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VIROLOGY 173, 736–742 (1989)

Chromatographic Analysis of the Aminoacyl-tRNAs Which Are Required for Translation of Codons at and around the Ribosomal Frameshift Sites of HIV, HTLV-1, and BLV

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Received May 10, 1989; accepted August 21, 1989

An examination of the frameshift signals or proposed signals within published sequences of retroviruses and other genetic elements from higher animals shows that each site utilizes a tRNA which normally contains Wybutoxine (Wye) base or Queuine (Q) base in the anticodon loop. We find experimentally that most of the Phe-tRNA present in HIV-1 infected cells lacks the highly modified Wye base in its anticodon loop and most of the Asn-tRNA in HTLV-1 and BLV infected cells lacks the highly modified Q base in its anticodon loop. Interestingly, Phe-tRNA translates a UUU codon within the ribosomal frameshift signal in HIV and Asn-tRNA translates a AAC codon within the proposed frameshift signals in HTLV-1 and BLV. Thus, the lack of a highly modified base in the anticodon loop of tRNAs in retroviral infected cells is correlated with the participation of these undermodified tRNAs in the corresponding frameshift event. This suggests that the "shifty" tRNAs proposed by Jacks *et al.* (Cell 55, 447–458, 1988) to carry out frameshifting may be hypomodified isoacceptors.

Many vertebrate retroviruses require ribosomal frameshifting to align different reading frames for expression of the *gag–pol* fusion protein (*1–6*). The ribosomal frameshift sites of three of these retroviruses, the *gag–pro* frameshift site in MMTV (*2*), and the *gag–pol* frameshift sites in HIV (*4, 6*) and in RSV (*5*) have been examined in detail. Each study suggests that leucine occurs at the frameshift site. Most certainly, the frameshift signal must also involve the codon or codons upstream of the frameshift site. Indeed, three conserved tetranucleotide sequences, which are present in the overlapping reading frames of many retroviruses as well as other genetic elements [(*5*) and see below], have been proposed as part of the frameshift signals (*5*). The signal appears to consist of a heptanucleotide sequence suggesting that at least two codons upstream of the frameshift site are also involved. A series of mutations within these proposed signals (*5–7*) have confirmed that they are involved in frameshifting and have led Jacks *et al.* (*5*) to propose the existence of specialized tRNAs [designated "shifty" tRNAs (*5*)] which mediate the frameshift event.

In the present study, we have examined the aminoacyl-tRNAs which are required for translation at and around the frameshift sites in HIV, HTLV-1, and BLV to determine if any alterations may occur in these isoacceptors. Interestingly, Phe-tRNA, which is required for translation within the ribosomal frameshift signal of HIV, lacks the highly modified Wyebutoxine (Wye) base (*8*) in its anticodon loop in HIV infected cells, while Asn-tRNA, which is required for translation within the ribosomal frameshift signal of HTLV-1 and BLV, lacks the highly modified Queuine (Q) base (*9*) in its anticodon loop in the corresponding infected cells. We also observed that each ribosomal frameshift signal or proposed signal within published sequences of retroviruses and other genetic elements from higher animals codes for an isoacceptor that normally contains Wye base (Phe-tRNA) or Q base (Asn- or Asp-tRNA) in its anticodon loop.

tRNA was isolated from HIV-1, HTLV-1, and BLV infected cells and from a corresponding set of uninfected cells and aminoacylated with rabbit reticulocyte aminoacyl-tRNA synthetases as described in the legend to Fig. 1. The chromatographic profiles of Asn-, Phe-, and Leu-tRNAs from cells infected with HIV-1 and from the corresponding uninfected cells are shown in Fig. 1. The Asn-tRNA isoacceptor which elutes first from the column contains Q base while the more hydrophobic, later eluting isoacceptor is deficient in Q base (*9*). Comparison of the elution profiles from infected and uninfected cells shows that virtually all of the Asn-tRNA from HIV infected cells is Q deficient while a greater proportion of the Asn-tRNA from the control cells contains Q base. Phe-tRNA profiles from infected and uninfected cells are also very different. The initial eluting phenylalanine isoacceptor, which lacks Wybutoxine (Wye) base (*8*), comprises most of the Phe-tRNA in HIV

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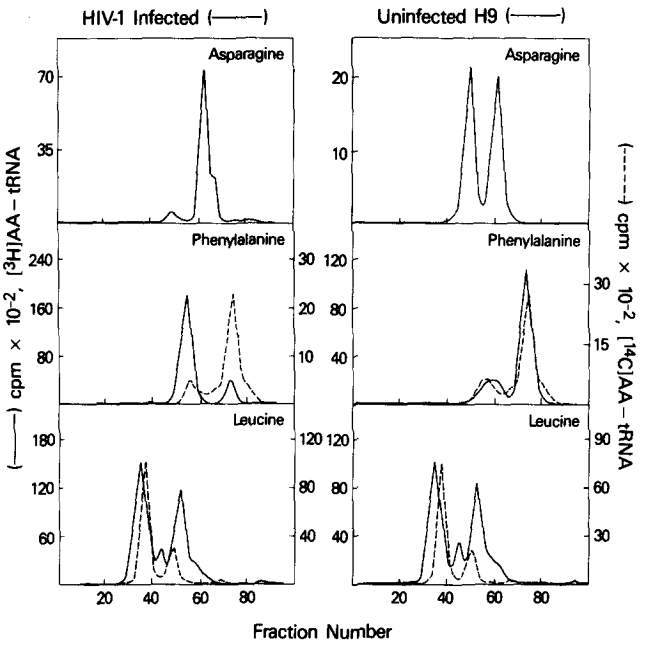

FIG. 1. Chromatography of aminoacyl-tRNAs from HIV infected and uninfected (H9) cells which are utilized at and around the frameshift site in HIV. HIV infected H9 cells and a matched set of uninfected H9 cells were grown under identical conditions by Dr. L. Arthur (Program Resources, Inc., NCI-FCRF, Frederick, MD), harvested, and stored at −80° until ready for use. tRNA was isolated from each cell line by phenol and chloroform extraction and the extract passed through a DE-52 column as described (*18*). Yields of tRNA from each cell line ranged from 15 to 20 A_{260} units. tRNA and aminoacyl-tRNA synthetases were prepared from rabbit reticulocytes, each tRNA preparation aminoacylated with labeled amino acid (purchased from Amersham Corp.), and the resulting labeled aminoacyl-tRNAs chromatographed on a RPC-5 column (*31*) as described (*11, 12*). To conserve tRNA from cell lines, these tRNAs were aminoacylated with ^{3}H-amino acid and in order that elution profiles of ^{3}H-aminoacyl-tRNAs from infected and uninfected cells may be readily compared to each other, the corresponding ^{14}C-aminoacyl-tRNAs were prepared from rabbit reticulocytes as a standard and cochromatographed with ^{3}H-aminoacyl-tRNAs. The figure shows the elution profiles of ^{3}H-aminoacyl-tRNAs (—) prepared from HIV infected (left set of panels) and uninfected, H9 (right set of panels) cells and cochromatography with the corresponding ^{14}C-aminoacyl-tRNAs (- - -) prepared from rabbit reticulocytes.

infected cells while the more hydrophobic, later eluting isoacceptor, which contains Wye base, comprises most of the Phe-tRNA in control cells. In contrast, the Leu-tRNA profiles from infected and uninfected cells appear to be very similar. In addition, no differences were observed in the elution profiles of Arg-tRNA [which decodes the AGG codon in the −1 frame of HIV (*4, 5*)] in infected and uninfected cells (data not shown).

The chromatographic properties of additional aminoacyl-tRNAs which have been shown to be altered in neoplastic cells [see review in (*10*)] were also examined in HIV infected and uninfected cells. No differences were detected in the chromatographic properties of Ala-, Gly-, and Val-tRNAs from HIV infected and uninfected cells (data not shown).

To determine if the levels of the undermodified isoacceptors in HIV infected cells are due to increased transcription of these isoacceptors or to alterations in the expressed tRNA, the levels of aminoacylation of 11 aminoacyl-tRNAs from infected cells were compared to those from uninfected cells as described previously (*11,12*). The levels of Ala-, Arg-, Asp-, Gly-, His-, Leu-, Lys-, Phe-, Ser-, Tyr-, and Val-tRNAs were similar in infected and uninfected cells (data not shown). Thus, the undermodified isoacceptors appear to result from alterations occurring in the expressed tRNA population and not from enhanced synthesis.

HTLV-1 (*13–15*) and BLV (*16,17*) require two ribosomal frameshift events, both of which are in the −1 direction, to align the different reading frames for expression of the *gag–pol* fusion protein. The *gag–pro* ribosomal frameshift signal in each virus presumably involves the homopolymeric A sequence, A AAA AAC, where lysine (AAA) and asparagine (AAC) are trans-

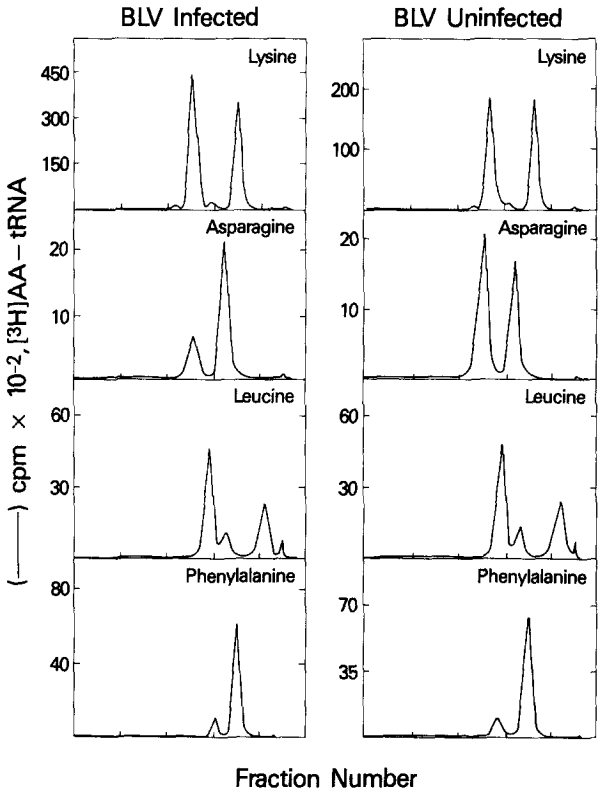

FIG. 2. Chromatography of aminoacyl-tRNAs from BLV infected and uninfected cells which are utilized at and around the proposed ribosomal frameshift sites in BLV. BLV infected and uninfected bat lung cells (*32*) were grown, the cells were harvested, and tRNA was prepared and aminoacylated as in the legend to Fig. 1. The figure shows the elution profiles of ^{3}H-aminoacyl-tRNAs prepared from BLV infected (left set of panels) and uninfected (right set of panels) cells.

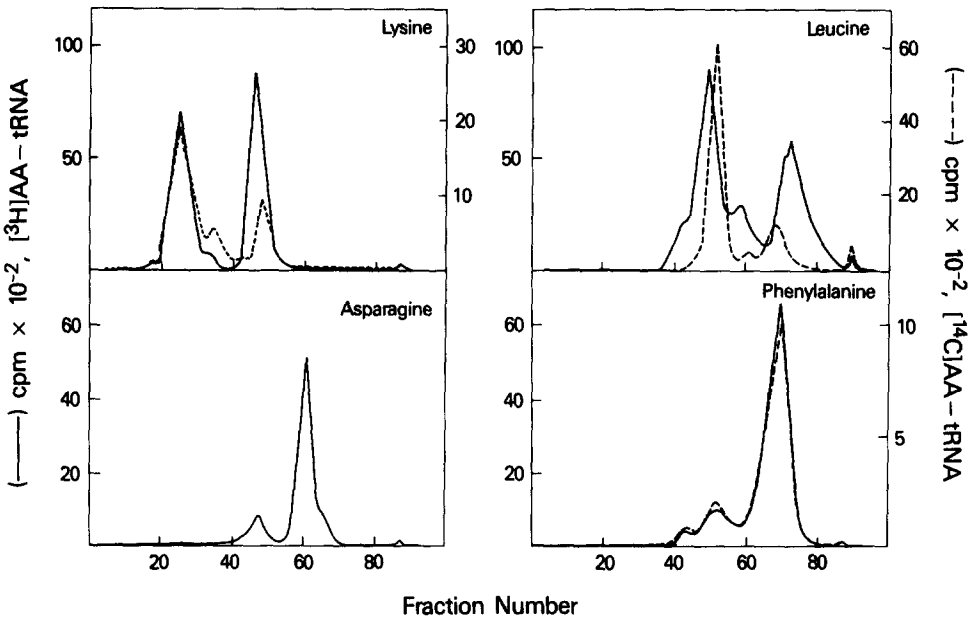

FIG. 3. Chromatography of aminoacyl-tRNAs from HTLV-1 infected cells which are utilized at and around the proposed ribosomal frameshift sites in HTLV-1. Several grams of MT-2 cells which were chronically infected with HTLV-1 were grown by Drs. M. G. Sarngadharan and Jerry Kern (Bionetics Research Laboratories, Rockville, MD), harvested, and stored at −80° until ready for use. tRNA was prepared and aminoacylated as given in the legend to Fig. 1. The figure shows the elution profiles of ^{3}H-aminoacyl-tRNAs prepared from HTLV-1 infected cells which are cochromatographed with ^{14}C-aminoacyl-tRNAs prepared rabbit reticulocytes (see legend to Fig. 1).

lated in the zero frame and the initial A represents the 3′ base of the proline codon, CCA, in HTLV-1 and the serine codon, UCA, in BLV (*13–17*). The *pro–pol* frameshift site may involve the heptanucleotide sequence, U UUA AAC, where leucine (UUA) and asparagine (AAC) are translated in the zero frame and the initial U represents the 3′ base of the proline codon, CCU, in both viruses. The shift to the −1 frame at the 3′ end of each heptanucleotide frameshift signal (*5*) results in translation of a leucine codon, either CUA or CUC, at the *gag–pro* and *pro–pol* frameshift sites of both viruses except at the *pro–pol* site of HTLV-1 where the shift results in translation of the proline codon, CCA (*13–17*). The elution profiles of several aminoacyl-tRNAs from BLV infected and uninfected cells (Fig. 2) and from HTLV-1 infected cells (Fig. 3) are shown. The aminoacyl-tRNAs examined are required for translation at and around the suspected ribosomal frameshift sites of each virus. The chromatographic profiles of Leu-, Lys-, and Phe-tRNAs (see Fig. 2), as well as those of Pro- and Ser-tRNAs (data not shown), from BLV infected and uninfected cells are very similar. The only tRNA which manifests a significantly different profile in the two sets of cells is Asn-tRNA where a much higher proportion of the Q deficient isoacceptor occurs in infected cells. Although a set of control cells was not available for comparison to the aminoacyl-tRNAs isolated from HTLV-1 infected cells, several conclusions can be made about the elution profiles of the tRNAs shown in Fig. 3. The chromatographic profiles of Phe- (see Fig. 3), Pro-, and Ser-tRNAs (data not shown) are similar to those observed in BLV infected and uninfected cells. The Leu-tRNA profile from HTLV-1 infected cells is similar to that observed from other mammalian tissues (*11*). The elution profile of Asn-tRNA, on the other hand, manifests a deprivation of Q base as is characteristic of tRNAs from other retroviral infected cells examined in this study.

An isoacceptor which is characteristically found to be elevated in rapidly dividing and/or neoplastic cells is Lys-tRNA$_4$ (*10,18*). Lys-tRNA$_4$, which is the minor isoacceptor that elutes second from the column, is not elevated in BLV (Fig. 2), HTLV-1 (Fig. 3), or HIV (Fig. 4) infected cells. However, Lys-tRNA$_3$, which is the major isoacceptor that elutes last from the column, is altered in HTLV-1 and in HIV infected cells. Cochromatography of the Lys-tRNA from HIV infected and uninfected cells shows that Lys-tRNA$_3$ from infected cells elutes slightly ahead of that from control cells (see Fig. 4). Similarly, Lys-tRNA$_3$ from HTLV-1 infected cells elutes slightly in front of that from rabbit reticulocytes (and in the same position as that from HIV infected cells) demonstrating that this isoacceptor is altered in HIV and HTLV-1 infected cells. Presumably the alteration is a hypomodification of one or both of the highly modified bases in the anticodon loop of this isoacceptor (see column 3 in Table 1). It is of interest to note that Lys-tRNA$_3$ is the primer for HIV reverse transcriptase (*19–*

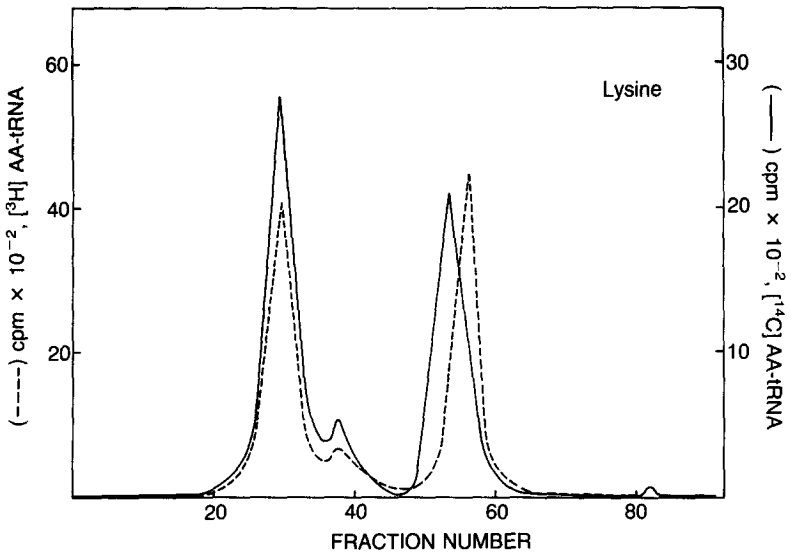

FIG. 4. Chromatography of Lys-tRNA from HIV infected and uninfected (H9) cells. ^{3}H-Lys-tRNA prepared from HIV infected cells (—) was cochromatographed with ^{14}C-Lys-tRNA prepared from uninfected (H9) cells (- - -). See legend to Fig. 1 for details.

21) and is utilized within the ribosomal frameshift signal in HTLV-1.

We also examined the elution profiles of Asp-tRNA, another tRNA which normally contains Q base in the 5′ position of its anticodon in mammalian tissues (*9*). tRNA from HIV, BLV, and HTLV-1 infected and uninfected cells was analyzed. As was observed in the studies with Asn-tRNA, a much greater proportion of the Asp-tRNA population in HIV and BLV infected cells was Q deficient and in HTLV-1 infected cells virtually all of the Asp-tRNA population lacked Q base (data not shown). In contrast, infection of mouse cells with Moloney murine leukemia virus, which expresses the *gag–pol* fusion protein by translational readthrough of an in-frame termination codon (*22*), does not result in a deficiency of Q base in Asn- and Asp-tRNAs (*23*). Thus, our present studies are consistent with the hypothesis that the occurrence of Q deficient tRNAs is a specific result of infection with retroviruses requiring ribosomal frameshifting to align their *gag–pol* reading frames.

The ribosomal frameshift signals or proposed signals within vertebrate retroviruses, within the non-retrovirus avian coronavirus and within other retrotransposons from higher animals, are shown in Table 1. The overlapping reading frames of each genetic element listed in the table contain one of three common consensus sequences (*5*): either A AAC, U UUA, or U UUU where asparagine (AAC), leucine (UUA), or phenylalanine (UUU) is read in the zero frame. Mutations within these sequences (*5–7*) have shown that the signal involves a heptanucleotide sequence (*5*) which is shown in the table. At least one, if not both, of the codons within each frameshift signal shown in the table corresponds to a tRNA which contains, under normal cellular conditions, a hypermodified base in its anticodon loop. Q base occurs normally in Asn-tRNA (*9*) which is coded by AAU or AAC in a number of the signals and in Asp-tRNA which is coded by GAU in the *pro–pol* signal of MMTV. Wye base occurs in Phe-tRNA (*8*) which is coded by UUU in numerous signals shown in the table. Interestingly, Leu-tRNA lacks a hypermodified base in its anticodon loop [see (24) and references therein]. We have grouped the signals into various classes based on the occurrence of Q base (Asn-tRNA, Class I), Wye base (Phe-tRNA, Class II), or lack of a highly modified base (Leu-tRNA, Class III) in the respective tRNA utilized (under normal cellular conditions) at the ribosomal A-site of each frameshift signal. Interestingly, whenever Leu-tRNA occurs at the A-site, either a tRNA with Q base (Asn-tRNA) or a tRNA with Wye base (Phe-tRNA) occurs at the P-site. Subclasses (i.e., IA, IB, etc.) are based on the occurrence of (or lack of occurrence of) hypermodified bases which are found normally in the anticodon loop of the tRNA utilized in the ribosomal P-site of each frameshift (see Table 1).

We have observed here that the tRNAs used in ribosomal frameshifting by retroviruses show a dramatic reduction in the level of hypermodified base in their anticodon loop in infected, as compared to uninfected, cells. For example, in the HIV frameshift signal, Phe-tRNA is utilized at the ribosomal P-site and Leu-tRNA at the ribosomal A-site. Phe-tRNA is deficient in Wye base and Leu-tRNA lacks a highly modified base in its anticodon loop. Interestingly, Asn-tRNA is utilized in translation within the heptanucleotide frameshift signal in HIV where the 3′ terminal base of the AAU codon is

TABLE 1

RIBOSOMAL FRAMESHIFT SIGNALS IN VERTEBRATE RETROVIRUSES AND IN OTHER GENETIC ELEMENTS FROM HIGHER EUCARYOTES AND THE OCCURRENCE OF HYPERMODIFIED BASES IN THE ANTICODON LOOP OF tRNAs AT THE RIBOSOMAL A- AND P-SITES

Class[a] and subclass[b]	Hypermodified base at A-site	Hypermodified base at P-site	Frameshift signal[c]	Location and source[d]
I A	Q	MCM5S2U and MS2T6A[b]	A AAA AAC	*gag–pro:* BLV, HTLV-1 and 2, MMTV, STLV-1
I B	Q	None	U UUA AAC	*pro–pol:* BLV, HTLV-1 and 2, STLV-1; *gag–pol:* EIAV; IBV
I C	Q	?	G GGA AAC	*gag–pro:* MPMV, SRV-1 and 2; *gag–pol:* VISNA
II A	Wye	Q	A AAU UUU	*pro–pol:* MPMV, SRV-1 and 2; *gag–pol:* 17.6
II B	Wye	None	G GGU UUU	*gag–pol:* mouse IAP
III A	None	Q	A AAU UUA	*gag–pol:* RSV
	None	Q	G GAU UUA	*pro:pol:* MMTV
III B	None	Wye	U UUU UUA	*gag–pol:* HIV-1 and 2, SIV, gypsy

[a] Class is based on whether a hypermodified base [Q (Class I) in Asn-tRNA or Wye (Class II) in Phe-tRNA] or lack of a hypermodified base [(Class III) Leu-tRNA] is normally found at the A-site of the frameshift signal.

[b] Subclass is based on whether a hypermodified base [Q base in Asn-, Asp-, and Tyr-tRNAs, Wye base in Phe-tRNA, MCM5S2U (5-methoxycarbonylmethyl-2-thiouridine), and MS2T6A (*N*-((9-β-D-ribofuranosyl-2-methylthiopurine-2-yl)carbamoyl)-threonine) in Lys-$tRNA_3$ (*33*) or unknown base in Gly-$tRNA_{UCC}$ (*33*) or lack of a highly modified base in Gly-$tRNA_{GCC}$ (*33*)] is normally found at the P-site of the frameshift signal.

[c] Heptanucleotide signal (*5*) found in the overlapping reading frames of retroviruses and other genetic elements requiring frameshifting to align the different reading frames.

[d] The location and source of the frameshift is given. Abbreviations and references to published work are BLV, bovine leukemia virus (*16, 17*) HTLV-1 and -2, human T-cell lymphotrophic virus-1 (*13–15*) and -2 (*34*); MMTV, mouse mammary tumor virus (*2, 3, 35*); STLV-1, simian T-cell leukemia virus (*15*); EIAV, equine infectious anemia virus (36, 37); MPMV, Mason-Pfizer monkey virus (*38*); SRV-1 and -2, simian acquired immunodeficiency virus syndrome [(SAIDS) designated as SRV (*39*) and SRV-2 (*40*)]; VISNA, VISNA virus (*41*); 17.6, transposable element in *Drosophila* designated 17.6 (*42*); mouse IAP, mouse intracisternal A-particle (*43*); RSV, Rous sarcoma virus (*1, 5, 44*); IBV, (coronavirus) infectious bronchitis virus (an avian nonretrovirus) (*45, 51*); HIV-1 and -2, human immunodeficiency virus-1 (*4, 12–14*) and -2 (*46*); SIV, simian immunodeficiency virus (*47, 48*); *gypsy,* transposable element in *Drosophila* designated *gypsy* (*49*).

the initial base of the signal; and Asn-tRNA manifests a much greater reduction in Q base in its anticodon loop in HIV infected, as compared to uninfected, cells. In the *gag–pro* frameshift signals of HTLV-1 and BLV, Lys-$tRNA_3$ is utilized at the ribosomal P-site and Asn-tRNA at the A-site. Lys-$tRNA_3$ is altered and presumably hypomodified in HTLV-1 infected cells, but appears normal in BLV infected cells. Asn-tRNA is hypomodified in both infected cell types. In the *pro–pol* frameshift signals of HTLV-1 and BLV, Leu-tRNA and Asn-tRNA are utilized and as noted above Leu-tRNA does not have a hypermodified base in its anticodon loop and Asn-tRNA is hypomodified in infected cells. The observation that alteration of the conserved sequence in the frameshift signal in RSV from U UUA to U UUU or to A AAC promoted efficient frameshifting, while alteration of this sequence to G GGG or to A AAA reduced frameshifting, led Jacks *et al.* (*5*) to propose that only certain, specialized "shifty" tRNAs participate in the frameshift event. This proposal is also supported by the observation that only three codons, UUA, UUU, and AAC, are found at the ribosomal A-site within the frameshift signals of each overlapping reading frame examined [(*5*) and see Table 1]. As noted above, the tRNA which translates one of these codons (UUA) does not contain a hypermodified base in its anticodon loop and the tRNAs which translate the other two codons normally contain a hypermodified base in their anticodon loop. However, we have found that the latter two tRNAs were deficient in this highly modified base in infected cells.

It seems reasonable that the frameshift event may be facilitated if the tRNA involved in frameshifting does not have a highly modified base in the anticodon loop; i.e., such a tRNA may be more "shifty". Clearly, the presence of G in place of the hypermodified Q base in the 5′ position of the anticodon ofAsn-tRNA [and other tRNAs normally containing Q base (9)] or of 1-methyl-G in place of the hypermodified Wye base in the 3′ position next to the anticodon of Phe-tRNA (*8*) would create more space in and around the frameshift site. Greater flexibility of movement of the respective tRNA antico-

don might be expected in absence of a highly modified base in the anticodon loop such as is found in Leu-tRNA and in hypomodified Asn- and Phe-tRNAs. It is of interest to note in this connection that the coding properties of tRNAs lacking Q base (*25–27*) and Wye base (*28*) in their anticodon loop are altered. Furthermore, the presence of the hypermodified base, N^6-(Δ^2-isopentenyl)-2-methylthioadenine, in the anticodon loop of *Escherichia coli* Phe-tRNA (which is in the same position as Wye base in mammalian Phe-tRNA) is known to stabilize the codon–anticodon complex and prevent misreading (*29*). It has also been shown recently that the presence of a modified base at the position just 3′ to the anticodon in proline tRNAs prevents frameshifting in bacteria (*50*). This study provides direct evidence that lack of a base modification in the anticodon loop of certain tRNAs may promote the frameshift event. Finally, it should be noted that human (*12*) and rabbit (*11*) reticulocytes are enriched for hypomodified species of Asn- and Phe-tRNAs which may account for the observation that HIV frameshifting occurs as efficiently in rabbit reticulocytes as in HIV infected cells (*4*). We do not know the mechanism by which infection with a retrovirus may alter the level of modification of certain cellular tRNAs. Studies with viral mutants might help gain insight into this problem. In any case, if alteration of a tRNA is required by some viruses for their expression, then inducing the cell to convert these tRNAs to the fully modified form may provide an avenue for inhibiting viral expression (*30*).

ACKNOWLEDGMENTS

This work was sponsored in part by NCI DHHS under Contract NO1-CO-74101 with BRI. We thank Janet Hanser for excellent technical assistance, Dr. L. Arthur for growth of HIV/H9 and H9 (uninfected) cells, and Drs. J. Kern and M. G. Sarngadharan for growth of the HTLV-1/MT2 cells.

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