Strand Displacement Activity of the Human Immunodeficiency Virus Type 1 Reverse Transcriptase Heterodimer and Its Individual Subunits*

Michael Hottiger†, Vladimir N. Podust, Roberta L. Thimmig§, and Ulrich Hubscher ‡

From the University Zürich-Irchel, Department of Veterinary Biochemistry, Winterturstrasse 190, CH-8057 Zürich, Switzerland and the §University of Colorado Health Science Center, Department of Biochemistry, Biophysics and Genetics, Denver, Colorado 80262

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By using a DNA substrate with defined gap size, we found that human immunodeficiency virus type 1 reverse transcriptase (HIV-RT) was able to perform strand displacement DNA synthesis. This activity was not affected first by calf thymus proliferating cell nuclear antigen and replication factor C and second by Escherichia coli single-stranded DNA-binding protein, which together allow DNA polymerase δ to perform strand displacement DNA synthesis (Podust, V., and Hubscher, U. (1988) Nucleic Acids Res. 21, 841–846). 3'-Azido-2',3'-dideoxythymidine triphosphate inhibited displacement completely, indicating that DNA synthesis is required for this reaction. The HIV-RT p66 polypeptide alone could perform limited strand displacement DNA synthesis, whereas the HIV-RT p51 polypeptide was completely inactive, likely due to its inability to replicate extensively on a M13 DNA template. On the other hand the HIV-RT p51 polypeptide enhanced the strand displacement activity of the HIV-RT p66 subunit at a molar ratio of 4:1, mainly by chasing short products into longer ones. Furthermore, kinetic experiments after complementation of HIV-RT p66 with HIV-RT p51 indicated that HIV-RT p51 can restore rate and extent of strand displacement activity by HIV-RT p66 compared with the HIV-RT heterodimer p66/p51, suggesting a function of the 51-kDa polypeptide.

The human immunodeficiency virus type 1 contains a reverse transcriptase which is crucial for replication of its RNA genome into a double-stranded DNA, which eventually is integrated in the host cell genome (see, e.g. Jacobo-Molina and Arnold, 1991). The reverse transcriptase consists of two polypeptides, a 66-kDa (p66) and a 51-kDa (p51) subunit (Di Marzo Veronese et al., 1986). HIV-RT1 p66 is cleaved by the viral-protease encoded in the HIV genome into a 61-kDa polypeptide. 3'-Azido-2',3'-dideoxythymidine triphosphate inhibited displacement completely, indicating that DNA synthesis is required for this reaction. The HIV-RT p66 polypeptide alone could perform limited strand displacement DNA synthesis, whereas the HIV-RT p51 polypeptide was completely inactive, likely due to its inability to replicate extensively on a M13 DNA template. On the other hand the HIV-RT p51 polypeptide enhanced the strand displacement activity of the HIV-RT p66 subunit at a molar ratio of 4:1, mainly by chasing short products into longer ones. Furthermore, kinetic experiments after complementation of HIV-RT p66 with HIV-RT p51 indicated that HIV-RT p51 can restore rate and extent of strand displacement activity of HIV-RT p66 compared with the HIV-RT heterodimer p66/p51, suggesting a function of the 51-kDa polypeptide.

The abbreviations used are: HIV, human immunodeficiency virus; RT, reverse transcriptase; PCNA, proliferating cell nuclear antigen; RF-C, replication factor C; SSB, single-stranded DNA-binding protein; pS1, reverse transcriptase which is crucial for replication of its RNA genome into a double-stranded DNA, which eventually is integrated in the host cell genome (see, e.g. Jacobo-Molina and Arnold, 1991). The reverse transcriptase consists of two polypeptides, a 66-kDa (p66) and a 51-kDa (p51) subunit (Di Marzo Veronese et al., 1986). HIV-RT1 p66 is cleaved by the viral-protease encoded in the HIV genome into a 61-kDa polypeptide. 3'-Azido-2',3'-dideoxythymidine triphosphate inhibited displacement completely, indicating that DNA synthesis is required for this reaction. The HIV-RT p66 polypeptide alone could perform limited strand displacement DNA synthesis, whereas the HIV-RT p51 polypeptide was completely inactive, likely due to its inability to replicate extensively on a M13 DNA template. On the other hand the HIV-RT p51 polypeptide enhanced the strand displacement activity of the HIV-RT p66 subunit at a molar ratio of 4:1, mainly by chasing short products into longer ones. Furthermore, kinetic experiments after complementation of HIV-RT p66 with HIV-RT p51 indicated that HIV-RT p51 can restore rate and extent of strand displacement activity of HIV-RT p66 compared with the HIV-RT heterodimer p66/p51, suggesting a function of the 51-kDa polypeptide.
DraI (Boehringer) was carried out at 37 °C for 4 h according to the manufacturer's protocol.

The homopolymers poly(rA) and poly(dA) (Pharmacia; concentrations were determined according to the manufacturer's protocol) were mixed at a weight ratio 10:1 to the oligomer oligo(dT)12-18 (Pharmacia) in 20 mM Tris-HCl (pH 8), containing 20 mM NaCl and 1 mM EDTA, heated at 75 °C for 10 min with subsequent slow cooling to room temperature.

Buffers

The following buffers were used. Buffer A: 50 mM Tris-HCl (pH 7.5), 10% (v/v) glycerol, 1 mM EDTA, 1 mM DTT, and 1 mg/ml phenylmethylsulfonyl fluoride; Buffer B: 50 mM Tris-HCl (pH 7.5), 50% (v/v) glycerol, 1 mM EDTA, and 25 mM KCl; Buffer C: 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM EDTA; Buffer D: 50 mM Tris-HCl (pH 7.5), 50% (v/v) formamide, 20 mM EDTA, 0.03% (w/v) xylene cyanol, and 0.03% (w/v) bromophenol blue; Buffer E: 20% (w/v) sucrose, 50 mM EDTA (pH 8), 1% SDS, 0.03% (w/v) xylene cyanol and 0.03% (w/v) bromophenol blue.

Enzymes and Proteins

HIV-RT heterodimer p66/p51 was overexpressed in Escherichia coli (gift of L. Loeb, Joseph Gottstein Memorial Cancer Research Laboratory, University of Washington) and purified based on a previous publication (Thimmig and McHenry, 1993). All enzymes were purified as described (Thimmig and McHenry, 1993). The fraction containing RT activity after the phenol/chloroform column was pooled, dialyzed against buffer A, and then applied to an anion-exchange liquid chromatography Mono Q column equilibrated with the same buffer. Enzyme was eluted from the column with a 0-0.6 M KCl gradient in buffer A. Fractions with RT activity were eluted at 200 mM KCl. Pooled fractions were dialyzed against buffer B and stored at −20 °C. HIV-RT p66 and HIV-RT p51 were purified as described (Thimmig and McHenry, 1993). All enzymes were purified to >95% purity and had a specific activity (tested on poly(A)oligo(dT)8) of: HIV-RT heterodimer p66/p51, 2500 units/ml, 17,400 units/mg; HIV-RT p66, 17,100 units/ml, 15,000 units/mg; and HIV-RT p51, 1800 units/ml, 1400 units/mg. Calf thymus DNA polymerases α and δ were isolated as described in Podust et al. (1992b) and Weiser et al. (1991). E. coli SSB was purified from the overproducer RLM 727 following the procedure of Loeman et al. (1986). PCNA and RP-C from calf thymus were purified according to Prelich et al. (1987) and Podust et al. (1992a, respectively).

Enzymatic Assays

DNA Polymerases—RT activity was determined in a final volume of 25 μl containing 50 mM Tris-HCl (pH 7.5), 5 mM MgCl2, 80 mM KCl, 1 mM DTT, 0.5 mM EDTA, 10 μM [3H]dATP (500 cpm/pmol), 1 μg of poly(rA)/oligo(dT)(30) (base ratio, 10:1), and enzyme fractions to be tested. Activity measurement on single-stranded primed M13 DNA contained, in a final volume of 40 μl, 50 mM Tris-HCl, 1 mM DTT, 1 mM ATP, dATP, dGTP, dCTP each at 50 μM, 10 μM [3H]dATP (500 cpm/pmol), 100 ng of single-stranded primed M13 DNA, and enzyme fractions to be tested. DNA polymerase δ was tested with activated calf thymus DNA and DNA polymerase δ with poly[d(A)poly(dT)] (base ratio, 10:1) in the presence of PCNA as described (Weiser et al., 1991). 1 unit of DNA polymerase activity corresponds to the incorporation of 1 nmol of dNMP into acid-precipitable material in 60 min at 37 °C.

Strand Displacement Activity—The assay to measure strand displacement activity contained, in a final volume of 25 μl, the following components: 40 mM Tris-HCl (pH 7.5), 1 mM DTT, 0.2 mg/ml bovine serum albumin, 10 mM MgCl2, 1 mM ATP, dATP, dGTP, dCTP, dTTP each at 50 μM, 50 ng of gapped [32P]M13 DNA, and enzyme fractions to be tested. The mixture was incubated for 1 h at 37 °C unless otherwise mentioned and the reaction terminated by addition of 5 μl of buffer E. Samples were then loaded on a 12% PAGE (12 cm × 21 cm × 0.8 mm) and electrophoresis performed at 30 V/cm for 60 min. Finally the gel was fixed in 10% acetic acid containing 12% methanol, dried at 80 °C, and exposed to a x-ray film (Kodak, X-Omat S).

PhosphorImager

Gels with labeled separated DNA products were exposed for 3 h to a special screen for a PhosphorImager (Molecular Dynamics). After exposure, the screen was scanned into the PhosphorImager and the indicated regions (see Figs. 3, 5, 6, and 7) quantified by using the Image Quant program of Molecular Dynamics.

RESULTS

DNA Substrates Used to Study Strand Displacement DNA Synthesis—To measure strand displacement DNA synthesis, we used single-stranded M13 DNA annealed to two oligonucleotides of 40 bases in length (substrate A in Fig. 1). The gap between the two 40-mers was 230 nucleotides. The 40-mer at the left represented the primer and is for this reason called primer, whereas the 40-mer at the right side was used to act as a potential obstacle for DNA synthesis and is called terminator. The terminator could also be used as a primer. This did not interfere with the analysis, since primer extension assays were exclusively performed with labeled primer.

To test whether the displacement activity is dependent on DNA synthesis the same oligonucleotides as in A were used, but in this experiment the terminator instead of the primer was labeled at its 5' end. After annealing, the substrate was digested with the restriction enzyme DraI, producing a primer and terminator each of 30 nucleotides in length (substrate B in Fig. 1). This substrate allowed the discrimination of strand displacement activity from 5' to 3' exonuclease activity and also from endonucleases.

HIV-RT Heterodimer p66/p51 Can Perform Strand Displacement—Strand displacement of HIV-RT heterodimer p66/p51 was tested on a substrate as indicated in Fig. 1A. From our previous observations (Podust and Hübsher, 1993) it was known that the eukaryotic DNA polymerase α is able to fill the gap of 230 nucleotides nearly completely, but has no strand displacement activity. Instead the DNA polymerase δ has the possibility in presence of the three proteins PCNA, RF-C, and E. coli SSB, not only to fill the gap, but also to displace DNA. Therefore the two DNA polymerases were used as positive or negative controls for strand displacement activity (Fig. 2, lanes 1, 8, and 9, respectively). Lane 8 indicates that the two proteins, PCNA and RF-C, are necessary for strand displacement.
by DNA polymerase δ. *Lanes 2–7* show DNA synthesis by HIV-RT heterodimer p66/p51. The length of the product is the same irrespective of whether a terminator was present or not (*lanes 2 and 3*). The fact that the enzyme stops at the same place, even in the absence of a terminator, is likely due to known termination sites at this place of the single-stranded single-stranded M13 DNA, one of these immediately before the 5' terminus (NNNNNCGNNANNNA/T) occurs in this region of single-stranded M13 DNA, which partially inhibited DNA synthesis by HIV-RT heterodimer p66/p51 (Abbotts et al., 1993). Three such sequences (NNNNNGNANNNNT) occur in this region of single-stranded M13 DNA, one of these immediately before the terminus starts and the other two within 80 nucleotides of the terminator region but there are some DNA products clearly longer than 270 nucleotides. DNA synthesis by HIV-RT heterodimer p66/p51 was not inhibited in the presence of E. coli SSB (lanes 4 and 6). Simultaneous addition of E. coli SSB, PCNA, and RF-C, however, inhibited DNA synthesis 50% (*lane 5*) as well as strand displacement more than 90% (*lane 7*). These results suggested that HIV-RT is able to perform strand displacement in the absence of PCNA, RF-C, and E. coli SSB (Podust and Hubscher, 1993).

Strand Displacement by HIV-RT Heterodimer p66/p51 Is Dependent on DNA Synthesis—To see whether strand displacement is dependent on DNA synthesis, we tested the HIV-RT heterodimer p66/p51 with the substrate B shown in Fig. 1. *Fig. 3, lane 2*, shows that the HIV-RT heterodimer p66/p51 was unable in the absence of the four dNTP's to displace the 30-mer, thus excluding the possibility that the activity is a bona fide DNA helicase. DNA helicase activity could not be detected under different conditions tested, *e.g.*, by varying nucleoside triphosphates and comparing it with various cellular DNA helicases (Thömmes et al., 1992). If, however, the four dNTP's were included the terminator was displaced (*lane 3*). 50 mM KC1 stimulated displacement 20% (*lane 4*), whereas 100 mM reduced the activity more than 40% (*lane 5*). In no case could labeled monophosphates be detected, indicating that the 30-mer terminator was displaced as a whole. When the DNA synthesis was inhibited with AZTTP, a specific HIV-RT chain termination inhibitor displacement is decreased more than 99% (*lane 6*), suggesting that DNA synthesis was required for strand displacement. The band below the bound fragment (*lanes 3–5*) likely represents DNA synthesis from the terminator with substrate that was not cut by DraI (see also Fig. 1).

**Limited Strand Displacement Synthesis Can Also Be Carried Out by HIV-RT p66**—To test whether the strand displacement activity is due to the HIV-RT heterodimer p66/p51 or to one of the subunits, we investigated each of the two subunits alone. For a meaningful comparison, we first tested the activity of HIV-RT heterodimer p66/p51, HIV-RT p66, and HIV-RT p51 in a standard RT assay on poly(rA)oligo(dT). The rationale to measure HIV-RT p51 is described by Thimmig and McHenry (1993). The same amounts of RT activity on poly(rA)oligo(dT) (*Fig. 4A*) were then tested on single-stranded primed M13 DNA (*Fig. 4B*). *Fig. 4B* shows that if the same amount of RT activity on poly(rA)oligo(dT) was taken, the HIV-RT heterodimer p66/p51 and, to a lesser extent, the HIV-RT p66 polypeptide could perform DNA synthesis on a natural DNA-primed DNA substrate. The HIV-RT p51, on the other hand, was inactive. Also after testing different buffers such as KPO4, imidazol, HEPES,
incorporation for HIV-RT heterodimer p66/p51, HIV-RT p66, and for three HIV-RT forms were first titrated on poly(rA)/oligo(dT) and compared with each other for activity. 100% corresponds to 530 pmol/80 min incorporation for HIV-RT heterodimer p66/p51, HIV-RT p66, and for HIV-RT p51. B, the same amount of enzyme as in A on poly(rA)/oligo(dT) was tested in a reaction mixture containing singly primed M13 DNA as described under "Materials and Methods," resulting in 88 pmol for HIV-RT heterodimer p66/p51, 59 pmol for HIV-RT p66, and <0.1 pmol for HIV-RT p51. C, reactions were carried out under conditions as described under "Materials and Methods" by using singly primed M13 DNA (substrate A in Fig. 1) without terminator (lanes 1, 2, 4, 6, and 9) or gapped M13 DNA with terminator (lanes 3, 5, 7, and 9). Each reaction contained the same amount of units as in A for HIV-RT heterodimer p66/p51, HIV-RT p66, and HIV-RT p51. Markers used were: 20 nt corresponding to the labeled primer (lane 1); 270-nt marker was polymerized on a gapped M13 DNA with terminator by 0.5 unit of DNA polymerase α (lane 3).

**Fig. 4. Strand displacement activity by three forms of HIV-RT.** Three forms of HIV-RT were tested: 1) the HIV-RT heterodimer p66/p51, 2) HIV-RT p66 polypeptide, and 3) HIV-RT p51 polypeptide. A, the three HIV-RT forms were first titrated on poly(rA)/oligo(dT) and compared with each other for activity. 100% corresponds to 530 pmol/80 min incorporation for HIV-RT heterodimer p66/p51, HIV-RT p66, and for HIV-RT p51. B, the same amount of enzyme as in A on poly(rA)/oligo(dT) was tested in a reaction mixture containing singly primed M13 DNA as described under "Materials and Methods," resulting in 88 pmol for HIV-RT heterodimer p66/p51, 59 pmol for HIV-RT p66, and <0.1 pmol for HIV-RT p51. C, reactions were carried out under conditions as described under "Materials and Methods" by using singly primed M13 DNA (substrate A in Fig. 1) without terminator (lanes 1, 2, 4, 6, and 9) or gapped M13 DNA with terminator (lanes 3, 5, 7, and 9). Each reaction contained the same amount of units as in A for HIV-RT heterodimer p66/p51, HIV-RT p66, and HIV-RT p51. Markers used were: 20 nt corresponding to the labeled primer (lane 1); 270-nt marker was polymerized on a gapped M13 DNA with terminator by 0.5 unit of DNA polymerase α (lane 3).

**Fig. 5. Strand displacement activity by HIV-RT p66 in the presence of increasing amounts of HIV-RT p51.** A, reactions were carried out under conditions as described under "Materials and Methods" by using gapped M13 DNA containing a terminator (substrate A in Fig. 1). Markers were: lanes 1 and 2, 46 nt corresponds to initial labeled primer (lane 1); 270-nt marker was polymerized on a gapped M13 DNA with terminator by 0.5 unit of DNA polymerase α (lane 2). Lanes 3-9 contained 0.15 unit of HIV-RT p51 and were supplemented with various molar amounts of HIV-RT p51: lane 3, none; lane 4, 1:4 (HIV-RT p51:HIV-RT p66); lane 5, 1:2; lane 6, 1:1; lane 7, 3:1; lane 8, 6:1; lane 9, 10:1; lane 10, the same amount of HIV-RT p51 was used as in lane 9, but in the absence of HIV-RT p66. The two subunits were preincubated for 5 min at 37 °C. B, the two regions, indicated by 1 and 2, were quantified by using a PhosphorImager, and the counts were plotted against the different amounts of HIV-RT p51 added to 0.15 unit of HIV-RT p66.

**HIV-RT p51 Can Influence the Strand Displacement Activity of HIV-RT p66**—Next we tested whether the limited strand displacement activity of HIV-RT p66 can be reconstituted by addition of HIV-RT p51. Different ratios of molar amounts of

| Enzyme | Terminator | Lane |
|--------|------------|------|
| no enzyme | DNA pol α | 1 |
| DNA pol α | DNA pol α | 2 |
| HIV-RT p66/p51 | HIV-RT p66/p51 | 3 |
| HIV-RT p66 | HIV-RT p66 | 4 |
| HIV-RT p51 | HIV-RT p51 | 5 |
| HIV-RT p66 | HIV-RT p51 | 6 |
| HIV-RT p66/p51 | HIV-RT p66/p51 | 7 |
| HIV-RT p66 | HIV-RT p51 | 8 |
| HIV-RT p66/p51 | HIV-RT p66/p51 | 9 |

**Fig. 4.** Strand displacement activity by three forms of HIV-RT.

**Fig. 5.** Strand displacement activity by HIV-RT p66 in the presence of increasing amounts of HIV-RT p51.
experiment at a ratio of 1:4 of HIV-RT p66 to HIV-RT p51 (lanes 4–6) and was compared with the synthesized products during the same time points of HIV-RT p66 alone (lanes 7–9). HIV-RT heterodimer p66/p51 (lane 3) and, as outlined earlier, DNA polymerase α served as controls (lane 2). From the quantification of the band intensities with the PhosphorImager (Fig. 6B), it was obvious that the region above the primer (=region 2) decreased in presence of HIV-RT p51 (ratio, 1:4 (HIV-RT p66:HIV-RT p51)) 10 times faster than in its absence. Strand displacement was stimulated by HIV-RT p51, and it appears that both rate and extent are affected by HIV-RT p51 (see below).

Rate and Extent of Strand Displacement Can Be Restored upon Addition of HIV-RT p51 to HIV-RT p66—In a further experiment we reconstituted strand displacement of HIV-RT p66 with HIV-RT p51 in excess of enzyme over template/primer. Fig. 7 shows a kinetic analysis where region 1 (see Fig. 6) was quantified. As expected, HIV-RT p66 was able to partially displace the terminator, and HIV-RT p51 was completely inactive. If HIV-RT p51 was preincubated with HIV-RT p66 for 8 h at 4 °C it appears that both rate and extent of strand displacement activity was observed. Since the curve superimposed the corresponding one obtained with the authentic HIV-RT heterodimer p66/p51, we conclude that the HIV-RT p51 facilitates strand displacement of HIV-RT p66.

**DISCUSSION**

In this paper we show that the HIV-RT heterodimer p66/p51 can perform strand displacement. The data for the HIV-RT heterodimer p66/p51 are in agreement with an earlier report by Huber et al. (1989). This activity is not affected by calf thymus PCNA and RF-C or E. coli SSB (or replication protein A, data not shown). In addition strand displacement was dependent on DNA synthesis. To test whether the activity is due to one of the individual subunits of HIV-RT, the HIV-RT p66 and HIV-RT p51 were each tested individually. HIV-RT p51 was unable to perform extensive DNA-dependent DNA synthesis on single-stranded primed M13 DNA, whereas on the synthetic poly(rA)/oligo(dT), activity could be detected. HIV-RT p66, on the other hand, showed DNA synthesis activity on both templates. To a lesser extent than the HIV-RT heterodimer p66/p51, the HIV-RT p66 was able to perform strand displacement. This activity is enhanced upon addition of HIV-RT p51. This enhancement of strand displacement activity could already be seen at a molar ratio of 4:1 (HIV-RT p66:HIV-RT p51). Rate and extent of strand displacement could be completely restored upon addition of HIV-RT p51 to HIV-RT p66. In sum, the function of HIV-RT p51 might be to enhance the rate of DNA syn-
Fig. 8. A template challenge experiment suggests that the heterodimeric HIV-RT p66/p51 and HIV-RT p66 alone catalyze the strand displacement in a single round of DNA synthesis. Reactions were carried out under conditions as described under "Materials and Methods" by using gapped M13 DNA containing a terminator (substrate A in Fig. 1). Lanes 1 and 2, 40 nt corresponds to initial labeled primer (lane 1); 270-nt marker was polymerized on a gapped M13 DNA with terminator by 0.5 unit of DNA polymerase α (lane 2); lane 3, HIV-RT p66 (0.2 unit) was first mixed with the labeled substrate (40 fmol of 3' OH ends) for 2 min at 37°C, followed by the addition of four dNTPs; lane 4, HIV-RT p66 (0.2 unit) was mixed with the labeled substrate (40 fmol of 3' OH ends) and the challenge DNA poly(rA)/oligo(dT) (20 pmol of 3' OH ends, 500-fold excess) for 2 min at 37°C, followed by the addition of the four dNTPs; lane 5, HIV-RT p66 (0.2 unit) was first mixed with the labeled substrate (40 fmol of 3' OH ends) for 2 min at 37°C, followed by the addition of the four dNTPs with subsequent incubation for 1 min at 37°C and second by addition of the challenge DNA poly(rA)/oligo(dT) (20 pmol of 3' OH ends, 500-fold excess); lanes 6-8, same as lanes 4-6 with the HIV-RT heterodimer p66/p51 (0.15 unit) instead of the HIV-RT p66.

thesis and the strand displacement activity of HIV-RT p66. Up to now, no function of HIV-RT p51 could be found. A possible role of HIV-RT p51 might be to act in the asymmetric dimer (Thimmig an McHenry, 1993) as an enhancer of the strand displacement activity of HIV-RT p66.

In the E. coli bacteriophage Φ29 it has been found that mutations near the Ex0III box of Ψ29 DNA polymerase inhibited strand displacement by this enzyme (Soengas et al., 1993). A similar sequence is also found in DNA polymerase δ (Cullmann et al., 1993), an enzyme possessing strand displacement activity in the presence of E. coli SSB, PCNA, and RF-C (Podust and Hubscher, 1993) and in HIV-RT (Ratner et al., 1995). Site-directed mutagenesis experiments of these domains will be required to test the hypothesis of a general strand displacement domain in certain DNA polymerases.

The strand displacement activity of HIV-RT heterodimer p66/p51 could also play a critical role in the replication cycle of HIV-1 for review see, e.g., Varumus et al., 1985; McHenry, 1989. Low molecular weight DNA extraction of cells infected by HIV-1 resulted in populations of three types of DNA molecules. The first is a linear band of 9.5 kilobase pairs, probably out of the cytoplasm which seems to appear already after 4 h after infection (Kim et al., 1989) and to undergo integration after 24 h (Robinson and Zinkus, 1990). Two additional populations of relaxed circular forms of 15 kilobase pairs and a supercoiled form of 6 kilobase pairs are only formed in the nucleus (Shank and Varmus, 1978; Kim et al., 1989). With the strand displacement activity of HIV-RT heterodimer p66/p51, it is likely that double-stranded linear full-length HIV DNA is generated by strand displacement events of the resulting plus strand segment on the 3' polypurine tract, as was shown in melittin-permeabilized virions (Boone and Skalka, 1981). These linear double-stranded DNA molecules could then be transported into the nucleus, where they are integrated into the host genome.

Charneau and Clavel (1991) found a population of gapped linear molecules where the synthesis of the 5' half of the viral plus strand is stopped near the central polypurin tract, the initiation site of the 3' half. This stop seems to be discrete, which could be due to a limited amount of brief strand displacement of HIV-RT, whereas the gap might be caused through a mutation in this polypurine tract. A similar event was found in cauliflower mosaic virus (Pfeiffer and Hohn, 1983). The gapped molecules are then also transported to the nucleus and circularized apparently by using the repair functions of the host cell.

In conclusion, it is conceivable that the strand displacement activity of HIV-RT might be required in later stages of the HIV replication cycle, where DNA-dependent DNA synthesis occurs on double-stranded DNA.

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