# Structural Constraints in the HIV-1 Reverse Trancriptase−Primer/Template Complex for the Initiation of DNA Synthesis from Primer tRNA$^{Lys3}$ †

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ABSTRACT: The topography and functional implications of the complex formed in vitro between human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) and its primer tRNA$^{Lys3}$ were studied in this work. On the basis of previous results showing the high affinity both of the native primer, tRNA$^{Lys3}$, as well as that of mismatched short oligonucleotide primers for HIV-1 RT, we synthesized chimeric primers containing tRNA$^{Lys3}$ linked to U and T residues of different lengths. We found that the affinity of the oligonucleotide primers for HIV-1 RT is dramatically increased when linked to primer tRNA. Our results also show that in the tRNA·RT complex, before annealing tRNA$^{Lys3}$ to the retroviral RNA genome, the 3′-terminal nucleotide of tRNA$^{Lys3}$ is positioned at a distance of one nucleotide unit away from the template in the active polymerization site of the enzyme.

The replication cycle of retroviruses involves the reverse transcription of the RNA genome into DNA prior to integration into the host cell genome (for a review see ref *1*). Reverse transcription is carried out by a retroviral encoded RNA-dependent DNA polymerase or reverse transcriptase (RT)[1] (*2*−*4*). HIV-1 RT utilizes cellular tRNA$^{Lys3}$ primer for the initiation of DNA synthesis as deduced from the viral genome nucleotide sequence (*5*). The 3′-end of primer tRNA is annealed to an 18-nucleotide region (primer binding site or PBS) located near the 5′-end of the viral genome (for reviews see refs *6*−*8*).

We can consider the idea that RT may interact directly with primer tRNA at different stages: during the selection of this tRNA between the whole population of cell tRNAs; during the initiation of the replication of the retroviral genome from the 3′ OH of the CCA end of primer tRNA; during the removal of tRNA from the nascent DNA strand. Hybridization of primer tRNA to the PBS fragment in the retroviral genome requires the partial unwinding of these two highly organized RNAs. Partial unfolding of both molecules allows the accurate annealing and consequent initiation of reverse transcription. Two crucial questions remain to be answered unambiguosly. First, is the primer tRNA selected by the RT, by the RT moiety in the polyprotein precursor Pr 160$^{gag-pol}$, by the *gag*-derived small basic nucleocapsid protein (NCp), by both proteins, or by other factors? Second, which are the factors involved in the annealing of 18 nucleotides from the CCA-end of primer tRNA to the PBS?

In addition to the interaction of the 3′ end of tRNA and the PBS, other contact sites between primer tRNA$^{Lys3}$ and the genomic HIV-1 RNA have been described. Thus, the interaction of the anticodon region of tRNA$^{Lys3}$ with an A-rich region upstream of the HIV-1 PBS is supposed to control the transition from the initiation to the elongation steps of HIV-1 replication (*9*). Besides these contacts other domains of tRNA$^{Lys3}$ may also interact with regions located at the 3′ or 5′ regions of the PBS (for references see ref *8*).

Although HIV-1 RT specifically and stably binds to its replication primer tRNA$^{Lys3}$, the in vitro complex formation is not sufficient to properly initiate reverse transcription. Apparently, even in the presence of the template, the 3′-end of tRNA cannot productively associate with the specific binding region of RT. However, when tRNA primer is preannealed to the RNA template, DNA synthesis starts with an initial buildup of 3−5 nucleotides (initiation phase), followed by a rapid conversion to longer DNA fragments (elongation phase) as proposed before (*10*). Both HIV-1 RT and the NCp have been implicated in unwinding the tRNA and its subsequent annealing to the PBS (*11*−*14*). Moreover, Mak et al. have provided strong evidence that the RT region of the *pol* domain from the *gag*−*pol* precursor (Pr160$^{gag-pol}$) is involved in HIV-1 encapsidation of tRNA$^{Lys3}$ (*15*). A

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[1] Abbreviations: HIV-1, human immunodeficiency virus type 1; RT, reverse transcriptase; PBS, primer binding site; PAGE, polyacrylamide gel electrophoresis; DMSO, dimethyl sulfoxide; ODN, oligodeoxynucleotide; ORN, oligoribonucleotide.

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speculative model has been proposed for the interaction between the two subunits of HIV-1 RT with the primer tRNA−template duplex (*16*). This model is supported by results obtained by chemical and UV cross-linking between primer tRNA and HIV-1 RT using the photoactivable nucleotide 4-thiouridine (*17*) or $Pt^{2+}$ (*18, 19*), as well as by kinetic evidence (*20*). But few data are available concerning the precise interaction of $tRNA^{Lys3}$ with RT at the molecular level.

An in vitro complex between $tRNA^{Lys3}$ and HIV-1 RT has been identified by different approaches such as centrifugation in glycerol gradient (*21*), gel shift (*13*), footprinting (*11, 22*), and cross-linking (*18*). The $K_d$ values of RT and $tRNA^{Lys3}$ complex formation have been estimated in the nM range (*20, 23, 24*). HIV-1 RT is a flexible heterodimeric protein, and significant conformational changes occur upon primer binding (*25*). The binding of primer/template induces a marked conformational change in RT and stabilized the dimeric form of the enzyme (*26*). We have shown that the interaction with $tRNA^{Lys3}$ induces structural changes in RT as followed by fluorescence or by the accessibility of the RT•tRNA complex to chymotrypsin (*27*). Acting as an enzyme effector and not as a primer, $tRNA^{Lys3}$ led to conformational changes of RT stimulating the enzyme activity (*28*).

According to Majumdar et al. (*29, 30*) HIV-1 RT is able to bind the primer before the template. Furthermore, our previous results showed that the 3′-end of the primer interacts with the same binding site of the enzyme either in the absence or presence of template (*31*). Thus, besides the normal binding site for the 3′-end of tRNA, crucial for the initiation of cDNA synthesis, other nucleotides in the 3′-terminal region of tRNA also interact with specific site(s) of the enzyme.

Assuming that primer tRNA is fixed first to HIV-1 RT, some important questions remain unanswered. Is the 3′-end nucleotide of the primer tRNA localized at a catalytically proficient position in the in vitro complex between HIV-1 RT and $tRNA^{Lys3}$? Does the catalytically efficient positioning take place only after unfolding of the acceptor stem of tRNA by RT, NCp, or other factors? Does this process change the conformation of the primer binding domain in RT?

To address these questions we have synthesized $tRNA^{Lys3}$ derivatives containing oligonucleotides in the 3′-end and studied their behavior as primers in the presence of a synthetic complementary RNA template. This approach allowed us both to show the stronger affinity of oligonucleotide primers when linked to tRNA and to determine the distance between the 3′ end of tRNA and the polymerizing active site of HIV-1 RT in the primer-enzyme complex.

## EXPERIMENTAL PROCEDURES

*Materials.* Unlabeled nucleotides and polynucleotides were obtained from Sigma or Pharmacia. Radioisotopes were purchased from Amersham or Radioisotop (Novosibirsk, Russia).

*Oligonucleotide Synthesis.* The synthesis and characterization of oligonucleotides have been described earlier (*32*). They were homogeneous according to reverse-phase and ion-exchange chromatography. Oligoribonucleotides were prepared as described before (*33*). The concentration of oligonucleotides was determined using the following extinction coefficients expressed as $mM^{-1}\ cm^{-1}$: $d(pT)_2$, 19.5; $d(pT)_4$, 34.6; $d(pT)_5$, 43.5; $d(pT)_6$, 52.0; $d(pT)_8$, 69.0; $d(pT)_{10}$, 87.0; and $d(pT)_{11}$, 95.7 (determined at 267 nm), and $(pU)_2$, 19.25; $(pU)_3$, 28.4; $(pU)_5$, 46.5; $(pU)_7$, 65.0; $(pU)_{10}$, 92.4; and $(pU)_{11}$, 102.75 (determined at 262 nm).

*Purification of Reverse Transcriptase.* Recombinant HIV-1 RT p66/p51 purified from transformed yeast was obtained as described earlier (*21*).

*3′-End tRNA Labeling.* tRNA was 3′-labeled with 5′-[$^{32}$P]-pCp and purified by PAGE (10%) in the presence of 8 M urea (*34*).

*Synthesis of tRNA Derivatives.* 1. *Derivatives Containing Extra Oligonucleotides.* Unlabeled or labeled $tRNA^{Lys3}$−$d(pT)_n$ and $tRNA^{Lys3}$−$(pU)_n$ derivatives were obtained by ligation of the corresponding ODNs or ORNs to the 3′-OH end of $tRNA^{Lys3}$ by T4 RNA ligase. The reaction mixture (50 $\mu$L) contained 50 mM Hepes (pH 7.5), 20 mM $MgCl_2$, 3.3 mM dithiothreitol, 15% DMSO, 100 $\mu$M ATP, 20 $\mu$M tRNA, 100 $\mu$M oligonucleotide, and T4 RNA ligase (4500 U/mL). The mixture was incubated 24−96 h at 4 °C. tRNA and tRNA derivatives were precipitated by ethanol−acetone (1:1), in the presence of 0.3 M sodium acetate, pH 5.0, and purified in 10% PAGE (8 M urea) or by gel filtration on a Fractogel TSK HW-50 followed by reverse-phase chromatography on a Lichrosorb RP-18. The yield of the product was 25−30%.

2. *Derivatives Containing Only One Extra Nucleotide: $tRNA^{Lys3}$−$(pU)_1$ and $tRNA^{Lys3}$−$d(pT)_1$. (a) $tRNA^{Lys3}$−$(pU)_1$.* This derivative was obtained from tRNA−$(pU)_2$ by removing the 3′-terminal nucleotide. For that purpose a procedure adapted from that described by Paulsen and Wintermeier (*35*) was used. Oxidation of tRNA was carried out as follows: 10 $A_{260}$ units of tRNA dissolved in 10−20 $\mu$L of $H_2O$ were incubated with 3-fold excess of $NaIO_4$ for 1−1.5 h at 4 °C. The oxidized tRNA was precipitated with 0.5 mL of ethanol−acetone (1:1), washed by 1 mL of ethanol, and then lyophilized. The oxidized tRNA was incubated with 0.3 M aniline (distilled twice over zinc dust), pH 5.0, for 2 h at 25 °C. Aniline was removed by two ether extractions, and the shortened tRNA was precipitated with 0.5 mL of ethanol−acetone (1:1). The precipitate was washed with 1 mL of ethanol and lyophilized. To remove the 3′-phosphate, tRNA (50−100 $A_{260}$/mL) dissolved in 0.1 M Tris HCl, pH 8.0, and 0.1 mM $MgCl_2$ was treated with 4 U/mL alkaline phosphatase for 2.5 h, at 37 °C. tRNA was precipitated twice from 100 $\mu$L of 0.1 M sodium acetate, pH 5.0, with ethanol−acetone (1:1). The pellet was washed with 1 mL of ethanol and lyophilized. The yield of the product was 40−60%.

*(b) $tRNA^{Lys3}$−$d(pT)_1$.* tRNA (100 $A_{260}$/mL) dissolved in 0.1 M Tris HCl, pH 8.0, and 0.1 mM $MgCl_2$ was treated with 4 U/mL alkaline phosphatase for 2.5 h at 37 °C to remove the 3′ phosphate group. The synthesis of $tRNA^{Lys3}$−$d(pT)_1$ was realized in the presence of 50 mM Hepes, pH 7.5, 20 mM $MgCl_2$, 3.3 mM dithiothreitol, 15% DMSO, 100 $\mu$M ATP, 20 $\mu$M tRNA, 100 $\mu$M pTp, and T4 RNA-ligase. After incubation for 24 h at 4 °C, the product was purified in 10% PAGE (8 M urea).

3. *Synthesis of tRNA−CTT-3′ and tRNA−CTTT-3′.* $tRNA^{Lys3}$ lacking the last two nucleotides (C and A) from the 3′-end was prepared by oxidation of $tRNA^{Lys3}$ as described in the previous paragraphs. The tRNA−C-3′ thus obtained

was used for the synthesis of either tRNA−CTT-3′ or tRNA−CTTT-3′ by ligation as described for other tRNA derivatives.

*PAGE−Urea.* Samples were suspended in buffer containing 50% glycerol, 0.2% bromophenol blue, 0.2% xylene cyanol, and 8 M urea. The 10% polyacrylamide−8 M urea gels were prerun at 1200 V for 30 min and submitted to migration for 3−4 h at 1200 V. Gels were then autoradiographed.

*Reverse Transcriptase Assay.* The reaction mixture (50−100 $\mu$L) contained 50 mM Tris-HCl buffer, pH 8.0, 5.0 mM $MgCl_2$, 80 mM KCl, 0.1 mM EDTA, 10 mM dithiothreitol, 50 $\mu$M dTTP, and 1 $\mu$Ci [$^3$H]dTTP (56 Ci/mmol). A saturating concentration of poly(A), which does not inhibit the polymerization reaction, was used: 1.5 $A_{260}$ units/mL. The optimal template concentration was determined using high concentrations of primers (5−10 times the $K_m$ values). The polymerization reaction was started by the addition of 5−20 nM RT.

*Determination of Kinetic Parameters.* The kinetic parameters for HIV-1 RT were determined at 30 °C. Samples of the reaction mixture were taken at 2−10 min intervals. Initial rates of the polymerization reaction were determined from the tangents of the data curves at zero time. The Michaelis−Menten parameters, $K_m$ and $V_{max}$, were determined according to Cornish-Bowden direct linear plots (*36*). The statistical error was estimated as described before (*37*) using the data of 3−4 experiments. The statistical errors of $K_m$ and $V_{max}$ were within 10−40%.

## RESULTS AND DISCUSSION

Previous observations of our group and others on the interaction between HIV-1 RT and native or synthetic primers showed that the enzyme can bind the primer either in the presence or in the absence of the template. This behavior is unusual since the interaction of primers with cellular DNA polymerases is always template-dependent. Such unique HIV-1 RT template-independent primer recognition could be due to the formation of additional contacts between the enzyme and the 5′-end of the oligonucleotide (*38*). On the basis of these interactions, we designed new molecules capable of acting as substrates in the polymerization reaction catalyzed by HIV-1 RT. Thus, tRNA-derivatives containing 3′ oligonucleotide tags complementary to the poly(A) template were synthesized.

*Synthesis of tRNA Derivatives.* tRNA$^{Lys3}$ derivatives with various $d(pT)_n$ or $(pU)_n$ oligonucleotides at the 3′-end were synthesized by ligation using T4 RNA ligase. After ligation the derivatives were purified by gel-filtration and reverse-phase chromatography or by electrophoresis. In all cases they were electrophoretically homogeneous. Optimal conditions were found allowing the synthesis of tRNA analogues with a yield of 25−30%. tRNA−$(pU)_1$ was obtained from tRNA−$(pU)_2$ by oxidation of the 3′-terminal nucleotide with $NaIO_4$, followed by the elimination of one nucleotide as detailed in the methods section. The kinetic properties of these primers were determined in the presence of a complementary poly(A) template.

*tRNA Derivatives Used as Primers by HIV-1 RT.* A first step in the study of the interaction between HIV-1 RT and the tRNA derivatives was to determine whether they can be used as primers. The polymerization reaction catalyzed by RT was performed in the presence of poly(A), a template complementary to the oligonucleotide. As shown in Figure 1, both types of derivatives, tRNA−$d(pT)_n$ and tRNA−$(pU)_n$ were used as primers.

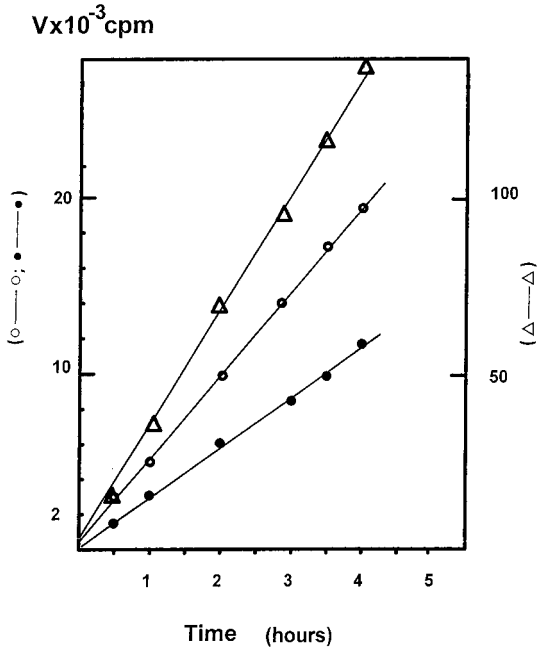


FIGURE 1: DNA synthesis catalyzed by HIV-1 RT in the presence of tRNA derivatives as primers. The rate of primer elongation was measured as described in Material and Methods, using a poly(A) template and the following primers: tRNA−$d(pT)_{13}$ (△), tRNA−$(pU)_{11}$ (○), and tRNA−$(pU)_2$ (●).

A control was necessary, however, to ascertain that we were measuring polymerization from the tRNA derivatives. We have previously shown that under certain conditions HIV-1 RT is able to catalyze DNA synthesis starting from mononucleotides used both as minimal primers and as nucleotide substrates in the presence of a complementary template. The rate of this *de novo* DNA synthesis is rather slow when compared to the polymerization primed by an oligonucleotide (*32*). To answer whether the polymerization observed in Figure 1 was the result of the synthesis primed from the tRNA derivative or from dTTP, an electrophoretic analysis of the polymerization products was performed. After incubation of RT with tRNA−[$^{32}$P]-$d(pT)_8$ in the presence of poly(A), high molecular weight products were formed (Figure 2, lanes 2 and 3). The same result was obtained with tRNA−[$^{32}$P]-$(pU)_{11}$ and tRNA−[$^{32}$P]-$(pU)_1$ (results not shown). A control using $d(pT)_{11}$ as primer gave a heterogeneous population of products (lanes 5 and 6). In the absence of tRNA derivatives no products were obtained (Figure 2, lane 4), thus showing that the *de novo* synthesis starting from α-[$^{32}$P]dTTP, if any, was negligible.

As the results showing the primer abilities of tRNA derivatives indicated that these molecules can initiate DNA synthesis, the kinetic parameters of the reactions were determined.

*$K_m$ Values of the tRNA Derivatives.* To analyze the primer efficiency of various tRNA derivatives in the reaction using a poly(A) template, we measured the Michaelis−Menten parameters for HIV-1 RT. As already shown for primers, the values of $K_m$ and of dissociation constants of the

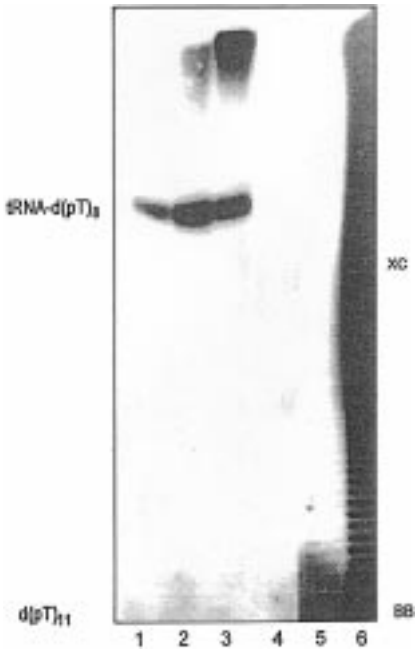


FIGURE 2: Electrophoretic analysis of the products synthesized by RT. RT was incubated in the presence of poly(A) as template, with different primers. Incubation was done in the presence of tRNA−[$^{32}$P]-d(pT)$_8$ incubated during 0, 10, and 20 min (lanes 1−3, respectively), [$^{32}$P]-dTTP for 20 min (lane 4), and d(pT)$_{11}$ for 5 (lane 5) and 10 min (lane 6). XC: xylene cyanol (Fluka). BB: bromophenol blue (Sigma).

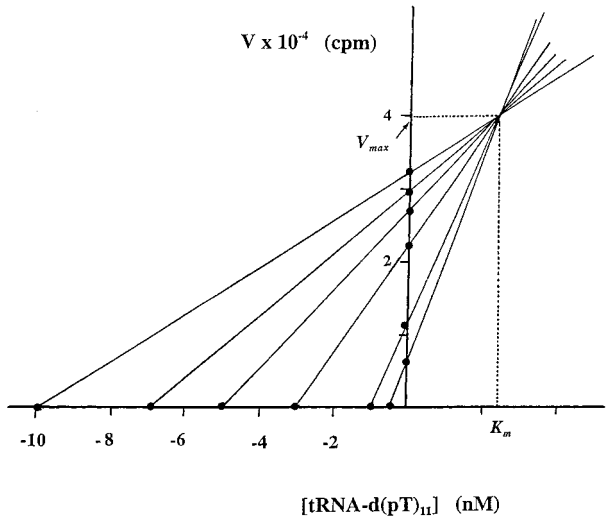


FIGURE 3: Initial rate of polymerization as a function of primer tRNA−d(pT)$_{11}$ concentration. $K_m$ and $V_m$ values were determined according to the representation of Cornish-Bowden. The common intersection point gives the values of $K_m$ and $V_{max}$ as indicated in the figure.

enzyme−template−primer complexes, $K_d$, were the same within a factor of 2 and, therefore, can be used to estimate the primer affinity (*39*). We determined the $K_m$ and relative $V_{max}$ according to the representation of Cornish-Bowden (Figure 3). These values for tRNA derivatives and the corresponding oligonucleotides are presented in Table 1. While the polymerization rate of tRNA derivatives was lower compared with the corresponding oligonucleotide primers, the affinity of tRNA derivatives was highly increased. This is a rather common situation since a higher affinity of HIV-1 RT for the template−primer generally correlates with a lower rate of DNA synthesis (*32, 40*).

Table 1. $K_m$ Values for tRNA Derivatives and Oligonucleotides Used as Primers

| tRNA−(pN)$_n$ | $K_m$ (nM) | relative $V_{max}$ (%)[a] | tRNA−(pN)$_n$ | $K_m$ (nM) | relative $V_{max}$ (%)[a] |
|---|---|---|---|---|---|
| tRNA−d(pT)$_1$ | 1.0 | 2 | tRNA−(pU)$_1$ | 1.5 | 3 |
| tRNA−d(pT)$_6$ | 2.0 | 4 | tRNA−(pU)$_2$ | 3.5 | 4 |
| tRNA−d(pT)$_8$ | 2.0 | 4 | tRNA−(pU)$_{11}$ | 4.5 | 5 |
| tRNA−d(pT)$_{11}$ | 2.0 | 5 | | | |
| tRNA−d(pT)$_{13}$ | 2.5 | 37 | | | |

| oligonucleotide | $K_m$ (nM) | oligonucleotide | $K_m$ (nM) |
|---|---|---|---|
| d(pT)$_2$ | 280 000 | (pU)$_2$ | 40 000 |
| d(pT)$_4$ | 140 000 | (pU)$_3$ | 18 000 |
| d(pT)$_5$ | 80 000 | (pU)$_5$ | 4 700 |
| d(pT)$_6$ | 38 000 | (pU)$_7$ | 860 |
| d(pT)$_8$ | 9 000 | (pU)$_{10}$ | 110 |
| d(pT)$_{10}$ | 1 600 | (pU)$_{11}$ | 100 |
| d(pT)$_{11}$ | 1 000 | | |

[a] $V_{max}$ values are relative to that of primer d(pT)$_{10}$ taken as 100%.

As shown in Table 1, a very interesting property of tRNA derivatives concerns their affinity for the enzyme. These compounds present $K_m$ values that are 2−5 orders of magnitude lower than the corresponding oligonucleotides. To ascertain if the differences found in affinity were not just due to tRNA-induced conformational changes in the enzyme, we measured the affinity of HIV-1 RT for oligonucleotides in the presence of tRNA$^{Lys3}$. In all cases the same $K_m$ and relative $V_{max}$ values were obtained for oligonucleotides, independent of the presence or the absence of tRNA.

With regard to the natural primer tRNA$^{Lys3}$, we have previously estimated its affinity for HIV-1 RT (*20*). Two $K_d$ values were found, 23 and 140 nM, corresponding to two sites of different affinity. Compared to different primers the affinity of HIV-1 RT for the tRNA derivatives is about 1 order of magnitude higher than the affinity for the natural primer, tRNA$^{Lys3}$, and 2−5 orders of magnitude higher than the corresponding oligonucleotides. This can be related to our previous results showing that HIV-1 RT has significant affinity toward mismatched primers (*38*). In the reactions catalyzed by most DNA polymerases, the $K_m$ values for partially complementary primers, such as (dT)$_n$(dC)(dT)$_m$, are practically the same as the $K_m$ values for (dT)$_m$. This means that complexes formed by these enzymes cannot recognize the 5′-end of the primers and that only the 3′-terminal nucleotide of the primer, in the template−primer duplex, interacts with the polymerase. In the case of HIV-1 RT we found a different situation: partially complementary oligonucleotides such as (dT)$_n$(dC)(dT)$_m$ presented higher affinity for RT that the corresponding complementary (dT)$_n$ or (dT)$_{n+m}$ primers. This indicates that HIV-1 RT besides interacting with the 3′-end is also able to interact with the 5′-terminal part of partially complementary primers. Furthermore, noncomplementary oligonucleotides cannot serve as primers for RT because their 3′-end base is sterically incompatible with the template. However, the presence of just an abasic unit in the 3′-terminal end of the oligonucleotide restores the capability to function as a primer for HIV-1 RT. As the noncomplementary part of the oligonucleotide cannot be complexed to the template, the interaction can only be due to additional contacts between the primer and the enzyme (*38*). Thus, we can assume that the 5′-end of oligonucleotide primers may interact with the tRNA-binding site of HIV-1 RT. To get further information about this

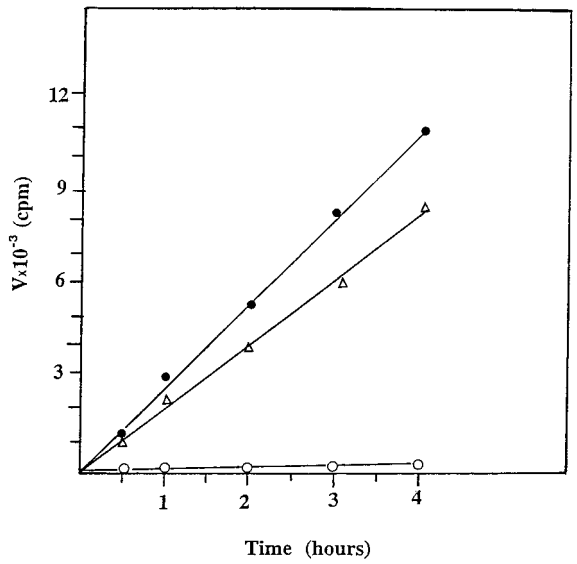


FIGURE 4: Polymerization catalyzed by HIV-1 RT in the presence of tRNA−CTT. The conditions were the same as in Figure 1. Key: tRNA−CTT (○); tRNA−CTTT (△); tRNA−d(pT)$_2$ (●).

interaction we determined the affinity of RT for mismatched primers in the presence or absence of tRNA. We found that in the absence of tRNA, the $K_m$ for a given mismatched primer, d(pC)$_7$d(pT)$_3$, was 20 $\mu$M, while in the presence of tRNA the affinity for d(pC)$_7$d(pT)$_3$ was highly reduced ($K_m$: 220 $\mu$M). This value was very similar to the $K_m$ for d(pT)$_3$ (200 $\mu$M), showing that in the presence of tRNA, only the 3′-terminal end of d(pC)$_7$d(pT)$_3$ interacted with HIV-1 RT. From these data we can conclude that the 5′ region of the mismatched primer cannot interact with the enzyme when tRNA is present.

Thus, the high affinity obtained with the tRNA derivatives may be explained considering that, in addition to the interaction with the 3′ terminal nucleotide of the primer, HIV-1 RT can form additional contacts with other regions of its natural primer tRNA$^{Lys3}$ (see scheme represented in Figure 5A,B).

*tRNA−CTT as a Possible Primer for RT.* To study the positioning of the terminal nucleotide of tRNA$^{Lys3}$ in the active site of the enzyme we prepared a tRNA in which the last two nucleotides C and A were replaced by TpT. Thus, this new tRNA, named tRNA−CTT, had the same length as normal tRNA$^{Lys3}$. We studied then the possible polymerization with this tRNA−CTT as primer and poly(A) as template. As shown in Figure 4, tRNA−CTT was not used as primer in the reaction catalyzed by HIV-1 RT. Interestingly enough, either tRNA−CTTT (a tRNA that is one unit longer than native tRNA$^{Lys3}$) or tRNA−d(pT)$_2$ were efficiently used as primers (Figure 4). In connection with that it is worth recalling that tRNA derivatives containing just one extra nucleotide served as primers (Table 1).

The inability of tRNA−CTT to primer DNA synthesis was an unexpected result considering the complementarity between poly(A) and the last two Ts present in tRNA−CTT. It can be hypothesized that, in the complex formed by RT and tRNA, the interaction between the primer binding domain and the 3′-terminus of native tRNA fixes the tRNA in such way that the 3′-OH cannot reach the polymerization site. Consequently, there is no polymerization. To have "normal polymerization" there must be translocation of the

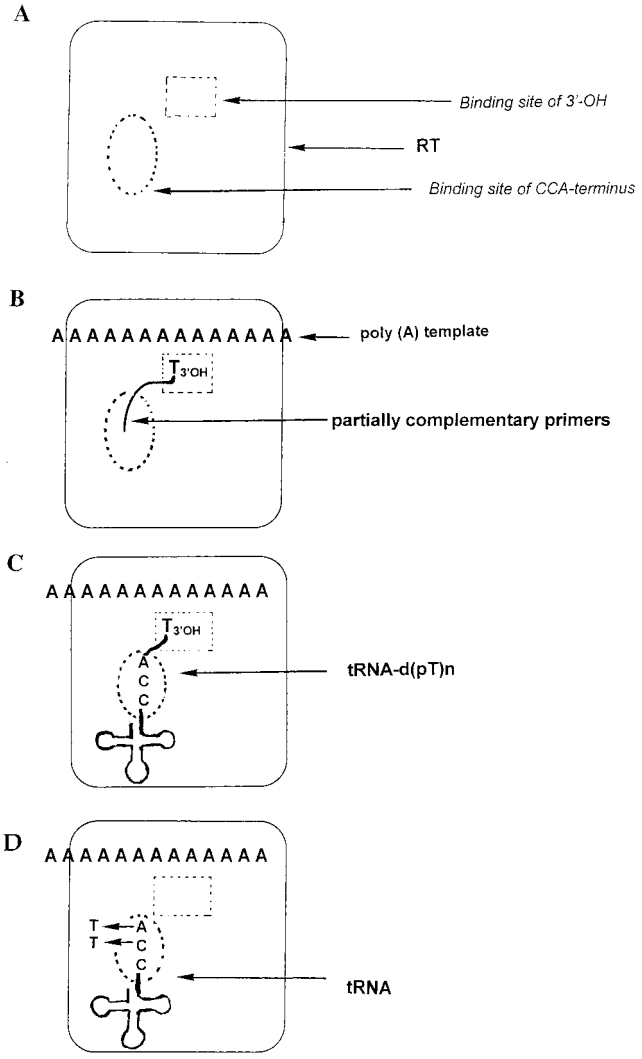


FIGURE 5: Schematic model representing the interaction between RT and the primers used in this work. (A) The binding site of the 3′-OH of any primer and the binding site of the CCA-terminus of tRNA are shown in the RT molecule. (B) A partially complementary primer interacts with RT in the presence of poly(A) template. Both binding sites are occupied. (C) the tRNA derivative in interaction with RT in the presence of poly(A). (D) RT in interaction with either normal tRNA or tRNA−CTT.

3′-terminus of tRNA allowing the efficient positioning of the A-terminal unit of tRNA on the enzyme. Such translocation may be difficult to obtain because of the need for partial melting of tRNA: the forces of interaction between the primer binding domain and the 3′-end of tRNA may not be as strong as to allow the melting and the following transition.

## CONCLUSION

HIV-1 RT can form an in vitro complex with tRNA$^{Lys3}$ before or after the enzyme interaction with the template. Previous to the annealing between tRNA and its complementary region in the retroviral template, native tRNA must undergo important structural changes. In native tRNA, the 3′- and the 5′-ends are at a short distance from each other, while this distance in the RT·tRNA complex depends on the degree of primer destabilization. It is certainly possible that RT in conjunction with NCp protein and/or the complementary regions of the template lead to destabilization of the

3′-end of the tRNA such that binding to the primer binding site can occur.

We have previously demonstrated that the binding of a template poly(A) does not prevent the interaction of HIV-1 RT with $tRNA^{Lys3}$ (*41*). Here we showed that, in the presence of poly(A), the tRNA derivatives were used as primers by HIV-1 RT. Although the template poly(A) is not complementary to primer tRNA, the addition of just one single nucleotide complementary to the template at the 3′-end of tRNA was sufficient to allow its utilization as primer as shown in the scheme presented in Figure 5C. Obviously the attachment of additional nucleotides to the 3′-end of tRNA allows the tRNA derivatives to be efficiently used as primers by the RT.

A very important observation, on the contrary, was that the replacement, from the 3′-terminal end of $tRNA^{Lys3}$, of the last two nucleotides, C and A by two Ts, gives a molecule with the same length as $tRNA^{Lys3}$ which is not elongated by RT in the presence of poly(A). It must be recalled that, unlike tRNA−CTT, tRNA−CTTT efficiently functions as a primer with poly(A). These unexpected results concerning the behavior of tRNA derivatives can be explained by assuming that, in the complex formed between HIV-1 RT and tRNA prior to binding the template, the tRNA is positioned one nucleotide away from the template in the active polymerization site of the enzyme (Figure 5D). This hypothesis would explain the inability of tRNA−CTT to be used as primer whereas the addition of just one extra nucleotide to the 3′-end of intact primer tRNA was sufficient for the primer to become active again.

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