DNA found in human immunodeficiency virus type 1 particles may not be required for infectivity

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We have studied the presence and significance of retroviral genome-derived DNA in the core of human immunodeficiency virus (HIV) particles produced from transfections of HXB2 expression vectors in COS-7 cells and from HIV type 1 IIIb chronically infected H9 cells. Viruses purified by sucrose cushion centrifugation and treated with DNase I contained 1000-fold more viral RNA than DNA. However protease-defective viruses that contained only p160\(^{\text{gp-pol}}\) had less than 100 times the amount of DNA in their cores than wild-type viruses suggesting that the p66/p51 form of reverse transcriptase was responsible for DNA transcription. Viruses produced by transfections in the presence of 3'-azido-3'-deoxythymidine (AZT) contained the viral RNA genome but only DNA of premature length because of the chain terminating effects of AZT. However such viruses were as infectious for CD4+ cells as wild-type virus. We conclude that retrovirus-derived DNA in HIV-1 particles is not required for infection and does not play a significant role in this process.

Introduction

The human immunodeficiency virus (HIV) contains two genomic RNA strands in its core, in association with the viral reverse transcriptase (RT) enzyme (Ferris et al., 1990; Lowe et al., 1988; Restle et al., 1990) and primer tRNA\(^{\text{lyS}}\) (Jiang et al., 1992; Kleiman et al., 1991). Reverse transcription of the genomic RNA template to form proviral dsDNA is thought to occur in the host cell cytoplasm after core entry (Weiss et al., 1985). RT exists in the virion as a heterodimer consisting of two subunits of 66K and 51K (Ferris et al., 1990; Larder et al., 1987; Lowe et al., 1988). This form of RT, p66/p51, binds to tRNA\(^{\text{lyS}}\) (Barat et al., 1989, 1991; Richter-Cook et al., 1992; Sarah-Cottin et al., 1992) to form a transcriptional complex on the primer binding site (PBS; Barat et al., 1989, 1991). Reverse transcription begins with RNA-dependent DNA polymerization to yield minus stop DNA (Haselton et al., 1976; Weiss et al., 1985). At the same time, the RNase H activity of RT degrades part of the unique (U)-5 and the repeat (R) region of the long terminal repeat (LTR; Gopalakrishnan et al., 1992). A template-switching event is then required for the near completion of minus strand DNA (Luo & Taylor, 1990; Panganiban & Fiore, 1988; Peliska & Benkovic, 1992). The plus strand appears to be primed by a polypurine-rich tract found 5' of the U3 region of the LTR (Omer et al., 1984).

Proviral DNA is believed to be generated as a consequence of reverse transcription of viral RNA in the cytoplasm. Recent studies described the presence of HIV DNA, synthesized from viral RNA templates, in HIV virions produced by chronically infected cells (Lori et al., 1992; Trono, 1992), and complemented earlier work on the presence of viral DNA in other retrovirus particles including avian leukosis and murine leukaemia viruses (Biswal et al., 1971; Levinson et al., 1970). In the case of HIV-1, both plus and minus DNA strands were detected by PCR amplifications and Southern blotting. In each case the amount of such material decreased in proportion to distance from the priming site. This suggests that reverse transcription can occur either in the cytoplasm, in immature virions prior to budding, or in mature virions. Although the form of RT responsible for these DNA transcripts is unknown, both groups (Lori et al., 1992; Trono, 1992) suggested that the p160\(^{\text{gp-pol}}\) precursor of RT might be responsible. Low RT activity (approximately 20% of wild-type) has been reported for p160\(^{\text{gp-pol}}\) using exogenous RT assays (Gottlinger et al., 1989; Jacobsen et al., 1992; Peng et al., 1989, 1991).

Interestingly, the HIV DNA found in virus particles appears to be of the same size and sequence as latent, labile, incomplete viral DNA found in quiescently infected lymphocytes (Zack et al., 1990, 1992). Therefore it is conceivable that the presence of HIV DNA in the virion core may be required for infectivity. The replication scheme of HIV and other retroviruses would then bear close resemblance to that of hepadnaviruses,
whose cores contain an asymmetric dsDNA necessary for completion of DNA elongation after the infection of host cells. Transcription of full-length viral DNA to RNA occurs in the nucleus; this RNA then acts as a template for reverse transcription prior to encapsidation and virus release (Ganem & Varmus, 1987).

We show here that treatment of COS-cells with 3'-azido-3'-deoxythymidine (AZT), prior to transfection with an HIV expression vector, also yields virus that contains 1000 times more genomic RNA than AZT chain-terminated DNA. If DNA in virions were required for infection, then virus produced in the presence of AZT should be non-infectious. The fact that such viruses were as infectious as wild-type viruses for H9 cells suggests that the packaging of HIV genome-derived DNA into viral particles is not required for infectivity.

**Methods**

**Plasmids.** The plasmids used for transfections were the wild-type HXB2 expression vector, SVC21 B410 and the protease-defective mutant pSVC-P1, kindly provided by Dr W. Haseloff (Dana Faber Cancer Institute, Boston, Mass., U.S.A.; Gottlinger et al., 1989). The pSVC-P1 plasmid contains sequences that code for a virus particle with only immature forms of p51/66 and other gag-pol gene products (determined by Western blotting; Gottlinger et al., 1989).

**Cell lines, virus stocks and virus infections.** Virus was obtained from the supernatant of either COS-7 cells transfected with HIV expression vectors or chronically infected human T lymphocyte H9 cells. The latter were obtained from the NIH AIDS Research and Reagent Program (Bethesda, Md., U.S.A.) and grown at 37 °C in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM-L-glutamine, 100 U/ml of penicillin G and 100 μg/ml of streptomycin. The COS-7 cells, derived from simian virus (SV)40-transformed African green monkey kidney cells, were grown at 37 °C as monolayer cultures in Dulbecco's modified Eagle's medium (containing high glucose and with glutamine but without sodium pyruvate) containing 10% FCS, 100 U/ml of penicillin G and 100 μg/ml of streptomycin.

Exponentially growing cells were harvested by trypsinization and replated at a density of 1 × 10⁶ cells per dish in 100 mm culture dishes, 24 h before transfection. Proviral plasmid DNA (10 μg) was added to each dish as a DNA–calcium chloride suspension. The method of CaCl₂-phosphate-mediated transfection was described previously (Jiang et al., 1992). In experiments performed with AZT, 2 μM of this drug was added to COS-7 cells 4 h prior to transfection and maintained until the virus was harvested. Triphosphorylation of AZT to the active form in cultured cells has previously been described (Furman et al., 1986; Gao et al., 1993). Viruses were isolated from the supernatant of transfected cells after 63 h of incubation. The culture fluids of transfected cells and chronically infected H9 cells were centrifuged in a Beckman SS-34 rotor at 3000 r.p.m., following which viruses were pelleted from the resulting supernatant by centrifugation in a Beckman Ti45 rotor at 26500 r.p.m. for 1 h. The viral pellet was purified by centrifugation at 26500 r.p.m. for 1 h through 15% sucrose onto a 65% sucrose cushion using a Beckman SW41 rotor. The viral band was removed, washed, passed through a 0.45 μm filter (Millipore) and resuspended in PBS. Viruses were then treated with 0.5 μg/ml of RNase-free DNase I (Canadian Life Technologies) at 37 °C for 30 min in the presence of 10 mM-MgCl₂. A sample of virus was used for p24 antigen capture analysis by ELISA (Abbott Laboratories).

H9 cells (2.5 × 10⁶ cells in 5 ml) grown in the culture conditions described above were exposed to dilutions of protease-defective virus, AZT-treated virus or wild-type virus (100 ng, 10 ng, and 0.1 ng of HIV p24) for 24 h at 37 °C. The AZT present in the transfection of COS-7 cells was removed from viral preparations by pelleting and sucrose cushion centrifugation. The cells were washed in PBS and serum-free RPMI-1640 medium, then resuspended at 5 × 10⁶ cells/ml in RPMI-1640 (with 10% FCS). Fresh medium was added over 11 days to keep the cell density at 5 × 10⁶ cells/ml. Culture fluids were removed at 12 days post-infection, clarified (centrifugation at 3000 r.p.m. for 30 min), and analysed for HIV p24 content by ELISA.

**Nucleic acid isolation.** The virus was lysed in TNE (10 mM-Tris–HCl pH 7.6, 100 mM-KCl and 10 mM-EDTA pH 8.0) containing 0.6% SDS at room temperature for 20 min. Proteins were removed by two phenol–s-aryl chloroform extractions (phenol equilibrated with 10 mM-Tris–HCl pH 7.6 and 10 mM-EDTA pH 8.0) followed by four s-aryl chloroform extractions. Yeast tRNA (10 μg; Pharmacia) per 200 μl of nucleic acid solution with a 1/10 volume of ammonium acetate and 3 volumes of ethanol was precipitated at −70 °C for 30 min. The pellet was resuspended in distilled water, then divided into two aliquots for preparation of the RNA and DNA. The RNA aliquot was treated with DNase I (0.5 μg/ml) in the presence of 10 mM-MgCl₂, 2 mM-DTT and 1 μl of recombinant RNasin (Canadian Life Technologies) for 30 min at 37 °C. The reaction was stopped with 100 mM-EDTA pH 8.0 and the RNA was extracted with phenol–chloroform, twice extracted with s-aryl chloroform and ethanol-precipitated under the conditions described above. This aliquot was used for RNA PCR amplification and the untreated aliquot was used for DNA PCR amplification. The concentration of viral nucleic acid could not be quantified by spectrophotometry. Therefore minor adjustments in the concentrations of viral RNA and DNA were made according to the levels of yeast tRNA, measured by spectrophotometry, after the purification steps.

**PCR amplification.** Quantitative PCR amplifications were used to estimate the initial copy number of both the DNA and RNA found in the virion. The RNA aliquot was reverse-transcribed prior to PCR amplification of DNA. The deoxyoligonucleotide primers used in the amplifications were designed for the HXB2 genome and modelled after those previously described (Zack et al., 1990). The following primer pairs were employed (A denotes antisense and S sense): A13 (nucleotide positions 635 to 614) and S1 (496 to 516) to amplify a 140 bp segment in the U5 region of the LTR, A2 (410 to 389) and S2 (221 to 242) to amplify a 207 bp segment in the U3 region of the LTR and AG4 (804 to 784) and SG4 (709 to 730) for a 95 bp segment upstream of the gag gene. The general locations of primers are represented schematically in Fig. 1. The primer pair Ga and Gs amplified a 110 bp segment in the cellular β-globin gene and was used as a control, with uninfected U-937 monocytic cell DNA (Zack et al., 1990).

The viral RNA aliquot was first reverse-transcribed using 100 pmol of the A13 primer and 200 U of Moloney murine leukaemia virus (MoMLV) RT (Canadian Life Technologies) in a solution containing 10 mM-dithiothreitol, 50 mM-Tris–HCl pH 7.8, 100 mM-KCl, 10 mM-MgCl₂ and 0.5 mM of each of the four dNTPs. The reaction was allowed to proceed at 37 °C for 1 h. The sense primer was labelled by a forward end-labelling exchange reaction using [γ-32P]ATP (1 μCi)/400 pmol of primer; Amersham; Chaconas & Van de Sande, 1980), before being added to a PCR cocktail containing 0.2 mM-dNTPs, 50 mM-KCl, 10 mM-Tris–HCl pH 8.3, 2.5 mM-MgCl₂ and 2 U of Tag polymerase (Canadian Life Technologies). The viral DNA aliquot and the reverse-transcribed RNA aliquot were subjected to 30 cycles of PCR (1 min at 95 °C, 1.5 min at 60 °C and 1 min at 72 °C). The plasmid pH2HXB2 was linearized with XhoI, diluted by 10-fold to copy numbers of
between \(10^7\) and one and then amplified by PCR for use as a quantification control for DNA amplifications. HIV RNA containing the LTR was obtained by in vitro transcription from a HIV RNA expression plasmid (Arts & Wainberg, 1994). Levels of RNA transcripts were quantified by spectrophotometry. RNA transcript preparations were then diluted, reverse-transcribed, amplified by PCR and used as approximate quantification controls for RNA amplifications. Yeast tRNA (2 \( \mu \)g) was added to each control amplification. The amplified products were separated by electrophoresis in a 7% denaturing polyacrylamide gel, which was then dried and autoradiographed. Quantification of product bands was performed by phosphor-imaging analysis (Bio-Rad). The copy number of linearized HXB2 or HIV RNA LTR was plotted against the c.p.m. of each product band as shown in Fig. 2. The equation of the lines generated by graphing \((R^2 > 0.94)\) was used to calculate the template copy number of the samples prior to PCR amplification (see legend to Fig. 2). Extrapolation of a curve of best fit then permitted the calculation of sample copy numbers. S.D.s were calculated from three independent PCR amplifications using the same viral RNA and DNA samples. The limit of detection in these PCR amplifications was less than 10 copies per reaction (Zack et al., 1992; data not shown).

**Results**

The presence of DNA in viruses produced by transfection of COS-7 cells

Transfection of COS-7 cells with an HIV expression vector provides a system with which to study HIV formation and budding, since the absence of surface CD4 receptors does not permit re-infection. To prevent contamination by viral DNA from lysed cells, the virus was pelleted by centrifugation, then purified by sucrose gradient centrifugation and treated with DNase I (see Methods). Using three HIV primer pairs (Fig. 1), PCR amplification of the HXB2 vector DNA in viruses produced by transfected COS-7 cells [COS(HXB2)] resulted in measurable levels of product (Fig. 3). The A13–S1 (Fig. 3a) and A2–S2 (Fig. 3b) primer pairs amplified a segment immediately downstream of the PBS in the U5 region of the LTR and following the first template switch in the U3 region of the LTR, respectively.

We found a slight decrease in the levels of products amplified by either of these primer pairs (Fig. 3a and b) in contrast to the AG4–SG4 primer pair (Fig. 3c), which amplifies a segment that is generated close to the end of full-length minus strand DNA synthesis (Fig. 3c). Uninfected U-937 cell DNA served as a negative control (Fig. 3b). PCR amplification with the \( \beta \)-globin primers (Ga and Gs) verified the presence of cellular DNA in our preparations. The copy number of A13–S1 template-derived samples, prior to amplification, was calculated as described in Methods (see Fig. 2 for a plot of the controls used in Fig. 3a). We found that approximately 500 copies of A13–S1 DNA (the limit of detection) were present in virus (2 pg of HIV p24) generated by COS-7 cells transfected by HXB2 plasmid (Table 1).

**DNA in viruses produced in the presence of AZT**

Experiments in which AZT (2 \( \mu \)m) was added to COS-7 cells 4 h before transfection yielded virus [COS(HXB2 + 2 \( \mu \)m-AZT)]. The DNA of these viruses was also amplified by PCR. Fig. 3(a) shows that virtually no differences were present in the levels of minus strong stop
Fig. 3. PCR amplifications, using various primer pairs, of HIV DNA from virus isolated from COS cell transfections with wild-type HXB2 virus in the absence or presence of 2 µM-AZT. In each set of amplifications using a specific primer pair, logarithmic increases in XhoI-linearized HXB2 plasmid copies were used as positive quantification controls. PCR-amplified virus DNA was isolated from virus purified by pellet centrifugation and sucrose cushion centrifugation, treated with DNase I and then lysed. Equal amounts of DNA in the COS(HXB2) and COS(HXB2 + 2 µM-AZT) were used in PCR amplifications using the A13-S1 (a), A2-S2 (b) and AG4-SG4 primer pairs (c). A negative cellular control is shown in (b) in which the B-globin primers, Ga-Gs, were included with the A2-S2 primers in PCR to show the presence of cellular DNA and absence of viral DNA using U-937 cells.

Table 1. Quantification of DNA and RNA molecules in viral particles

<table>
<thead>
<tr>
<th>Virus</th>
<th>Amount of p24 (pg) per PCR reaction</th>
<th>DNA copy number*</th>
<th>RNA copy number*</th>
<th>Ratio of DNA to RNA with A13-S1 primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>COS(HXB2)</td>
<td>2 ± 0</td>
<td>500 ± 50</td>
<td>800 ± 200</td>
<td>2 ± 0.3</td>
</tr>
<tr>
<td>COS(HXB2 + 2 µM-AZT)</td>
<td>1 ± 4</td>
<td>700 ± 100</td>
<td>100 ± 30</td>
<td>1 ± 0.1</td>
</tr>
<tr>
<td>COS(HXB2−protease)</td>
<td>210</td>
<td>2600 ± 800</td>
<td>1600 ± 300</td>
<td>1000 ± 300</td>
</tr>
<tr>
<td>H9(HXB2)</td>
<td>8 ± 2</td>
<td>2300 ± 500</td>
<td>2600 ± 600</td>
<td>4 ± 0.8</td>
</tr>
</tbody>
</table>

*Copy numbers from various amplifications were calculated from an equation derived from the line of a plot of known original template copy numbers of HXB2 versus c.p.m. of amplified product. The s.d. was calculated from three separate PCR amplifications on the same viral DNA or RNA preparations.
†ND, Not determined.

DNA (A13-S1) in viruses generated in the presence or absence of AZT or in estimated copy numbers (Table 1). In contrast, a sharp drop was observed in levels of DNA transcribed after template switching in viruses generated in the presence of AZT (Fig. 3b and c). It should be noted that the same concentration of AZT (2 µM) in the active triphosphorylated form efficiently blocks both HIV reverse transcription (Huang et al., 1990) and replication (Mitsuay et al., 1985). The relative copy number of the A2-S2 PCR amplification product was fivefold less in virus derived from AZT-treated COS-7 cells than in that from untreated cells (Table 1). However, there was a significant decrease in AG4-SG4 copy number in viruses harvested from both the COS(HXB2
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Fig. 4. PCR amplifications of HIV DNA from protease-defective virus isolated from COS cell transfections. The DNA of HIV-infected monocytic cells (U937-IIIb) amplified with the A2–S2 primer pair (b) was used as a positive control. Equivalent amounts of the DNA of the protease-defective virus COS(HXB2–protease) were used for PCR amplifications with the primers A13–S1 (a) and A2–S2. Linearized HXB2 plasmid copies were used as a positive quantification control.

Fig. 5. PCR amplification of viral RNA after the reverse transcription step. RNA from the wild-type viruses COS(HXB2) and COS(HXB2 + 2 µM-AZT) and RNA from the protease-defective virus COS(HXB2–protease) were amplified by PCR using the A13–S1 primer pair. HIV LTR RNA obtained from an in vitro RNA expression vector (data not shown) was used as a positive PCR quantification control. RNA from all samples was first reverse-transcribed with the A13 primer and MoMLV RT before PCR amplification (see Methods). Total cellular RNA isolated from chronically infected cells (U937-IIIb) was used as a positive control.

+2 µM-AZT) and COS(HXB2) cell types. This decrease was probably due to diminished reverse transcription in virus particles following elongation of viral DNA (Lori et al., 1992; Trono, 1992).

The presence of DNA in protease-defective virus produced by transfection of COS-7 cells

We next determined the ability of p160pg-poi to synthesize DNA by studying a protease-defective virus, which lacks the mature form of RT (p66/p51; Gottlinger et al., 1989). This virus does however contain p55pg that is recognized by p24 monoclonal antibodies in both immunoprecipitations and antigen capture assays (Peng et al., 1989, 1991). Lysates of such protease-defective particles have been reported to have about 20% of the RT activity found in wild-type viruses using exogenous oligo(dT)–poly(rA) primer templates (Gottlinger et al., 1989). PCR amplifications performed on nucleic acids extracted from p160pg-poi-containing particles (2 pg of p24) yielded no DNA product (the limit of detection per reaction was fewer than 10 DNA copies; data not shown). Viral DNA could be amplified when higher concentrations of virus particles (200 pg of p24) were employed (Fig. 4). A slight decrease was observed between levels of minus strong stop DNA (Fig. 4a) and minus strand DNA generated after the first template switch (Fig. 4b) for protease-defective virus [COS(HXB2–protease)] but not for wild-type virus [COS(HXB2)].

Quantification of RNA and DNA in HIV-1

The same nucleic acid samples used for DNA amplification were digested with DNase I and residual viral RNA was reverse-transcribed using the A13 primer. The generated DNA was used for PCR amplification using the A13–S1 primer pair (Fig. 5). Because of the extra reverse transcription step, the efficiency of amplification was lower than in the studies described above. It was possible to compare relative amounts of nucleic acid in these various viruses because the ratios of DNA to RNA were calculated on the basis of template copy numbers derived from internal PCR amplification controls. Although considerable variability (a range of 20%) was observed with regard to PCR amplification efficiency, the plots generated from positive control standards (for example, in Fig. 5) showed significant correlation ($R^2 > 0.95$).

All three viruses studied, COS(HXB2), COS(HXB2 + 2 µM-AZT) and COS(HXB2–protease) possessed much
more RNA than DNA (Fig. 5 compared with Fig. 3a and 4a, respectively). Quantitative analysis (based on the calculations described above) revealed DNA to RNA ratios of $10^{-4}$ for wild-type viruses COS(HXB2) and COS(HXB2+2 μM-AZT) and $10^{-6}$ for the protease-defective virus COS(HXB2–protease), as shown in Table 1.

Detection of viral DNA and RNA in HIV-1 from chronically infected H9 cells

Previous studies described the presence of DNA in viruses isolated from chronically infected H9 cells (Lori et al., 1992; Trono, 1992). We also examined the presence of DNA and RNA in such particles in order to relate the results described above using COS-7 cells to a system that did not involve transfection. Fig. 6 shows the results of DNA PCR amplifications of H9-derived virus (HXB2). As with wild-type virus derived from COS-7 cells [COS(HXB2)], the amount of A2–S2 product (Fig. 6b) did not differ significantly from that of A13–S1 product (Fig. 6a).

The RNA in H9(HBX2) was reverse-transcribed and the DNA product amplified by PCR using the A13–S1 primer pair (Fig. 6d). The amount of HIV RNA calculated to be present in these particles far exceeded levels of DNA (DNA to RNA ratio of $5 \times 10^{-4}$; Table 1).

Ratio of p24 antigen to genomic HIV RNA

The ratio of pg of p24 antigen to copies of HIV RNA was $1 \times 10^{-4}$, $1.4 \times 10^{-4}$, $2.1 \times 10^{-4}$ and $2.1 \times 10^{-4}$ for viruses COS(HXB2), COS(HXB2+2 μM-AZT), COS(HXB2–protease) and H9(HBX2) respectively. The expected value for infectious virions was $1 \times 10^{-7}$ pg of p24 per RNA copy, based on the approximate value of 2000
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Fig. 7. HIV p24 antigen production, after 12 days of infection, measured by ELISA. H9 cells (2.5 x 10^6 cells in 5 ml) were exposed for 24 h to 100, 10, 1 and 0.1 ng of HIV p24 from COS(HXB2) (■), COS(HXB2 + 2 μM-AZT) (□) or COS(HXB2 – protease) (□) virus stocks. Cells were maintained at a density of 5 x 10^5 cells/ml throughout the 12 days of incubation.

Infection of H9 cells

We infected H9 cells with similar dilutions of wild-type COS(HXB2) virus, COS(HXB2 + 2 μM-AZT) virus and COS(HXB2 – protease) virus (100, 10, 1 and 0.1 ng viral p24 onto 2.5 x 10^6 cells). Similar levels of productive HIV infection were obtained with most dilutions of wild-type virus and virus generated in the presence of AZT based on levels of p24 antigen in culture fluids (Fig. 7). The protease-negative virus was non-infectious for H9 cells. Although the cells were washed after viral adsorption, the low levels of p24 detected are most probably attributable to residual virus inoculum (fig. 7). Similar findings were obtained when these various viral preparations were used to infect fresh cultures of human peripheral blood mononuclear cells (data not shown).

Discussion

We have investigated whether viral DNA found in HIV particles (Lori et al., 1992; Trono, 1992) plays a functional role in HIV-1 infection. If this DNA were functionally significant, reverse transcription could be completed rapidly following core entry, allowing for integration and new viral synthesis. This would assume that viral DNA was bound to both RT and viral RNA, thus supporting further transcription.

To ascertain the significance of DNA in HIV, we first calculated the ratio of DNA to RNA by quantitative PCR amplifications, using virus obtained from culture fluids of HIV-1 strain IIIb chronically infected H9 cells and from COS-7 cells transfected with HIV expression vectors. We found that HIV RNA was at least 1000 times more abundant in virions than DNA, and that virtually no viral DNA was present in protease-defective viral particles. Recently, we have used the same method of quantitative PCR amplification to demonstrate chain termination by AZT during HIV infection of activated peripheral blood lymphocytes, brain macrophages (Geleziunas et al., 1993) and Jurkat cells (Arts & Wainberg, 1994).

We have shown that HIV-1 DNA does not play a role in infection. Initial viral RNA sequences transcribed by RT are at least 1000 times more abundant than equivalent DNA sequences, implying that most particles do not contain this DNA. In general, retroviral infection leads to a far higher proportion of non-infectious than infectious particles. This may be interpreted to mean that only the small fraction of HIV particles that contain DNA are infectious. We found that AZT, administered prior to transfections of COS-7 cells, prematurely terminated viral DNA elongation when compared with wild-type. AZT chain-terminated DNA is incapable of promoting further elongation, because of the absence of a 3' hydroxyl group (Zack et al., 1992). We have also shown, by quantitative PCR amplification, that AZT can chain-terminate elongating viral DNA in activated peripheral blood lymphocytes, quiescent brain macrophages (Geleziunas et al., 1993) and Jurkat cells (Arts & Wainberg, 1994). We found that chain termination by AZT in viral particles occurred only after the first template switch, which is consistent with the mode of action of nucleoside analogues in HIV-infected cells (Arts & Wainberg, 1994). However, virus generated in the presence of AZT was as infectious as wild-type for H9 cells, suggesting that HIV genome-derived DNA is not involved in infection. Of course short DNA fragments, not detected by the primer pairs used in the PCR amplifications, might have been
present and extended following infection. Our system did not permit us to determine the fraction of virus particles that were infectious for H9 cells.

Since HIV assembly occurs with precursor proteins that are processed during virion formation and budding, it is likely that synthesis of viral DNA might be catalysed by the p160\textsuperscript{gag-pol} RT precursor. Indeed, the RT activity of the Gag–Pol precursor was substantially less than that of wild-type processed RT (between 0 and 50% activity) when lysates of both HIV (Gottlinger \textit{et al.}, 1989; Jacobsen \textit{et al.}, 1992; Park \& Morrow, 1992; Peng \textit{et al.}, 1989, 1991) and other retroviruses were studied (Craven \textit{et al.}, 1991; Crawford \& Goff, 1984). In our experiments, PCR analysis revealed 100 times less DNA in protease-defective viruses than in wild-types and at least 10\textsuperscript{9} times less viral DNA than RNA. Because protease-defective viruses lack the virus-encoded enzyme that cleaves and processes p160\textsuperscript{gag-pol}, this suggests that mature RT (p66/p51) is involved in the synthesis of the DNA found in virus particles. The very low levels of DNA in protease-defective particles could be synthesized by p160\textsuperscript{gag-pol} or by traces of p66/p51 or p66/p66, processed from p160 by cellular proteases (Ferris \textit{et al.}, 1990; Lowe \textit{et al.}, 1988).

Viral DNA could be made by RT in the cytoplasm during viral formation, in the immature virion prior to budding, or in the virion after budding. The first of these alternatives is not supported by the lack of p66/p51 and the presence of p160\textsuperscript{gag-pol} in protease-defective particles (Gottlinger \textit{et al.}, 1989; Peng \textit{et al.}, 1989, 1991). The incomplete, heterogeneous size of the minus- and plus-strand viral DNA transcripts and very low levels of transcriptional initiation are consistent with the low nucleoside triphosphate pool found in virions (Gao \textit{et al.}, 1993).

In summary, viral DNA is present in viral particles but at levels at least 1000-fold less than viral RNA. The mature p66/p51 form of RT and not p160\textsuperscript{gag-pol} is apparently responsible for the synthesis of this DNA, which is present in fewer than 0.01% of virions and not required for the infection of susceptible cells.

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