the expression of the three NP epitopes. Owing to the location of the initiation and terminal codons involved, scanthrough was determined to be the most likely mechanism operating in the expression of these epitopes.

We wished to confirm the potential of scanthrough in a controlled system that is not subject to alternative translation mechanisms such as reinitiation, in which the ribosome terminates translation and starts again at a downstream AUG (28–30). Kozak (23) has shown, using standard biochemical techniques, that the degree of scanthrough is dependent upon the context surrounding the first AUG (defined by the following optimal sequence: CCA(F2r)−6CAUGG+4, in which the −3 purine and the +4 guanine are most important) and that scanthrough initiation is limited to the upstream region of the message (31). We created a truncated NP gene in which the region between the NP50-57 and NP147-155 epitopes was eliminated, leaving them separated by 5 nt (Fig. 1a). This had the effect of putting the two epitopes in alternate RFS: NP50-57 in R F +1, NP147-155 in R F 0, with no alternative R F stop codons that could influence the expression of these epitopes. Mutation of the residues required for binding to the MHC class I molecule has confirmed that H-2k and H-2d responses are limited to NP50-57 and NP147-155, respectively (24). In front of the two epitopes we placed a translation initiation cassette consisting of tandem start codons in the same reading frames as the two epitopes. The second ATG (R F +1, excellent context) is in frame with NP50-57. This start codon is preceded by an ATG in R F 0 with varying contexts that are predicted to influence significantly the efficiency of translation initiation. Based upon established results (32), four contexts were provided that we designated excellent (A at −3, G at +4), good (A at −3, T at +4), fair (G at −3, T at +4), and poor (T at −3, T at +4). In this range, the level of initiation is predicted to vary over 10-fold. The AUG in the same frame as the NP147-155 epitope. To confirm that the epitopes depend upon the R F 0 and R F +1 AUGs for expression we mutated either the R F 0 or R F +1 ATG while keeping the remaining ATG in excellent context. A predicted outcome for the translation of this cassette is that a scanning ribosome should first encounter the AUG8R 0 for translation of NP147-155. However, a percentage of scanning ribosomes will bypass it, with some level of initiation occurring at the second AUG, resulting in the expression of NP50-57. We anticipated that, as the initiation context surrounding AUG147-155 was improved, more ribosome would be competed away from translating in the R F +1 and directed into R F 0, resulting in decreased presentation of NP50-57.

All of our constructs were recombined into the vac ge-
nome to assay for the expression of these two epitopes by infected target cells in a standard 51Cr release assay. To measure presentation of both epitopes in the same target cell, we utilized L929 (H-2k) cells transfected with the gene encoding the Kd class I molecule (26). Presentation of the NP147–155 epitope was quite strong when driven by initiation codons in either good or excellent contexts (Fig. 1b).

Levels of specific lysis were comparable to those associated with infection by a wild-type NP-expressing vac. Of note, presentation was diminished when the RF0 ATG was placed into fair and poor contexts. Mutation of the RF0 ATG resulted in complete loss of lysis above control levels, confirming its essential role in expression of the NP147–155 epitope.

It is generally understood that TCD8+ are exquisitely sensitive, requiring very little antigen expression and very few peptide-class I complexes at the cell surface to trigger effector function (13, 33–35). In this light, the diminution of NP147–155 presentation resulting from downgrading of the R0 ATG may be unexpected. However, we and others have noted the relative inefficiency with which NP147–155 is presented (24, 36, 37), probably because of a major site for proteasome attack within the epitope (36). Antón et al. (37) have estimated that only 30 NP147–155–Kd complexes result from the processing of wild-type NP. This compares with 1,800 NP50–57–Kk complexes. Further supporting the notion that NP147–155 expression is close to, if not below, levels associated with maximal T cell stimulation, we have recently noted that a 2- to 5-fold reduction in wild-type NP expression within target cells is sufficient to reduce NP147–155-specific lysis appreciably (Yellen-Shaw, A.J., K.A. Puorro, and L.C. Eisenlohr, unpublished data). Using proinsulin synthesis as a readout, Kozak observed that a change from excellent to good context results in a 2-fold decrease in expression, from good to fair, a 3.7-fold decrease, and from fair to poor, a decrease of at least 3.5-fold (32). Together, this information provides an explanation for the lower presentation of NP147–155 in the fair and poor contexts.

Presentation of the NP 50–57 epitope, which depends upon bypass of the R0 AUG by a sufficient portion of ribosomes, followed a pattern predicted by the scanning hypothesis. When the RF0 ATG is in poor, fair, and good contexts, specific lysis was not significantly different from that associated with the ATG147–155 null construct. When the RF0 ATG is in excellent context, presentation of NP50–57 was clearly compromised, though consistently present. This is reinforced by data from a separate assay shown in Fig. 2, which provides specific lysis figures over several effector/target ratios. The importance of the RF+1 AUG for this expression is confirmed by the low lysis associated with the
AUGRF-1 null construct. In this assay, levels were above those associated with control virus, but in other assays (see Fig. 2) the two were comparable. We also note the complete loss of NP50–57-specific lysis with a single mutation within the NP50–57 epitope. In our previous publication, we observed only partial loss of lysis with this mutant (24). As we used NP50–57-expressing vac to prime mice for effector populations in the studies reported here, and full-length NP vac for this purpose in the previous work, we strongly suspect the existence of a weaker H-2-k–restricted epitope within NP. Such an epitope has been suggested by Daly et al. (38) who generated a K- restricted T cell hybridoma with reactivity to NP that does not map to NP50–57. In accord with the scanning hypothesis, we reasoned that multiplying the number of RF0 start codons in excellent context would track a higher proportion of ribosomes to RF0, further reducing presentation of NP50–57. Therefore, the single start codon module was replaced by a string of triple RF0 start codons, each in excellent context. This strategy significantly reduced levels of NP50–57 presentation. This construct is also shown in Fig. 2, where no lysis above that associated with negative control virus was observed.

These results demonstrate that even when the preceding start codon is in excellent context, there is a degree of scanthrough that permits presentation of an epitope in an alternative reading frame. As the context surrounding the primary AUG becomes more favorable, the potential for epitope expression in the alternative reading frame decreases. Epitope expression in the alternative reading frame was clearly compromised when the RF0 AUG was in excellent context. An extensive survey of vertebrate mRNAs revealed that only 25% have primary start codons in excellent context. Good and fair contexts are each present in 36% (72% total) of messages (20). Thus, the majority of messages are predicted to feature a level of scanthrough that would permit strong expression of an epitope in an alternative reading frame. The results further demonstrate that the level of scanthrough is also influenced by the number of upstream start codons in alternative RFs.

Reversal of the Cassette. As discussed above, the NP147–155 epitope is inefficiently generated compared with NP50–57. Therefore, conditions that permit activation of NP147–155-specific TCD8+, via scanthrough may be more limited. To test this, the pairing of the first and second start codons with NP147–155 and NP50–57 was reversed as depicted in Fig. 3a and described in Materials and Methods. This placed the first AUG and NP50–57 in RF0 and the second AUG and NP147–155 in RF-1. Whereas NP50–57 in an alternative RF could be presented in the face of an upstream RF0 AUG in excellent context (see Fig. 2b; Fig. 3), presentation of NP147–155 in the same circumstance could not be detected (Fig. 3b). Thus, in addition to the context and number of upstream AUGs, the epitope itself can determine the potential for TCD8+ activation via scanthrough.

Altogether, our data indicate that scanthrough and reading of alternative ORFs that likely accompanies all translation to some degree is sufficient to activate TCD8+. The frequency with which epitopes are expressed in this manner, and the extent to which they play a role in the total response to foreign and self antigens, remains to be determined. However, we note that the cassette system employed may have underestimated the potential for this mechanism. The alternative ORFs in this study are much larger than those that naturally occur. For example, the average alternative ORF within NP encodes a peptide of 14 amino acids. Epitopes within such small products may be processed and loaded onto class I molecules hundreds- to thousands fold more efficiently than their equivalents that are part of larger proteins (37).

Figure 3. Reversal of the cassette. (a) Two base insertions, indicated by underline, were made to the original cassette resulting in pairing of RF0 ATG with NP50–57 and the RF-1 ATG with NP147–155. (b) Scanning beyond an AUG in excellent context is not sufficient to allow presentation of NP147–155. The indicated reverse and control constructs were tested for target cell sensitization in a standard 51Cr release assay. Specificity of the effector populations is indicated above each data set.

Translation Termination Codons Are Efficient at Preventing the Expression of Downstream Epitopes. A genetic region that has great potential for supplying additional substrate for class I–restricted epitopes is the 3' untranslated region. Part of this region could be available for translation if the translation termination codon is bypassed to a certain extent. In a manner similar to optimal initiation context, the sequence surrounding a stop codon can significantly influence translation termination (25, 39, 40), with certain sequences permitting a degree of leakthrough. Given the extreme sensitivity of TCD8+, even small amounts of stop codon readthrough could make the 3' UTR of a gene available as a substrate for epitope production. To test this notion, we inserted a stop codon, with either weak or strong termination efficiency, in RF0 of the full-length, wild-type NP between NP147–155 and NP366–374 (Fig. 4a). In S. cerevisiae, the weak context stop codon has been shown to allow 3.35% readthrough, whereas the strong context stop codon allows only 0.36% (25). We intentionally placed these stop codons far into the NP ORF to elim-
inate the confounding mechanisms of scanning and reinitiation that are possible in upstream regions of the message (19, 28, 30, 41). As seen in Fig. 4b, both stop codons were very efficient at preventing NP 366–374 presentation. As expected, NP 50–57 presentation was unaffected by these manipulations because this epitope lies upstream of the insertion site.

Therefore, it seems unlikely that translating ribosomes can productively penetrate stop codons in this region of the gene in this system. From our previous results showing presentation of epitopes from biochemically undetectable levels of substrate (24), we would have predicted that 3.35% readthrough of the termination codon would have produced sufficient amounts of NP 366–374 to sensitize target cells. In our hands, NP 366–374 is efficiently generated, resembling NP 50–57 much more than NP 147–155 by several criteria (Yellen-Shaw, A.J., K.A. Puorro, and L.C. Eisenlohr, unpublished data). However, it may be that the vac expression system employed is less permissive in translation termination or that a more efficiently generated epitope would indicate a low level of readthrough.

The unavailability of NP 366–374 for presentation from these constructs is compatible with previous data in which frameshifts were introduced into the distal region of NP. These frameshifts were predicted to produce termination codons in regions of the mRNA that would not be available for reinitiation of translation (19, 41). This was confirmed by the loss of NP 366–374 presentation (24). This termination codon efficiency is also in concurrence with the work of Shastri and Gonzalez (13), who demonstrated that placing a termination codon directly in front of an epitope was highly efficient at preventing the expression of that epitope. Given these results, we speculate that cryptic epitope expression due to unconventional translation may be limited to the early regions of the open reading frame. Further investigation will be required to address this possibility, including an analysis of frameshifting, in which the actively translating ribosome shifts into a new reading frame. This is a mechanism that would not be limited to a particular region of the open reading frame and one that we have begun to investigate.

With these studies, we have demonstrated the potency of scanthrough in the 5′ region of a gene. In contrast, stop codon readthrough is apparently not available for epitope production. These studies have further elucidated the role of cryptic translation events in the production of substrate for immune responses. Such epitopes could play important roles in thymic education, T CD8+ -mediated autoimmunity, cancer immunotherapy, and vaccine design.

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Address correspondence to L.C. Eisenlohr, Thomas Jefferson University, BLSB Rm 726, 233 South 10th St., Philadelphia, PA 19107. Phone: 215-503-4540; FAX: 215-923-4153; E-mail: L_Eisenlohr@lac.jci.tju.edu.

The current address for T.N.J. Bullock is Beirne Carter Center for Immunology Research, Health Sciences Center, Box 4012, University of Virginia, Charlottesville, VA 22908. The current address for L.N. Franklin is Merck & Co., Inc., Merck Research Laboratories, Laura Franklin WP26A-3000, West Point, PA 19486.

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References

1. Bjorkman, P.J., M.A. Saper, B. Samraoui, W.S. Bennett, J.L. Strominger, and D.C. Wiley. 1987. Structure of the human class I histocompatibility antigen, HLA-A2. Nature (Lond.). 329:506–512.

2. Townsend, A., and H. Bodmer. 1989. Antigen recognition by class I–restricted T lymphocytes. Annu. Rev. Immunol. 7: 601–624.

3. Yewdell, J.W., and J.R. Bennink. 1992. Cell biology of antigen processing and presentation to major histocompatibility complex class I molecule-restricted T lymphocytes. Adv. Immunol. 52:1–123.

4. Heemels, M.-T., and H. Ploegh. 1995. Generation, translocation, and presentation of MHC class I–restricted peptides. Annu. Rev. Biochem. 64:463–491.

5. York, I.A., and K.L. Rock. 1996. Antigen processing and presentation by the class I major histocompatibility complex. Annu. Rev. Immunol. 14:369–396.

6. Townsend, A.R.M., F.M. Gotch, and J. Davey. 1985. Cytotoxic T cells recognize fragments of the influenza nucleoprotein. Cell. 42:457–467.

7. Sweetser, M.T., L. Morrison, V.L. Braciale, and T.J. Braciale. 1989. Recognition of pre-processed endogenous antigen by class I but not class II MHC-restricted T cells. Nature (Lond.). 342:180–182.

8. Eisenlohr, L.C., J.W. Yewdell, and J.R. Bennink. 1992. Flanking sequences influence the presentation of an endogenously synthesized peptide to cytotoxic T lymphocytes. J. Exp. Med. 175:481–487.

9. Uenaka, A., T. Ono, T. Akisawa, H. Wada, T. Yasuda, and E. Nakayama. 1994. Identification of a unique antigenic peptide pRL1 on BALB/c RLF1 leukemia recognized by cytotoxic T lymphocytes and its relation to the Akt oncogene. J. Exp. Med. 180:1599–1607.

10. Guilloux, Y., S. Lucas, V.G. Brichard, A. Van Pel, C. Viret, E.D. De Plaen, F. Brassier, B. Lethé, F. Jotereau, and T. Boon. 1996. A peptide recognized by human cytotoxic T lymphocytes on HLA-A2 melanoma is encoded by an intron separating the upstream open reading frame and by intercistronic distance in the MHC class I heavy chain gene. J. Exp. Med. 183:1173–1183.

11. Gooding, L.R., K.A. O’Connell, R. Geib, and J.M. Pipas. 1988. Cytotoxic T cell recognition of the SV40 tumor antigen: a note of caution. In Processing and Presentation of Antigens. B. Pernis, S. Silverstein, and H. Vogel, editors. Academic Press, New York. 87–95.

12. Fetten, J.V., N. Roy, and E. Gilboa. 1991. A frameshift mutation at the NH2 terminus of the nucleoprotein gene does not affect generation of cytotoxic T lymphocyte epitopes. J. Immunol. 147:2697–2705.

13. Shastri, N., and F. Gonzalez. 1993. Endogenous generation and presentation of the ovabumin peptide/Kb complex to T cells. J. Immunol. 150:2724–2736.

14. Hahn, Y.S., V.L. Braciale, and T.J. Braciale. 1991. Presentation of viral antigen to class I major histocompatibility complex-restricted cytotoxic T lymphocytes. Recognition of an immunodominant influenza hemagglutinin site by cytotoxic T lymphocytes is independent of the position of the site in the hemagglutinin translation product. J. Exp. Med. 174:733–736.

15. Wang, R.-F., M.R. Parkhurst, Y. Kawakami, P.F. Robbins, and S.A. Rosenberg. 1996. Utilization of an alternative open reading frame of a normal gene in generating a novel human cancer antigen. J. Exp. Med. 183:1131–1140.

16. Kozak, M. 1978. How do eucaryotic ribosomes select initiation regions in messenger RNA? Cell. 15:1109–1123.

17. Kozak, M. 1989. The scanning model for translation: an update. J. Cell Biol. 108:229–241.

18. Kozak, M. 1992. A consideration of alternative models for the initiation of translation in eukaryotes. Crit. Rev. Biochem. Mol. Biol. 27:385–402.

19. Kozak, M. 1984. Compilation and analysis of sequences upstream from the translational start site in eukaryotic mRNAs. Nucl. Acid. Res. 12:857–872.

20. Kozak, M. 1987. An analysis of 5′-noncoding sequences from 699 vertebrate messenger RNAs. Nucl. Acid. Res. 15:8125–8148.

21. Kozak, M. 1989. Context effects and inefficient initiation at non-AUG codons in eucaryotic cell-free translation systems. Mol. Cell. Biol. 9:5073–5080.

22. Morá, F., B. Lopez, T. Henri, and J. Godet. 1985. α-Thalassemia associated with the deletion of two nucleotides at position -2 and -3 preceding the AUG codon. EMBO J. (Eur. Mol. Biol. Organ.) 4:1245–1250.

23. Kozak, M. 1987. At least six nucleotides preceding the AUG initiator codon enhance translation in mammalian cells. J. Mol. Biol. 196:947–950.

24. Bullrock, T.N.J., and L.C. Eisenlohr. 1996. Ribosomal scanning past the primary initiation codon as a mechanism for expression of CTL epitopes encoded in alternative reading frames. J. Exp. Med. 184:1319–1330.

25. Bonetti, B., L. Fu, J. Moon, and D.M. Bedwell. 1995. The efficiency of translation termination is determined by a synergistic interplay between upstream and downstream sequences in Saccharomyces cerevisiae. J. Mol. Biol. 251:334–345.

26. Eisenlohr, L.C., I. Back, J.R. Bennink, K. Bernstein, and J.W. Yewdell. 1992. Expression of a membrane protease enhances presentation of endogenous antigens to MHC class I–restricted T lymphocytes. Cell. 71:963–972.

27. Yewdell, J.W., J.R. Bennink, G.L. Smith, and B. Moss. 1985. Influenza A virus nucleoprotein is a major target antigen for cross-reactive anti-influenza A virus cytotoxic T lymphocytes. Proc. Natl. Acad. Sci. USA. 82:1785–1789.

28. Luukkonen, B.G.M., W. Tan, and S. Schwartz. 1995. Efficiency of initiation of translation on human immunodeficiency virus type 1 mRNAs is determined by the length of the upstream open reading frame and by intercistronic distance. J. Virol. 69:4086–4094.

29. Abastado, J.-P., P.F. Miller, B.M. Jackson, and A.G. Hinnenbusch. 1991. Suppression of ribosomal reinitiation at upstream open reading frames in amino acid-starved cells from the basis for GCN4 translational control. Mol. Cell. Biol. 11: 486–496.

30. Kozak, M. 1987. Effects of intercistronic length on the efficiency of translation by eucaryotic ribosomes. Mol. Cell. Biol. 7:3438–3445.

31. Kozak, M. 1995. Adherence to the first AUG codon when a second AUG codon follows closely upon the first. Proc. Natl. Acad. Sci. USA. 92:2662–2666.

32. Kozak, M. 1988. Point mutations define a sequence flanking the upstream open reading frame of a normal gene in generating a novel human cancer antigen. J. Exp. Med. 183:1131–1140.
compatibility complex class I molecule to alloreactive T cells. J. Exp. Med. 182:1739–1750.

34. Christinck, E.R., M.A. Luscher, B.H. Barber, and D.B. Williams. 1991. Peptide binding to class I MHC on living cells and quantitation of complexes required for CTL lysis. Nature (Lond.). 352:67–70.

35. Eisen, H.N., Y. Sykulev, and T.J. Tsomides. 1996. Antigen-specific T-cell receptors and their reactions with complexes formed by peptides with major histocompatibility complex proteins. Adv. Prot. Chem. 49:1–56.

36. Yellen-Shaw, A.J., E.J. Wherry, G.C. Dubois, and L.C. Eisenlohr. 1997. Point mutation flanking a CTL epitope ablates in vitro and in vivo recognition of a full-length viral protein. J. Immunol. 158:3227–3234.

37. Antón, L.C., J.W. Yewdell, and J.R. Bennink. 1997. MHC class I-associated peptides produced from endogenous gene products with vastly different efficiencies. J. Immunol. 158:2535–2542.

38. Daly, D., P. Nguyen, D.L. Woodland, and M.A. Blackman. 1995. Immunodominance of major histocompatibility complex class I-restricted influenza virus epitopes can be influenced by the T-cell receptor repertoire. J. Virol. 69:7416–7422.

39. Fearon, K., V. McClendon, B. Bonetti, and D.M. Bedwell. 1994. Premature translation termination mutations are efficiently suppressed in a highly conserved region of yeast Ste6p, a member of the ATP-binding cassette (ABC) transporter family. J. Biol. Chem. 269:17802–17808.

40. Kozak, M. 1986. Regulation of protein synthesis in virus-infected animal cells. Adv. Virus Res. 31:229–292.

41. Donzé, O., P. Damay, and P.-F. Spahr. 1995. The first and third uORFs in RSV leader RNA are efficiently translated: implications for translational regulation and viral RNA packaging. Nuc. Acids Res. 23:861–868.