Initiation of in vitro reverse transcription from $tRNA^{Lys3}$ on HIV-1 or HIV-2 RNAs by both type 1 and 2 reverse transcriptases

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Abstract HIV reverse transcription is initiated from a cellular tRNA partially associated with the retroviral genome. Here we studied homologous HIV-2 cDNA synthesis using natural or synthetic primers. With natural $tRNA^{Lys3}$, synthesis of early products comprising nucleotides +5 to +7 preceded the elongation step leading to synthesis of (−) strong-stop cDNA. In the presence of a poly(A)·oligo(dT) trap, no full-length product was observed while early products were still present, showing a transition between initiation and elongation. With DNA primers only an unspecific elongation was found. Our data show a similar mechanism of reverse transcription initiation by HIV-1 and HIV-2 reverse transcriptases. Furthermore, using a heterologous system we found that HIV-1 RNA, in contrast to data reported in the literature, was an excellent template for HIV-2 reverse transcriptase.

Key words: Human immunodeficiency virus type 1; Human immunodeficiency virus type 2; $tRNA^{Lys3}$; Reverse transcription; Initiation

1. Introduction

In addition to its role in protein synthesis and several other functions in various cellular processes, tRNA is crucial in initiation of retroviral DNA synthesis. Human immunodeficiency viruses type 1 and type 2 (HIV-1 and HIV-2) use the same primer, cellular $tRNA^{Lys3}$, to initiate reverse transcription of the (−) strand of proviral DNA [1–3]. The replication of HIV-2 has not been studied as extensively as that of HIV-1. Most of the reported data concerning HIV-2 have been obtained using synthetic primer/template complexes [4–6]. The HIV-2 genome, besides being 451 nucleotides longer than HIV-1 RNA, presents a higher complexity of its secondary structure, most specifically in the long terminal repeat (LTR) regions. HIV-1 and HIV-2 isolates have about 61% homology in the conserved *gag* and *pol* genes and 30–40% of homology in their LTRs sequences [7]. Fig. 1 shows schematically the secondary structure of the primer binding site (PBS) region of HIV-2 determined using chemical and enzymatic approaches [8]. The secondary structure of the HIV-2 PBS domain is different from that of HIV-1 [9]. The PBS seems more accessible in HIV-2 than in HIV-1: only three out of 18 nucleotides are involved in a base pair, while in the case of HIV-1, 12 out of 18 nucleotides are found in a double stranded structure.

The annealing between tRNA and PBS prior to reverse transcription implies interactions between two highly structured nucleic acids. Evidence, first reported in the case of avian retroviruses, demonstrated that regions in the genomic RNA other than the PBS interact with the primer tRNA. Indeed, contacts between the TΨC arm of the primer $tRNA^{Trp}$ and a region outside the avian retroviral PBS have been proposed [10,11]. In this model, avian reverse transcriptase (RT), $tRNA^{Trp}$ and the RNA genomic template form a complex in which the three components are maintained in a specific orientation allowing an efficient initiation of reverse transcription.

In HIV-1 several observations indicated that, in addition to the 3′ acceptor stem domain, other regions of $tRNA^{Lys3}$ are able to interact with HIV-1 viral RNA. Hybridization between an A-rich loop in the 5′ region of the PBS and the U-rich anticodon domain of primer $tRNA^{Lys3}$ has been described [12–14]. The importance of this interaction was also shown in HIV-1 infected cells [15–17]. The HIV-1 A-rich region is placed in the U_5 domain, close to the PBS, in a single stranded region of a stem loop. Sequence analysis of the regions around the PBS revealed a second A-rich cluster in 3′, putatively capable of interacting with the anticodon of $tRNA^{Lys3}$ [18]. Lanchy et al. suggested that the functional implication of this loop-loop interaction was to facilitate the switch between the initiation and the elongation steps during synthesis of the (−) cDNA strand [19]. In HIV-2 RNA four consecutive A bases, partially single stranded (positions 287–290 of $HIV\text{-}2_{ROD}$ RNA), are also present in the 5′ region near the PBS (see the empty black box in Fig. 1). This A-rich region may be a good candidate for interacting with the anticodon domain of $tRNA^{Lys3}$. Further studies of the interaction between $tRNA^{Lys3}$ and HIV-2 RNA are necessary to know if similar secondary primer-template interactions as those described for HIV-1 take place in the type 2 retrovirus.

Compared to HIV-1, very little is known concerning the initiation of cDNA synthesis by HIV-2 RT using a $tRNA^{Lys3}$ primer/HIV-2 RNA template duplex. Here, we studied HIV-2 reverse transcription initiation, and more specifically the synthesis of the cDNA early products. Natural and synthetic forms of $tRNA^{Lys3}$, as well as synthetic $tDNA^{Lys3}$, were used as primers of HIV-2 reverse transcription. Our results showed a pattern of cDNA synthesis initiation similar to HIV-1. Next we addressed the question whether HIV-2 RT was able to reverse transcribe in a heterologous systems. While it has been reported that only HIV-1, avian myeloblastosis virus, and simian immunodeficiency virus (SIV) RTs were able to elongate $tRNA^{Lys3}$ in the presence of HIV-1 RNA [20], we observed cDNA synthesis catalyzed by HIV-2

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Abbreviations: HIV, human immunodeficiency virus; RT, reverse transcriptase; PBS, primer binding site; ODN, oligodeoxyribonucleotide; ORN, oligoribonucleotide; LTR, long terminal repeat; SIV, simian immunodeficiency virus

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RT from $tRNA^{Lys3}$ on both HIV-1 and HIV-2 RNAs. Also, reverse transcription from the natural tRNA was obtained with HIV-1 RT on HIV-2 RNA.

2. Materials and methods

2.1. Materials

The pRT2 plasmid encoding the genes for the HIV-2 RT p68/p54 heterodimer (a kind gift of Prof. R. Goody, Max Planck Institute, Dortmund, Germany) was expressed in *Escherichia coli* and purified as previously described [21]. T7 RNA polymerase was from Stratagene, *Hin*cII and RNase-free DNase I from Gibco-BRL Life Technologies, and the human placental RNase inhibitor (RNasin) from Promega. Natural $tRNA^{Lys3}$ was purified from beef liver as already described [22]; the purified tRNA showed an aminoacylation level of 1200–1600 pmol/A_{260} unit. In vitro synthetic $tRNA^{Lys3}$ was prepared by T7 RNA polymerase from the pLys plasmid which contains the coding sequence (a kind gift from Dr. S.H. Wilson, North Carolina [23]) under the control of the T7 bacteriophage promoter. Radioisotopes [α-^{32}P]dATP (3000 Ci/mmol), [α-^{32}P]dCTP (3000 Ci/mmol) and [α-^{32}P]CTP (3000 Ci/mmol) were purchased from Amersham (Buckinghamshire, UK). Unlabeled nucleotides and 1 kb DNA ladders were from Gibco-BRL Life Technologies. Poly(A) and oligo(dT)$_{12-18}$ were from Sigma. The synthetic primer/template duplex was prepared as previously described [24].

2.2. In vitro HIV RNA synthesis

Plasmids pHIV-2 containing the nucleotides 1–545 from HIV-2_{ROD} strain and pmCG6 containing the fragment 1–4005 from HIV-1_{MAL} strain under the control of T7 promoter were a kind gift from Dr. J.-L. Darlix, INSERM, Lyon, France. *E. coli* HB 101 (1035) rec A$^-$ was used for plasmid amplification. After digestion of the HIV-2 clone by *Nco*I (position 545) and of the HIV-1 clone by *Hin*cII (position 521), both were transcribed by T7 RNA polymerase. 3 μg of linearized plasmid DNA was transcribed in a final volume of 0.1 ml containing 40 mM Tris-HCl (pH 8.0), 8 mM $MgCl_2$, 10 mM spermidine, 25 mM NaCl, 10 mM dithiothreitol, 0.5 mM of each ribonucleoside triphosphate, 100 units of T7 RNA polymerase and 20 units of placental ribonuclease inhibitor RNasin, for 1.5 h at 37°C. After treatment with 12 U of RNase-free DNase I for 10 min at 37°C, RNA transcripts were extracted with one volume of phenol/chloroform/isoamyl alcohol (24/24/1), then with one volume of chloroform and precipitated in 2.5 volumes of ethanol and 0.3 M ammonium acetate. The size and the integrity of the RNAs were checked by UV light after electrophoresis on a 1% agarose gel with ethidium bromide. RNA fragments containing the ROD and MAL sequences start both at position +1. Nucleotide sequences were checked and shown to be identical with the original clones [8,25].

2.3. Reverse transcription assay

Reverse transcription was initiated by preincubation, in a final volume of 10 μl containing 50 mM Tris-HCl (pH 8.0), 6 mM $MgCl_2$, 2 mM dithiothreitol, 15 mM NaCl, 100 nM HIV-1 or HIV-2 RNA, 100 nM $tRNA^{Lys3}$, 0.2 unit of RNasin and 150 nM HIV-2 or HIV-1 RT. With HIV-2 RNA as template, 5 μCi of [α-^{32}P]dATP (3000 Ci/mmol) was added, while [α-^{32}P]dCTP (3000 Ci/mmol) was used with HIV-1 RNA. Preincubation was carried out during 10 min at 37°C. Natural, synthetic $tRNA^{Lys3}$ and $tDNA^{Lys3}$ (1 μM) were prehybridized to the primer binding site of HIV RNA (1 μM) by heating 2 min at 95°C in the presence of 150 mM NaCl and slow cooling to 4°C. The four unlabeled dNTP precursors (200 μM each with HIV-2 RNA or 100 μM each with HIV-1 RNA), and 100 μM extra dATP or dCTP (respectively with HIV-2 or HIV-1 RNA) were then added and the incubation was continued for variable times at 37°C. When indicated 2.4 A_{260}/ml poly(A)·oligo(dT) (corresponding to 1.6 μM of oligo(dT)$_{12-18}$ primer) was used as a trap and added together with the dNTPs to bind free RT. The reaction was stopped by addition of 3 μl of gel loading buffer solution (10 mM Tris-HCl pH 8.0, 1 mM EDTA, 0.1% bromophenol blue, 0.1% xylene cyanol and 30% glycerol) and the tubes were kept in ice. When the reaction was performed in the presence of synthetic $tRNA^{Lys3}$, we used a uniformly ^{32}P-labeled tRNA, the yield of primer tRNA obtained by in vitro transcription by T7 RNA polymerase being rather low. In this case, neither labeled dNTP nor any excess of cold dNTP was added during the incubation. Reaction products were analyzed by electrophoresis on 6% polyacrylamide-TBE-7 M urea denaturing gels and autoradiographed.

3. Results and discussion

Experiments performed in this work were designed to study initiation during the first step of in vitro HIV-2 reverse transcription, as well as the cDNA synthetic ability of HIV-2 RT when using homologous or heterologous primer/template duplexes, involving natural or synthetic primers.

3.1. HIV-2 reverse transcription with RNA primers

Reverse transcription primed by $tRNA^{Lys3}$ with HIV-2 RT using HIV-2 RNA as template during different incubation times is shown in Fig. 2. Two series of intermediate cDNA bands corresponding to nucleotides +5 to +7 and +20 to +24 were observed between 15 s and 4 min of incubation (lanes 2–6). The strong-stop band (377 nt) was visible at 10 min (lane 8) and its intensity was maximal at 45 min (lane 10) of incubation. Interestingly, when poly(A)·oligo(dT) was used as a trap (lanes 11–14), no strong-stop band was visible even after 45 min at 37°C. Only three faint, but significant bands ap-

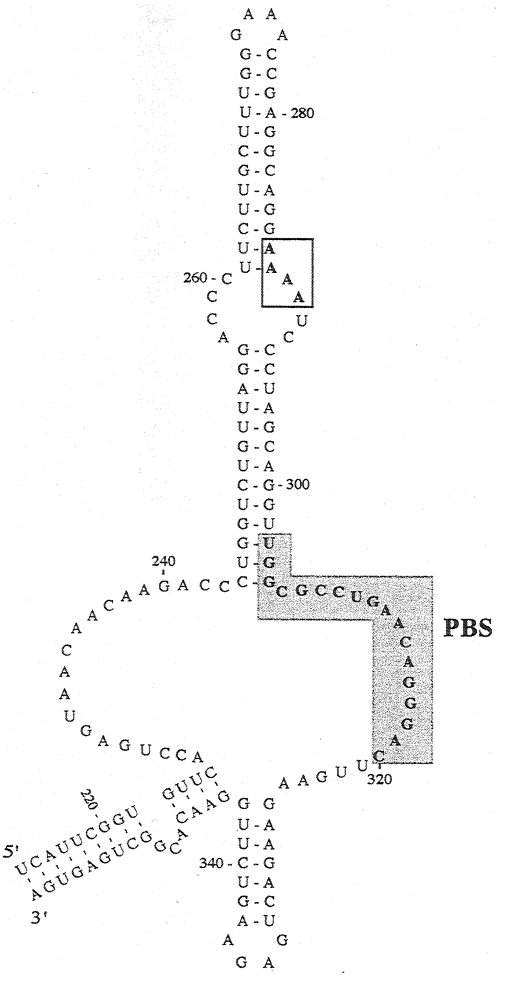

Fig. 1. Schematic model of the secondary structure of the PBS region of HIV-2 RNA (adapted from [8]). The gray box corresponds to the PBS sequence, the empty black box shows the A-rich region 5′ of the PBS.

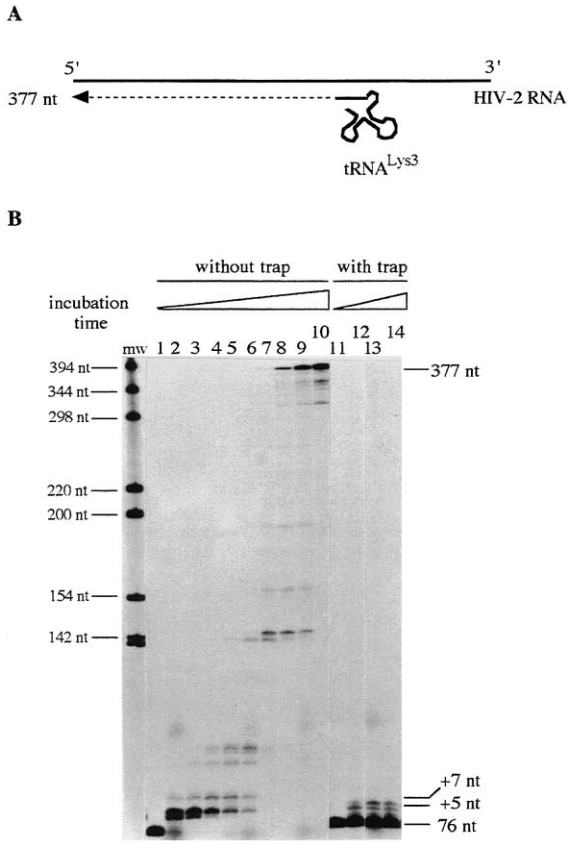

Fig. 2. Reverse transcription by HIV-2 RT of HIV-2 RNA template primed by $tRNA^{Lys3}$, in the presence or absence of a trap poly(A)·oligo(dT). A: Schematic representation of the expected size of full-length cDNA from $tRNA^{Lys3}$ primer. B: The reaction products were analyzed by gel electrophoresis as described in Section 2. Lanes 1–10: respectively, 0, 15 s, 30 s, 1, 2, 4, 7, 10, 30 and 45 min of incubation starting with the dNTPs; lanes 11–14: 0, 15 s, 10 and 45 min at 37°C starting with the dNTPs mixture and a poly(A)·oligo(dT) trap. Labeled 1 kb DNA ladders were used as size markers.

peared at 15 s, corresponding to the incorporation of nucleotides +1 to +5. These results are very similar to those described in the HIV-1 homologous system [26], but in the latter case the longest products observed in the presence of the trap corresponded to the addition of three nucleotides (data not shown). These results strongly suggest that the transition process from initiation to elongation in the early phase of reverse transcription is similar in HIV-1 and HIV-2 replication. As for HIV-1, the same molecule of HIV-2 RT is not able to initiate cDNA synthesis from HIV-2 RNA and to elongate it until the complete strong-stop band, as described previously in the case of $tRNA^{Lys3}$ primed homologous HIV-1 reverse transcription [27].

To further understand the mechanism of reverse transcription by HIV-2 RT, we studied the initiation primed with synthetic $tRNA^{Lys3}$. This in vitro synthesized primer does not have the modified bases present in native $tRNA^{Lys3}$. By chemical treatment of native tRNA leading to the conversion of a modified thio-uridine into cytidine, as well as by other experimental approaches, it has been reported that modified bases may play an important role in the $tRNA^{Lys3}$/HIV-1 RNA interactions [12–14].

As shown in Fig. 3, HIV-2 RT was able to use synthetic $tRNA^{Lys3}$ as primer and the intensity of the strong-stop cDNA band, which appeared after 7 min incubation (lane 7), increased regularly with time (lanes 8–10). However, no elongation of the labeled primer was observed in the presence of the poly(A)·oligo(dT) trap (lanes 11–14). Even the incorporation of 5–7 nucleotides observed with natural $tRNA^{Lys3}$ did not take place with the synthetic primer, suggesting that the transition between initiation and elongation steps did not occurred with the synthetic $tRNA^{Lys3}$. The same result was observed when a synthetic 18-mer ORN primer, complementary to the PBS sequence (anti-PBS), was used (results not shown). The data obtained with these two synthetic RNA primers were different from those previously reported where +3 and +5 nucleotide intermediate products were observed, but no complete cDNA synthesis [27].

In our in vitro system, in the presence of the poly(A)·oligo(dT) trap, only the natural $tRNA^{Lys3}$ was elongated by five nucleotides on HIV-2 RNA (three bases on HIV-1 template, see Fig. 5A), whereas the synthetic RNA primers were not (Figs. 2 and 3). Our results (Figs. 2 and 3) support the hypothesis that modified bases in natural tRNA are implicated in the recognition of the primer-template duplex by RT, and in a two-step mechanism in the early cDNA synthesis. However, the presence of post-transcriptional modifications in natural $tRNA^{Lys3}$, as well as the interaction between the anticodon of the tRNA primer and the A-rich region of HIV-1 RNA, may not be necessary for the strong-stop cDNA synthesis, since an anti-PBS ORN primer is efficiently elongated on HIV-1 and HIV-2 RNAs. It has been recently shown that deletion of the A-rich loop present in the U_5 region of HIV-1 RNA has no effect on the elongation of the annealed $tRNA^{Lys3}$ [28]. Our results are supported by recent evidence, including an elegant phylogenetic analysis, showing that a significant level of reverse transcription was observed in a murine leukemia virus [29] and in a SIV [30] in the absence of interaction between the anticodon of the tRNA primer and the A-rich region 5′ to the PBS.

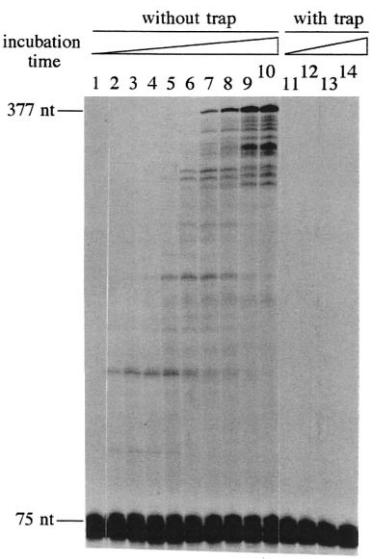

Fig. 3. Reverse transcription of HIV-2 RNA template by the homologous RT and a synthetic $tRNA^{Lys3}$ as primer. Same conditions as Fig. 2 were used. A similar result was obtained when a non-labeled synthetic $tRNA^{Lys3}$ was used in the same conditions described for natural tRNA primer.

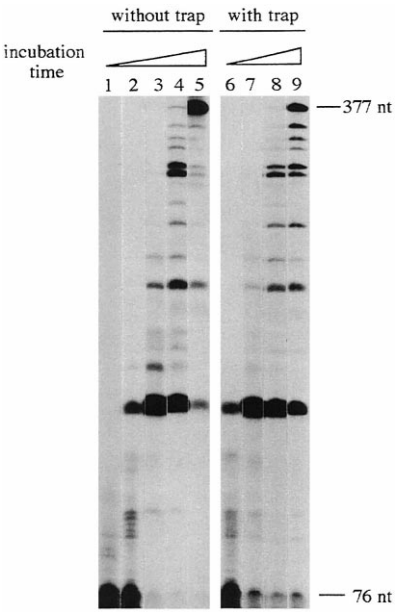

Fig. 4. Reverse transcription from a synthetic tDNA^{Lys3} primer in a homologous HIV-2 in vitro system. Lanes 1–5: respectively, 0, 15 s, 1, 7 and 45 min of incubation at 37°C. Lanes 6–9: 15 s, 1, 7 and 45 min of incubation in the presence of the trap.

3.2. HIV-2 reverse transcription with DNA primers

Compared to natural tRNA primer, a faster rate of cDNA synthesis was obtained (Fig. 4) when the reaction was primed by synthetic tDNA^{Lys3}, a molecule which is also devoid of modified bases. Similar results were obtained with a synthetic anti-PBS ODN. In the presence of these DNA synthetic primers, only 15 s were necessary to obtain an important cDNA band of about 100 nucleotides from HIV-2 RNA (45 nt in the case of HIV-1 template). Moreover, in reverse transcription primed by DNA, only a low decrease in the cDNA synthesis efficiency was observed in the presence of the poly(A)·oligo(dT) trap, showing that the same RT molecule was able to initiate and elongate the DNA primer without any transition step. Thus with DNA primers, initiation resulted from a non-specific mechanism. Similar conclusions have been reported previously, showing that ODN primers are very efficiently elongated by heterologous RTs [27].

Can the differences found between RNA or DNA primers be explained only by the affinity between RT and primer? Data obtained with HIV-1 RT showed that the enzyme presented a similar high affinity for anti-PBS ODN and natural tRNA primers, even if the observed processivity was very different [19]. In contrast, a low affinity of HIV-1 RT for synthetic RNA primers (anti-PBS ORN or synthetic tRNA^{Lys3}) was reported. However, gel retardation experiments and fluorescence determination showed similar affinities of HIV-1 RT towards the natural and synthetic tRNA^{Lys3} [31,32]. To explain our results, we can hypothesize that the conformation of the primer/template heteroduplex involving a DNA primer in interaction with HIV RT is different from the RNA/RNA natural duplex. This can be correlated to our previous data on cDNA synthesis using synthetic primer/template duplexes and HIV-1 RT [33]. Our results concerning riboprimers and deoxyriboprimers demonstrated a reverse correlation between the primer affinity and the rate of DNA synthesis. In the presence of a synthetic RNA template, while riboprimers presented a high affinity for HIV-1 RT, deoxyriboprimers gave the faster polymerization rate. Analysis of the binding and elongation of various template/primer duplexes showed that the optimal conformation of a template/primer for HIV-1 RT takes place when the template presents an A-like chain conformation (RNA) and the primer a B-like chain conformation (DNA) in the duplex. In this case a higher affinity and translocation rate is favored.

Furthermore, recent results showed that most (>95%) of

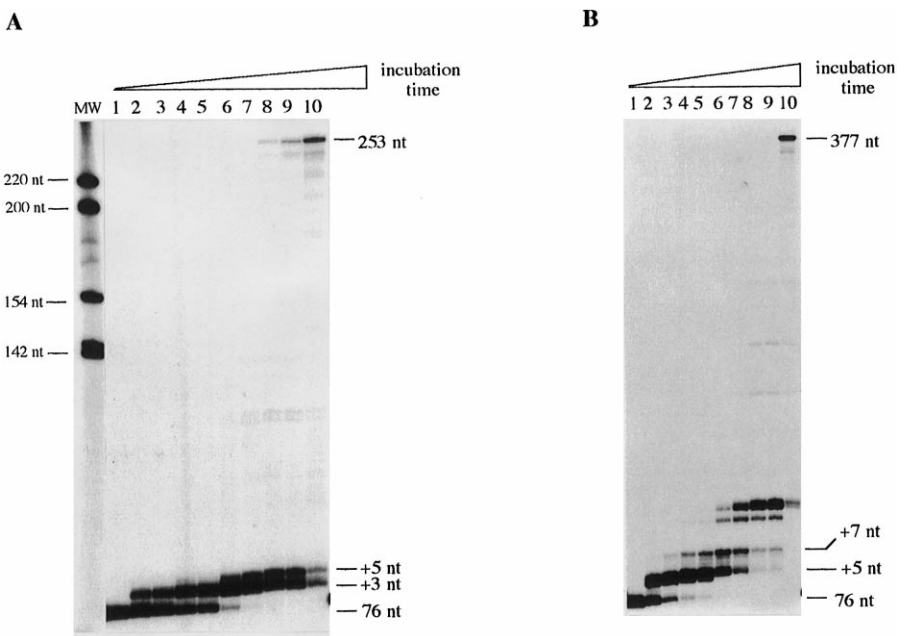

Fig. 5. Heterologous in vitro reverse transcription in the presence of natural primer tRNA^{Lys3}. A: cDNA synthesis by HIV-2 RT on HIV-1 RNA. Lanes 1–10: respectively, 0, 15 s, 30 s, 1, 2, 4, 7, 10, 30 and 45 min of incubation with the dNTPs. B: Reverse transcription by HIV-1 RT of HIV-2 RNA template. Identical incubation times as A were used.

the virion-extracted tRNA/viral RNA duplexes were found to be already elongated in HIV-2 and HIV-1 particles, implying that partial cDNA synthesis may take place in intact virions [26,34]. They suggested that extended $tRNA^{Lys3}$ could be more efficiently elongated by the RT enzyme, since its 3′ end corresponds to a DNA primer, which is characterized by a fast elongation. This could explain the differences observed between the distributive initiation and the fully processive elongation steps, only observed in natural $tRNA^{Lys3}$ primed reverse transcription.

3.3. Reverse transcription in a heterologous system

As mentioned above, both the initiation and elongation steps seem to operate in a similar way in HIV-1 or HIV-2 homologous cDNA synthesis systems. In the case of a heterologous system, however, previous reports showed that HIV-2 RT was unable to copy a HIV-1 RNA template in a natural $tRNA^{Lys3}$ primed reverse transcription reaction [20]. We addressed the question whether there will be heterologous reverse transcription in our experimental conditions. Results from Fig. 5A show that reverse transcription initiated by HIV-2 RT from a $tRNA^{Lys3}$ primer, using a HIV-1 RNA fragment corresponding to the 5′ retroviral genome as template, was very efficient. In this in vitro heterologous system, the 253 nt strong-stop cDNA was obtained after 10 min of incubation (lane 8). Incorporation of nucleotides +3 to +5 and faint intermediate bands were also visualized. Similarly, an efficient $tRNA^{Lys3}$ primed cDNA synthesis reaction using a HIV-2 RNA template and HIV-1 RT was observed ([26] and Fig. 5B). As both enzymes, HIV-1 and HIV-2 RTs, present similar processivity on the same template (Figs. 2 and 5B), we can assumed that the cDNA 'pausing' bands are due to the secondary structure of the template, rather than to differences in the processivity of the two retroviral enzymes.

Thus, the results presented here show that HIV-2 RT was able to reverse transcribe both HIV-1 and HIV-2 RNA at similar rates. This is in contradiction with previously reported data [20] where it was suggested that the inability to synthesize cDNA was related to a C-terminal deletion in the small subunit of the HIV-2 RT recombinant form [35]. However, using the same recombinant construction described in [21], i.e. an RT with a p54 subunit of 439 amino acids, we observed a significant elongation from natural $tRNA^{Lys3}$ on HIV-2 or HIV-1 RNA templates (Figs. 2 and 5, respectively). Similar results were obtained when we used different forms of HIV-1 and HIV-2 RTs, RNase H (+) or (−). Moreover, a p66/p66 homodimer HIV-1 RT was also able to elongate $tRNA^{Lys3}$ on HIV-1 RNA (results not shown).

In conclusion, two original observations have been described in this work. First, we showed that initiation of HIV-2 reverse transcription is a specific process with a transition between initiation and elongation. The mechanism is very similar to that reported for HIV-1. Both HIV-1 and HIV-2 share the same primer tRNA and possess an A-rich region 5′ to the PBS sequence. Such an interaction between $tRNA^{Lys3}$ and HIV-2 RNA has only been suggested [8,12,30]. Work is in progress using the antisense strategy on the A-rich mutated HIV-2 RNA region in order to obtain further proof of this interaction. Second, in contrast to previous reports [20], we showed that the HIV-1 RNA/$tRNA^{Lys3}$ complex was efficiently extended not only by the homologous HIV-1 RT, but also by a heterologous enzyme, HIV-2 RT.

Experiments designed to obtain a better understanding of the mechanism of HIV cDNA initiation synthesis seem very important to set up an efficient inhibitory strategy to block this key step of the retroviral replication cycle.

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References

[1] Litvak, S. (1996) in: Retroviral Reverse Transcriptases. R.G. Landes, Austin, TX.
[2] Coffin, J.M., Hughes, S.H. and Varmus, H.E. (1997) in: Retroviruses, Cold Spring Harbor Laboratory Press, Cold spring Harbor, NY.
[3] Mak, J. and Kleiman, L. (1997) J. Virol. 71, 8087–8095.
[4] Hizi, A., Tal, R., Shaharabany, M. and Loya, S. (1991) J. Biol. Chem. 266, 6230–6239.
[5] Shahabarani, M. and Hizi, A. (1991) AIDS Res. Hum. Retroviruses 7, 883–888.
[6] Fan, N., Rank, K.B., Poppe, S.M., Tarpley, W.G. and Sharma, S.K. (1996) Biochemistry 35, 1911–1917.
[7] Guyader, M., Emerman, M., Sonigo, P., Clavel, F., Montagnier, L. and Alizon, M. (1987) Nature 326, 662–669.
[8] Berkhout, B. and Schoneveld, I. (1993) Nucleic Acids Res. 21, 1171–1178.
[9] Baudin, F., Marquet, R., Isel, C., Darlix, J.-L., Ehresmann, B. and Ehresmann, C. (1993) J. Mol. Biol. 229, 382–397.
[10] Cobrinik, D., Aiyar, A., Ge, Z., Katzman, M., Huang, H. and Leis, J. (1991) J. Virol. 65, 3864–3872.
[11] Aiyar, A., Ge, Z. and Leis, J. (1994) J. Virol. 68, 611–618.
[12] Isel, C., Marquet, R., Keith, G., Ehresmann, C. and Ehresmann, B. (1993) J. Biol. Chem. 268, 25269–25272.
[13] Isel, C., Ehresmann, C., Keith, G., Ehresmann, B. and Marquet, R. (1995) J. Mol. Biol. 247, 236–250.
[14] Skripkin, E., Isel, C., Marquet, R., Ehresmann, B. and Ehresmann, C. (1996) Nucleic Acids Res. 24, 509–514.
[15] Wakefield, J.K., Wolf, A.G. and Morrow, C.D. (1995) J. Virol. 69, 6021–6029.
[16] Wakefield, J.K., Kang, S.M. and Morrow, C.D. (1996) J. Virol. 70, 966–975.
[17] Liang, C., Li, X., Rong, L., Inouye, P., Quan, Y., Kleiman, L. and Wainberg, M.A. (1997) J. Virol. 71, 5750–5757.
[18] Kohlstaedt, L.A. and Steitz, T.A. (1992) Proc. Natl. Acad. Sci. USA 89, 9652–9656.
[19] Lanchy, J.M., Ehresmann, C., Le Grice, S.F.J., Ehresmann, B. and Marquet, R. (1996) EMBO J. 15, 7178–7187.
[20] Arts, E.J., Stetor, S.R., Li, X., Rausch, J.W., Howard, K.J., Ehresmann, B., North, T.W., Wohrl, B.M., Goody, R.S., Wainberg, M.A. and Le Grice, S.F. (1996) Proc. Natl. Acad. Sci. USA 93, 10063–10068.
[21] Müller, B., Restle, T., Kuhnel, H. and Goody, R.S. (1991) J. Biol. Chem. 266, 14709–14713.
[22] Bordier, B., Tarrago-Litvak, L., Sallafranque-Andréola, M.L., Robert, D., Tharaud, D., Fournier, M., Barr, P.J., Litvak, S. and Sarih-Cottin, L. (1990) Nucleic Acids Res. 18, 429–436.
[23] Kumar, A. and Wilson, S.H. (1990) Biochemistry 29, 10717–10722.
[24] Tarrago-Litvak, L., Viratelle, O., Darriet, D., Dalibart, R., Graves, P.V. and Litvak, S. (1978) Nucleic Acids Res. 5, 2197–2210.
[25] Alizon, M., Wain-Hobson, S., Montagnier, L. and Sonigo, P. (1986) Cell 46, 63–74.
[26] Oude Essink, B.B., Das, A.T. and Berkhout, B. (1996) J. Mol. Biol. 264, 243–254.

[27] Isel, C., Lanchy, J.M., Le Grice, S.F., Ehresmann, C., Ehresmann, B. and Marquet, R. (1996) EMBO J. 15, 917–924.
[28] Li, X., Quan, Y., Arts, E.J., Li, Z., Preston, B.D., De Rocquigny, H., Roques, B.P., Darlix, J.L., Kleiman, L., Parniak, M.A. and Wainberg, M.A. (1996) J. Virol. 70, 4996–5004.
[29] Fossé, P., Mougel, M., Keith, G., Westhoff, E., Ehresmann, B. and Ehresmann, C. (1998) J. Mol. Biol. 275, 731–746.
[30] Berkhout, B. (1997) Nucleic Acids Res. 25, 4013–4017.
[31] Thrall, S.H., Reinstein, J., Wöhrl, B.M. and Goody, R.S. (1996) Biochemistry 35, 4609–4618.
[32] Weiss, S., König, B., Müller, H.J., Seidel, H. and Goody, R.S. (1992) Gene 111, 183–197.
[33] Nevinsky, G.A., Andréola, M.L., Jamkovoy, V.I., Levina, A.S., Barr, P.J., Tarrago-Litvak, L., Tharaud, D. and Litvak, S. (1992) Eur. J. Biochem. 207, 351–358.
[34] Huang, Y., Wang, J., Shalom, A., Li, Z., Khorchid, A., Wainberg, M.A. and Kleiman, L. (1997) J. Virol. 71, 726–728.
[35] Fan, N., Rank, K.B., Leone, J.W., Heinrikson, R.L., Bannow, C.A., Smith, C.W., Evans, D.B., Poppe, S.M., Tarpley, W.G., Rothrock, D.J., Tomasselli, A.G. and Sharma, S.K. (1995) J. Biol. Chem. 270, 13573–13579.