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Identification of tRNAs Incorporated into Wild-Type and Mutant Human Immunodeficiency Virus Type 1

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We have identified the tRNAs which are incorporated into both wild-type human immunodeficiency virus type 1 strain IIIB (HIV-1IIIB) produced in COS-7 cells transfected with HIV-1 proviral DNA and mutant, noninfectious HIV-1Δat particles produced in a genetically engineered Vero cell line. The mutant proviral DNA contains nucleotides 678 to 8944; i.e., both long terminal repeats and the primer binding site are absent. As analyzed by two-dimensional polyacrylamide gel electrophoresis, both mutant and wild-type HIV-1 contain four major-abundance tRNA species, which include tRNA125*5, tRNA129* (the putative primer for HIV-1 reverse transcriptase) and tRNA1556. Identification was accomplished by comparing the electrophoretic mobilities and RNase T1 digests with those of tRNA125* and tRNA129* purified from human placenta and comparing the partial nucleotide sequence at the 3′ end of each viral tRNA species with published tRNA sequences. Thus, the absence of the primer binding site in the mutant virus does not affect tRNA129* incorporation into HIV-1. However, only the wild-type virus contains tRNA125* tightly associated with the viral RNA genome. The identification of the tightly associated tRNA as tRNA125* is based upon an electrophoretic mobility identical to that of tRNA129* and the ability of this RNA to hybridize with a tRNA125*-specific DNA probe. In addition to the four wild-type tRNA species, the mutant HIV-1-like particle contains two tRNA1556 species and three tRNA-sized species that we have been unable to identify. Their absence in wild-type virus makes it unlikely that they are required for viral infectivity.

In the early stages of viral infection, the conversion of the retroviral RNA genome into DNA which can be integrated into the infected host cell genome is catalyzed by a virus-specific protein, reverse transcriptase (RT). This enzyme requires a primer for activity, which in retroviruses is a tRNA (9, 10, 12, 15, 20, 21, 29, 33, 36, 37). The 3′-terminal 18 nucleotides of primer tRNA bind to a complementary region just 3′ of the 5′-UT region, which is termed the primer binding site. In addition, recent evidence has indicated that six to seven nucleotides within the 5′-UT region may also bind to a corresponding number of nucleotides in the Ty6C loop (2) and that genomic RNA sequences 3′ of the primer binding site may play a role in facilitating primer tRNA binding to the RNA genome (21). The sequence of the primer binding site in human immunodeficiency virus type 1 (HIV-1) DNA suggests that the primer tRNA in this virus is tRNA1556 (30), one of the three major tRNA1556 isoacceptors in mammalian cells (29). Purified bovine tRNA1556 has also been shown to have the ability to interact with HIV-1 RT and prime reverse transcription in vitro (3, 34). In this report, we show that this tRNA is one of a few select tRNAs incorporated into HIV-1 during viral assembly.

The primer tRNA is selected for incorporation into the virus from over 100 different host cell tRNA species. The mechanism by which this occurs is largely unknown, and it is not clear whether other tRNAs found in retroviruses are also incorporated by the same mechanism. The number of abundant tRNA-sized RNA species reported to be found in retroviruses is quite variable, ranging from more than 20 (9, 11, 14, 26, 33) to only 2 (27). Even for a single-type virus, HIV-1, we have found that the number of major-abundance tRNAs incorporated ranges from 4 to 25, depending upon the cell type producing the virus (18). HIV-1 produced from transfected COS cells contains only four major tRNAs (18) and has been shown to be infectious (1, 31). The infectivity of the virus produced by the particular HIV-1 proviral DNA vector and COS-7 cell line used in this work has been documented previously (13, 42). Since this virus contains only four major-abundance tRNA species, the greater complexity of the tRNA population seen in HIV-1 produced in other cell types may not reflect the actual tRNA requirements for the viral life cycle. Are the tRNAs in COS cell-produced virus selected because of unique functions that they perform in the viral life cycle, or are these tRNAs incorporated into the virus because of common structural properties recognized by the selection mechanism, irrespective of having a viral function? The results in this report indicate that the tRNAs incorporated probably share common structural properties, since in the COS-7 cell-produced virions, the most abundant tRNAs incorporated are the major tRNA1556 isoacceptors. One of these, tRNA125*, behaves as a primer tRNA; i.e., it is the major viral tRNA species found tightly associated with the viral genome.

Is primer tRNA first placed onto the primer binding site and then carried into the virus with the genomic RNA, or is it packaged independently of genomic RNA? It has been observed that in defective murine leukemia virus (23) and avian sarcoma virus (SE21Q1 [28]) lacking genomic RNA, the patterns of tRNA incorporated match those of the wild-type virus. In this work, we have examined the ability of an HIV-1 mutant lacking the primer binding site (as well as both long terminal repeats) to incorporate the normal...
pattern of tRNAs, including primer tRNA\textsuperscript{\text{LYS}}, and show that select tRNA incorporation in HIV-1 does not depend upon the presence of the primer binding site.

**MATERIALS AND METHODS**

**Virus.** HIV-1 produced in COS-7 cells was obtained by transfecting these cells with HIV-1\textsubscript{HXB2} proviral DNA as previously described (18). Virus was isolated from the supernatant 63 h posttransfection. The supernatant was first centrifuged in a Sorvall SS-34 rotor at 3,000 rpm for 30 min, and the virus was then pelleted from the resulting supernatant by centrifugation in a Beckman Ti45 rotor at 35,000 rpm for 1 h. The viral pellet was then purified by centrifugation at 26,500 rpm for 1 h through 15% sucrose onto a 65% sucrose cushion, using a Beckman SW41 rotor.

The construction of plasmid pMT-HIV and the establishment and characterization of a stable Vero monkey cell line expressing the mutant virus have been described elsewhere (16). Briefly, a proviral fragment from HIV-1\textsubscript{LAI}, containing only protein-coding information (nucleotides 678 to 8944) was expressed in monkey kidney Vero cells by using the inducible human metallothionein promoter. Noninfectious HIV-like particles were secreted into the culture supernatant. These mutant virions contained all of the HIV-1 structural proteins and were purified by sucrose cushion centrifugation.

**Viral RNA isolation and fractionation.** Total viral RNA was extracted from viral pellets by the guanidinium isothiocyanate procedure (5). Total viral RNA containing both high-molecular-weight genomic RNA and low-molecular-weight tRNA was fractionated by using commercial Nucleobond AX-20 columns (Nest Group, Southboro, Mass.). This column binds total viral RNA in a low-salt buffer (0.2 M KCl, 15% ethanol [EtOH], 100 mM Tris-PO\textsubscript{4} [pH 6.3]). Low- and high-molecular-weight RNAs are eluted sequentially with higher-salt buffers (18). tRNA is eluted with a 0.8 M KCl buffer (0.8 M KCl, 15% EtOH, 100 mM Tris-PO\textsubscript{4} [pH 6.3]), while genomic RNA and any tRNA associated with it are eluted next, using a 1.3 M KCl buffer (1.3 M KCl, 15% EtOH, 100 mM Tris-PO\textsubscript{4} [pH 6.3]). The fractionation of free and total associated tRNA was done as follows. An AX-20 column was equilibrated with 1 ml of 0.2 M KCl buffer. Solutions were passed through the column by gravity. Twenty micrograms of total viral RNA was dissolved in 500 μl of 0.2 M KCl buffer and loaded on the column. The column was then sequentially washed, first with 2 ml of 0.8 M KCl buffer, which elutes free viral tRNA, and then with 2 ml of 1.3 M KCl buffer, which elutes viral genomic RNA and the tRNA associated with it, termed total associated tRNA. Each 2-ml wash was collected as 4- to 500-μl fractions in 1.5-ml microcentrifuge tubes. After the addition of 10 μg of carrier DNA (pBR322), 400 μl of isopropanol was added to each tube, and the tubes were immediately centrifuged at top speed in an Eppendorf microcentrifuge for 30 min. (The addition of carrier DNA to the sample prior to loading it on the AX-20 column is unnecessary, since it does not improve recovery of the sample from the column.) Pelleted samples were washed once with 70% EtOH and once with 95% EtOH and then dried in vacuo. With this procedure, >80% of total viral RNA is recovered. To isolate tightly associated tRNA, total viral RNA was heated for 3 min at 65°C in dissociation buffer (20 mM Tris [pH 7.5], 200 mM NaCl, 2 mM disodium EDTA), which will cause the release of loosely associated tRNA from the genomic RNA. After quick cooling on ice, the RNA sample was fractionated with an AX-20 column, isopropanol precipitated in the presence of 10 μg of carrier DNA, washed, and dried as described above. The 0.8 M KCl buffer will elute the freed loosely associated tRNA along with the initially free RNA, while the 1.3 M KCl buffer will elute the genomic RNA along with the tRNA still tightly associated with it.

**Purification of human tRNA\textsuperscript{\text{LYS}} and tRNA\textsuperscript{\text{LYS}}.** Ten to 20 mg of total RNA can be isolated from 200 g of human placenta by using the method of Roe (32), which uses standard phenol-chloroform extractions to isolate total RNA, followed by DEAE-cellulose chromatography to isolate total tRNA. The tRNA was aminoacylated with [\text{\textsuperscript{3H}}]lysine, and tRNA\textsuperscript{\text{LYS}} and tRNA\textsuperscript{\text{LYS}} fractions were isolated by using standard chromatographic methods for tRNA purification (sequentially, DEAE–Sephadex A-50, reverse-phase chromatography [RPC-5], and Porix C4 chromatography [8, 29]). A final purification step used two-dimensional (2D) polyacrylamide gel electrophoresis (PAGE) as described below. The Porix C4 tRNA\textsuperscript{\text{LYS}} fraction was resolved into two lysine tRNAs, while the Porix C4 tRNA\textsuperscript{\text{LYS}} fraction remained a single species during electrophoresis. The tRNA\textsuperscript{\text{LYS}} isoacceptor spots were eluted from gel slices by soaking in water overnight and were concentrated by ethanol precipitation. Their identity as lysine tRNAs was confirmed through partial RNA sequencing (see below) and hybridization with DNA probes specific for tRNA\textsuperscript{\text{LYS}}.

In this report, we refer to two tRNA species representing tRNA\textsuperscript{\text{LYS}}. The term tRNA\textsuperscript{\text{LYS}} refers to a population of two tRNA\textsuperscript{\text{LYS}} species which differ by one base pair in the anticodon stem (29). The first major peak eluting from RPC-5 contains these two tRNA species, and these are not resolved by Porix C4 chromatography. We do, however, resolve this population into two tRNA\textsuperscript{\text{LYS}} species by the final step of 2D PAGE. Since we have not determined which spot is tRNA\textsuperscript{\text{LYS}} and which is tRNA\textsuperscript{\text{LYS}} , we call both spots tRNA\textsuperscript{\text{LYS}}.

**RNA labeling.** The fractionated RNA samples were labeled by the (\textsuperscript{32P})PCp 3′-end-labeling technique (4). (\textsuperscript{32P})PCp was made as follows. Five microcuries of (\textsuperscript{32P})ATP (specific activity, 3,000 Ci/mmole; Dupont Canada) was dried down in a microcentrifuge tube, using N\textsubscript{2}. Then 100 μl of reaction solution (50 mM Tris-HCl [pH 9.2], 5 mM MgCl\textsubscript{2}, 3 mM dithiothreitol, 5% bovine serum albumin, 1 μM 3′-CMP, 10 U of T4 kinase) was added. The reaction mixture was incubated at 37°C for 3 h, and the conversion of 3′-CMP to (\textsuperscript{32P})PCp was monitored by polyethyleneimine thin-layer chromatography in 0.8 M NH\textsubscript{4}SO\textsubscript{4}, which separates (\textsuperscript{32P})PCp from (\textsuperscript{32P})ATP.

Labeling of RNA with (\textsuperscript{32P})PCp was done as previously described (4, 19). Free viral RNA was labeled without further treatment. Total associated tRNA was first heated to 65°C for 3 min, quickly cooled, and then labeled. Labeled tightly associated tRNA was obtained by fractionating a similar amount of labeled, total associated tRNA on an AX-20 column as described above. After labeling of the various RNA fractions, free (\textsuperscript{32P})PCp was removed from the labeled macromolecules either by using Sephadex G-50 (Pharmacia) homemade spin columns, equilibrated with TE buffer (10 mM Tris [pH 7.5], 1 mM EDTA), or during the electrophoresis run. Before analysis by PAGE, the samples were heated at 90°C for 2 min.

**RNase T\textsubscript{1}, fingerprinting and RNA sequencing.** Partial RNase T\textsubscript{1}, digests are analyzed by using 1D PAGE to separate the RNA fragments (25). To sequence the nucleotides at the 3′ end of the tRNA molecule, the enzymatic sequencing methodology was used. This method, first developed by Donis-Keller et al. (7) and reviewed in reference 22,
uses enzymes which cut 3'- or 5'-end-labeled RNA at specific bases. The radioactive fragments are separated by 1D PAGE, and the sequence is read from the electrophoretic band pattern in a manner analogous to reading DNA sequencing gels. Specific enzymatic cleavage is altered in the presence of modified bases, and other methodologies are required to identify them. However, modified bases are normally missing within the 18-nucleotide region at the 3' end of tRNAs, and this method can result in the rapid identification of tRNAs.

1D and 2D PAGE. Electrophoresis of viral RNA was carried out at 4°C in a the Hoefer SE620 gel electrophoresis apparatus. Gel size was 14 by 32 cm. The first dimension was run in a 10% polyacrylamide–7 M urea gel for approximately 16 h at 800 V, until the bromophenol blue dye was beginning to elute from the bottom of the gel. After autoradiography, the piece of gel containing RNA was cut out, embedded in a second gel (20% polyacrylamide–7 M urea), run for 46 h (25-W limiting), and then subjected to autoradiography. All electrophoretic runs were carried out in 0.5× TBE (1× TBE is 5 mM Tris, 5 mM boric acid, and 1 mM disodium EDTA). The electrophoretic gel patterns presented in this report show only low-molecular-weight RNA, since the high-molecular-weight viral genomic RNA cannot enter the polyacrylamide gels. Furthermore, these patterns represent only the most abundant tRNA species present, since the high specific activities of the labeled tRNAs used will reveal many more minor-abundance species with longer film exposures.

The amount of 32P radioactivity in each major-abundance tRNA relative to other tRNA-sized species seen in the 2D PAGE was determined either by excising each spot from the gel and directly counting its radioactivity in a liquid scintillation counter, using Cerenkov counting, or by analyzing the 2D PAGE tRNA pattern via phosphor imaging (Bio-Rad, Toronto, Ontario, Canada). The two methods gave similar results.

Detection of tRNA^{59} isoacceptors by RNA-DNA hybridization. To detect the presence of tRNA_{Lys}^{59} and tRNA_{Lys}^{59} in viral RNA, we have synthesized 18-mer DNA oligonucleotides complementary to the 3' 18 nucleotides of tRNA_{Lys}^{59} (5'-TGCGCTAATAGTGGG-3') or tRNA_{Lys}^{59} (5'-TGCG GCCGCCAGGAC-3'). These DNA probes hybridize specifically with tRNA_{Lys}^{59} or tRNA_{Lys}^{59} (see Fig. 7A) and were hybridized to dot blots of RNA samples on Hybond N (Amersham). The DNA oligomers were 5' end labeled with T4 polynucleotide kinase and [γ-32P]ATP (3,000 Ci/mmol; Dupont Canada), and specific activities of 10^8 to 10^9 cpm/μg were generally reached. Approximately 10^7 cpm per oligomer was used for hybridization reactions.

RESULTS

Figure 1A shows the 2D PAGE pattern of tRNA incorporated into HIV-1 transiently produced in transfected COS-7 cells, and Fig. 2A shows the tRNA species incorporated into the mutant virions produced in Vero cells established with pMT-HIV-1. COS-7 cell-produced virus contain four major-abundance tRNA species, spots 1, 2, and 4 being most abundant. Figure 1B shows that in 1D PAGE, species 1 and 2 have electrophoretic mobilities similar to those of the two lysine tRNAs that we have isolated from the tRNA_{Lys}^{59} isoacceptor family, while species 3 and 4 have mobilities similar to that of purified tRNA_{Lys}^{59}. Species 1 to 4 isolated from the mutant virus present a pattern similar to that of the wild-type viral tRNAs in 2D PAGE (Fig. 2A) and migrate in

FIG. 1. tRNA incorporated into HIV-1 produced in transfected COS-7 cells. (A) 2D PAGE (performed as described in the text) of free viral tRNA; (B) comparison of electrophoretic mobilities of pure human tRNA_{Lys}^{59} and tRNA_{Lys}^{59} with that of HIV-1 tRNA species found in the free tRNA population. Lanes A to C represent purified human placental tRNA_{Lys}^{59} and the two components of tRNA_{Lys}^{59}, respectively; lanes 1 to 4 correspond to the like-numbered spots in panel A.

FIG. 2. tRNA incorporated into the mutant HIV-1 produced in chronically infected Vero cells. (A) 2D PAGE (performed as described in the text) of free viral tRNA; (B) comparison of electrophoretic mobilities of pure human tRNA_{Lys}^{59} and tRNA_{Lys}^{59} with HIV-1 tRNA species found in the free tRNA population. Lanes A to C represent purified human placental tRNA_{Lys}^{59} and the two components of tRNA_{Lys}^{59}, respectively; lanes 1 to 9 correspond to the like-numbered spots in panel A.
The fingerprints of partial T1 digests of purified human tRNA\(^{Lys}\) isoacceptors and viral tRNAs isolated from COS-7 cell-produced HIV-1 were compared (Fig. 3). It is clear that spots 1 and 2 produce the same fingerprint pattern as does tRNA\(^{Lys}\), while spot 4 (but not spot 3) produces the same fingerprint pattern as does tRNA\(^{Lys}\).

Figure 4 shows similar T1 digest fingerprints for the RNA species present in the mutant virus. The T1 digest fingerprints of species 1 to 4 of the mutant virus are identical to those of tRNAs 1 to 4 found in the wild-type virus. The identity of these mutant viral RNA species is further confirmed in Fig. 5, which shows 1D PAGE RNA sequencing gels of spots 1, 2, and 4. The sequences of the last 18 nucleotides of the 3' ends of these viral tRNAs again indicate that spots 1 and 2 are tRNA\(^{Lys}\) and spot 4 is tRNA\(^{Lys}\) (29). The partial sequences of the 3' termini of all nine RNA species found in the mutant virus are listed in Table 1. Spot 3, found in both wild-type and mutant viruses, appears to be a tRNA\(^{Leu}\) isoacceptor, while the sequences of spots 8 and 9 match that of mammalian tRNA\(^{His}\). We have not been able to match the partial sequences of spots 5 to 7 with any known RNA listed in the current data bases.

The tRNA incorporated into wild-type HIV-1 contains three, and possibly four, major-abundance species (with the three tRNA\(^{Lys}\) isoacceptors being the most abundant). Simple tRNA patterns of incorporation have also been found for mouse mammary tumor virus (27) and avian myeloblastosis virus and Rous sarcoma virus as well (20). In all retroviruses examined, including HIV-1, there are also more minor-abundance species whose appearance is dependent upon film exposure. In HIV-1, their amount relative to the tRNA\(^{Lys}\) isoacceptors also varies from one viral preparation to another. Analysis of the electrophoretic patterns of free tRNA in wild-type HIV-1 reveals that, on average, the radioactivity in the four major-abundance species represents 80% of the total radioactivity on the gel. Among the four major tRNA species, the relative abundances are as follows: spot 1 (tRNA\(^{Lys}\)), 46%; spot 2 (tRNA\(^{Lys}\)), 16%; spot 3 (tRNA\(^{His}\)), 8%; and spot 4 (tRNA\(^{Lys}\)), 30%. The 2:1 ratio of tRNA\(^{Lys}\) to tRNA\(^{Lys}\) is similar to the concentration ratio of these tRNAs in the cell.

The primer tRNA for reverse transcription in HIV-1 is believed to be tRNA\(^{Lys}\). In other retroviruses examined, the primer tRNA is distinguished from other viral tRNAs by...
being found tightly associated with the viral RNA genome. Figure 6 shows 1D PAGE of tightly associated tRNA isolated from COS-7 cell-produced HIV-1. A single tRNA-sized species which moves with the electrophoretic mobility of tRNA$^{3\text{YS}}$ is found, supporting the premise that this tRNA is the primer. This tRNA also hybridizes with a tRNA$^{3\text{YS}}$-specific probe. We have developed 18-mer DNA probes which specifically hybridize with either tRNA$^{3\text{YS}}$ or tRNA$^{3\text{YS}}$. These probes are complementary to the last 18 nucleotides at the 3' ends of these tRNAs. The tRNA dot blots in Fig. 7A demonstrate that these probes hybridize only to their respective tRNA$^{3\text{YS}}$ isoacceptors and to the tRNA$^{3\text{YS}}$-rich fraction obtained during the purification of human placental tRNA$^{3\text{YS}}$ by DEAE-cellulose chromatography. These oligomer probes also do not hybridize to the tRNA$^{3\text{YS}}$-poor fraction from the DEAE-cellulose column or to Escherichia coli tRNA$^{\text{Phe}}$, which shows a 67% homology to tRNA$^{3\text{YS}}$ in the last 3' 18 nucleotides. Using these probes, we find (Fig. 7B) that the free tRNA populations of both wild-type and mutant HIV-1 contain tRNA$^{12\text{YS}}$ and tRNA$^{3\text{YS}}$. 

FIG. 5. RNA sequence gels showing the partial nucleotide sequences at the 3' termini of human placental tRNA$^{3\text{YS}}$ (A), mutant viral spot 4 (B), human placental tRNA$^{12\text{YS}}$ (C), and mutant viral spot 1 (D). Lanes: OH⁻, alkaline hydrolysis; T₁, U₂, PhyM, and B.c., RNases used for enzymatic sequencing (T₁, U₂, PhyM, and Bacillus cereus, respectively).
The tightly associated tRNA from COS-7 cell-produced HIV-1 hybridizes only with the tRNA135-\(^\text{ys}\)-specific probe, while neither tRNA135-\(^\text{ys}\) isoacceptor is detected in the tightly associated fraction isolated from similar amounts of mutant virus.

Figure 6 shows that in addition to tRNA135-\(^\text{ys}\), a larger RNA species is found tightly associated with the viral genome. We have previously reported the presence of this RNA in the tightly associated fraction of RNAs isolated from HIV-1 produced from chronically infected H9 cells. It has an approximate size of 185 nucleotides (18). We also find this RNA species in mouse mammary tumor virus (19) but do not find it in the mutant virus RNA reported here. The identity of this RNA is unknown. In addition to this 7S-sized RNA, HIV-1 low-molecular-weight RNA contains a discrete DNA migrating as a 5S RNA, i.e., approximately 124 bp in size (18). Both 5S and 7S RNAs have also been found in avian retrovirus (35, 39).

**DISCUSSION**

In retroviruses, tRNAs are found both in a free population and in a subfraction associated with the genomic RNA, which can be further fractionated into loosely and tightly associated tRNA (39). In this report, we have characterized the free and tightly associated tRNA populations found in infectious, wild-type HIV-1 and in a noninfectious mutant HIV-1 particle which lacks both long terminal repeats and the primer binding site in the proviral DNA genome. (The loosely associated fraction was not characterized in this work.) Primer tRNA is found in the free fraction and has also been found to be more tightly associated with the RNA genome than are viral tRNAs (11, 26, 27, 38, 40). In these studies, we have found that the free tRNA populations of both wild-type and mutant HIV-1 contain two species of tRNA135-\(^\text{ys}\), tRNA135-\(^\text{ys}\), and tRNA135-\(^\text{ye}\). tRNA135-\(^\text{ys}\) is the putative primer for reverse transcription; in support of this hypothesis, we have found that the COS-7 cell-produced virus contains a tRNA tightly associated with the viral genome which migrates with the same electrophoretic mobility as does tRNA135-\(^\text{ys}\) and hybridizes to a tRNA135-\(^\text{ys}\)-specific DNA probe. The mutant virus does not contain this tightly associated tRNA. Our data show that neither a different cell type nor the absence of the primer binding site alters the select incorporation of the tRNA135-\(^\text{ys}\) isoacceptors. We conclude that the primer binding site is not required for tRNA135-\(^\text{ys}\) incorporation, unless the mechanism for primer tRNA incorporation in Vero cells is different from that in COS-7 cells. This seems unlikely, since our result supports previous work which indicates that the RNA genome is not required for primer tRNA incorporation in either murine leukemia virus (23) or avian sarcoma virus (28).

The incorporation of the tRNA135-\(^\text{ys}\) isoacceptors into HIV-1 need not reflect a viral function that they perform but may occur because the recognition signal for selecting primer tRNA135-\(^\text{ys}\) is shared with the other tRNA135-\(^\text{ys}\) isoacceptors. Of course, one cannot eliminate the possibility of a viral function for tRNAs present in more minor amounts in wild-type virus. However, we feel that the variation that we observe in the abundance, or even detectability, of some tRNA-sized species present in HIV-1 produced in different cell types (18) argues against their playing an essential role in the viral life

### Table 1. Sequences of 3'-termini of low-molecular-weight viral RNAs

<table>
<thead>
<tr>
<th>Spot</th>
<th>Sequence (3'-5')</th>
<th>Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ACCGGCGGGTUGGACCCCG</td>
<td>tRNA135-(^\text{ys})</td>
</tr>
<tr>
<td>2</td>
<td>ACCGGCGGGTUGGACCCCG</td>
<td>tRNA135-(^\text{ys})</td>
</tr>
<tr>
<td>3</td>
<td>ACCACGGCCAGGCGCCCU</td>
<td>tRNA135-(^\text{ys})</td>
</tr>
<tr>
<td>4</td>
<td>ACCCGCGGGTUGGACCCCG</td>
<td>tRNA135-(^\text{ys})</td>
</tr>
<tr>
<td>5</td>
<td>ACCGGCGGGTUGGACCCCG</td>
<td>?</td>
</tr>
<tr>
<td>6</td>
<td>ACCACGGACUGGGTUGGACCCCG</td>
<td>?</td>
</tr>
<tr>
<td>7</td>
<td>ACCACGGACUGGGTUGGACCCCG</td>
<td>?</td>
</tr>
<tr>
<td>8</td>
<td>ACCACGGACUGGGTUGGACCCCG</td>
<td>tRNA135-(^\text{ys})</td>
</tr>
<tr>
<td>9</td>
<td>ACCACGGACUGGGTUGGACCCCG</td>
<td>tRNA135-(^\text{ys})</td>
</tr>
</tbody>
</table>

![1D PAGE of tightly associated tRNA found in HIV-1 produced in COS-7 cells (lane 1) and purified human tRNA135-\(^\text{ys}\) (lane 2).](image-url)
cycle. Such variability is also demonstrated by the absence of mutant spots 8 and 9 (tRNA<sup>A<sub>12</sub></sup>) and spots 5, 6, and 7 (unidentified) in the wild-type virus, and we therefore feel it unlikely that the abundance of these species in the mutant virus reflects a viral function.

tRNA<sup>A<sub>12</sub></sup> is believed to be the primer tRNA for HIV-1 RT. We have in fact found only one tRNA tightly associated to the viral RNA genome in wild-type virus. It has the electrophoretic mobility of tRNA<sup>A<sub>12</sub></sup>, and it hybridizes with a tRNA<sup>A<sub>12</sub></sup>-specific probe but not with a tRNA<sup>A<sub>12</sub></sup>-specific probe. This finding indicates that the two tRNA<sup>A<sub>12</sub></sup> isoacceptors incorporated into the virus probably do not also serve as primer tRNA. Their incorporation along with tRNA<sup>A<sub>12</sub></sup> may therefore reflect a recognition signal for incorporation shared by all three species. This phenomenon is also observed in mouse mammary tumor virus, a virus that uses tRNA<sup>A<sub>12</sub></sup> as a primer but also incorporates tRNA<sup>A<sub>12</sub></sup> as the only other major tRNA species (27).

In considering what feature these tRNAs have in common which allows for them to be selected for incorporation, one must consider what process might be used to bring the tRNAs into the virus. This mechanism is largely unknown, but a candidate viral protein which may play a role in this process is the p160<sup>pro-pol</sup> precursor. This protein contains the RT sequences, and the role of RT in selective tRNA incorporation has been reported. In mutant avian sarcoma virus (28) and murine leukemia virus (24) lacking RT protein, the incorporation of host cell tRNA becomes nonselective. Mature HIV-1 RT (p66/p51) has also been shown to interact with tRNA<sup>A<sub>12</sub></sup> in vitro (3). However, p160<sup>pro-pol</sup> rather than mature RT is implicated in tRNA selection, since the precursor is not cleaved until after viral budding (6, 41). (The p160<sup>pro-pol</sup> precursor is cleaved by a viral protease, and we have recently observed that in a protease-negative mutant HIV-1 which is unable to process these precursors, tRNA<sup>A<sub>12</sub></sup> is both incorporated into the virus and placed onto the viral genome, indicating that these processes do not depend upon precursor processing [17].)

It is unlikely that p160<sup>pro-pol</sup> would interact with naked tRNA, since tRNA is found complexed in the cell with proteins such as EF-1α and aminocyl tRNA synthetases. Therefore, a specific interaction between p160<sup>pro-pol</sup> and tRNA<sup>Lys</sup> might involve an initial interaction with the protein that the tRNAs are complexed with. If this protein is lysine tRNA<sup>Lys</sup> synthetase, the specificity of binding might result from an initial protein-protein, rather than protein-tRNA, interaction. This interaction could occur during p160<sup>pro-pol</sup> synthesis, since all of these components would be in close proximity around the translational apparatus. Further experiments will be needed to determine whether p160<sup>pro-pol</sup> demonstrates special interaction with either deproteinized tRNA<sup>Lys</sup> or tRNA<sup>A<sub>12</sub></sup>-protein complexes.

The popularity of HIV-1 as an object of study has resulted in the availability of a large number of well-characterized mutant proviral DNAs. Through transfection, these DNAs can be used to produce mutant virus in the COS cell system. The ability to now measure both the incorporation and genomic placement of primer tRNA<sup>A<sub>12</sub></sup> in HIV-1 produced in transfected COS cells will facilitate the elucidation of the mechanism underlying these processes through a study of the effects of different mutations on these processes.

ACKNOWLEDGMENTS

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