JOURNAL OF VIROLOGY, Aug. 1992, p. 5067–5074
0022-538X/92/085067-08$02.00/0

Vol. 66, No. 8

Viral DNA Carried by Human Immunodeficiency Virus Type 1 Virions

FRANCO LORI,[1] FULVIA DI MARZO VERONESE,[2] ANTHONY L. DE VICO,[2] PAOLO LUSSO,[1] MARVIN S. REITZ, JR.,[1] AND ROBERT C. GALLO[1]*

Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20892,[1] and Advanced BioScience Laboratories, Inc., Kensington, Maryland 20895[2]

Received 26 February 1992/Accepted 11 May 1992

A fundamental step in the replication of retroviruses is the reverse transcription of the viral RNA genome into a double-stranded DNA provirus. Retroviruses are believed to carry genomic information only as RNA, and synthesis of DNA is thought to start only after virus entry into the infected cell. We report here that infectious mature human immunodeficiency virus type 1 virions contain viral DNA of heterogeneous size. This heterogeneity seems to result from random stops of reverse transcription during minus- and plus-strand synthesis. The DNA carried by human immunodeficiency virus type 1 virions presumably originates from reverse transcription which takes place prior to or during formation of the mature virus particle.

Reverse transcriptase (RT) is a versatile enzyme which modulates several activities: RNA-dependent DNA polymerase (which is unusual among DNA polymerases), DNA-dependent DNA polymerase (similar to cellular DNA polymerases), and an RNase H activity which removes the transcribed RNA (1, 30, 32). Human immunodeficiency virus type 1 (HIV-1) RT uses $tRNA_3^{Lys}$ as a primer to initiate the synthesis of a DNA minus strand complementary to the genomic RNA (32). While reverse transcription is proceeding, the RNase H activity digests the transcribed RNA, with the exception of two polypurine-rich stretches which are used by RT as primers for the synthesis of the DNA plus strand (32). The reverse transcription mechanism may involve RNA or DNA binding proteins, which for HIV-1 likely include the nucleocapsid proteins p7 and possibly p6 (2). However, the interactions which occur between the RT and other viral components during virion assembly and maturation are poorly understood. Reverse transcription is believed to occur only after a retrovirus has entered the target cell (32). At this time the mature form of RT converts the viral RNA into a DNA provirus which integrates into the host cell DNA and acts as a template for the production of viral RNA and proteins. These proteins are generally expressed as precursor molecules, including the Gag/Pol precursor, which is cleaved to yield mature RT during or after release of nascent virus (32). Since the Gag/Pol precursors of HIV-1 and other retroviruses have been shown to be enzymatically active (5, 12, 14, 20), reverse transcription could conceivably be mediated by either the Gag/Pol precursor or mature RT before or during the formation of a virus particle. Indeed, some RT-dependent DNA viruses have been shown to use reverse transcription during their replication cycle (8, 15, 29). In these viruses the viral RT catalyzes the synthesis of an incomplete DNA form which is then carried by the mature viral particle (3, 29). In this report, we asked whether HIV-1 RT could also operate at these early stages of the HIV-1 life cycle and, consequently, whether viral DNA could be carried by mature viral particles.

* Corresponding author.

MATERIALS AND METHODS

Purification of HIV-1 virions and HIV-1 RT. HIV-1(HTLV-IIIB) virions were double banded by sucrose gradients from the supernatant of chronically infected H9-IIIB cells as described previously (7). RT was purified from HIV-1_{IIIB} by immunoaffinity chromatography as described previously (7). Briefly, purified immunoglobulin G from the anti-HIV-1 RT monoclonal antibody M3364 was coupled to activated CH-Sepharose 4B by incubation for 16 h at 4°C in 40 ml of 0.1 M sodium bicarbonate, pH 8.0, containing 0.5 M NaCl. The gel was washed and further incubated for 1 h at room temperature in 40 ml of blocking medium to block remaining protein binding sites on the gel. The gel was packed into a column and extensively washed and equilibrated with phosphate-buffered saline (PBS) containing 0.1% Triton X-100. An extract of HIV-1_{IIIB} was applied to the immunoglobulin G-Sepharose column. The unbound proteins were collected, and the column was washed extensively with PBS. Proteins bound to the column were eluted with 0.2 M ammonium hydroxide and neutralized and concentrated by dialysis against 30% polyethylene glycol 6000 in 50 mM Tris-HCl, pH 8.0, containing 0.1 M NaCl, 8 mM dithiothreitol, and 0.1 mM phenylmethylsulfonyl fluoride. For enzyme assays, 1 mg of bovine serum albumin per ml was added to an aliquot of the concentrated column eluate as a preservative.

Sucrose density analysis of HIV-1. One milliliter of 500× concentrated HIV-1_{IIIB} was DNase treated, layered on a 20 to 60% (by weight) sucrose gradient in TNE (10 mM Tris-HCl, 100 mM NaCl [pH 8.0], 10 mM EDTA), and centrifuged overnight at 35,000 rpm in a SW47 rotor. Twenty fractions of 0.5 ml were collected from the bottom of the gradient, resuspended in TNE, and centrifuged for 2 h at 35,000 rpm in a SW47 rotor. Pelleted virus was lysed in 125 mM Tris (pH 8.0)–0.25% Triton X-100–0.25 M NaCl, and portions from the same fractions were assayed for RT, quantitative polymerase chain reaction (PCR) using primers M667/AA55, and immunoblot (using serum from an HIV-1-infected individual as the source of antibodies).

DNase and RNase treatment of samples. DNase digestions of intact virus samples were performed in TN (TNE without EDTA) by incubating the samples for 1 h at 37°C with 10 U of RNase-free DNase (Boehringer) per ml in the presence of 6 mM $MnCl_2$. After DNase digestion, the viral samples were

washed twice in TNE to eliminate DNase. DNase or RNase digestions of viral extracts were performed for 1 h at 37°C with 10 U of RNase-free DNase (Boehringer) per ml or 10 U of DNase-free RNase per ml, respectively. Before PCR analysis was performed, DNase or RNase was digested with 100 μg of proteinase K per ml for 1 h at 56°C, and proteinase K was inactivated for 20 min at 95°C.

PCR procedures. DNA was extracted from all samples as described previously (22). PCR amplifications were performed as described previously (33). Quantitation of HIV-1 DNA during PCR amplification was obtained by comparison with a standard curve of serial dilutions of pHXB2(Rip7) plasmid DNA (16). The copy number used for the standard curve ranged from 10 to 1,000 copies, as indicated in the figures. The samples were loaded onto a 10% polyacrylamide gel and run at 50 V for 2 h. The gels were exposed to autoradiography for 3 h at −70°C.

Southern blot experiments. Ten micrograms of pHXB2 (Rip7) was digested with *Sst*I, *Hin*dIII, *Pvu*II, *Bgl*II, *Ssp*I, or *Xho*II, electrophoresed on a 1% agarose gel, and transferred onto nitrocellulose paper. The filter was prehybridized at room temperature with 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–10% Denhardt's solution–0.1% sodium dodecyl sulfate (SDS) for 1 h and hybridized under the same conditions for 3 h. To obtain HIV-1 RT endogenous probes, 100 μl of purified RT was pretreated with bovine pancreatic RNase by incubation in 50 mM Tris-HCl (pH 8.0)–100 mM NaCl–8 mM dithiothreitol–0.1 mM phenylmethylsulfonyl fluoride–1 mg of bovine serum albumin per ml–100 μg of RNase A per ml–20 U of RNase T_1 at 37°C for 30 min; 10 mM $MgCl_2$ and 50 μCi each of [^{32}P]dATP, [^{32}P]dCTP, [^{32}P]dGTP, and [^{32}P]TTP (3,000 Ci/mmol; Amersham) were then added to the mixture, and it was incubated at 37°C for 1 h. The proteins were digested with 100 μg of proteinase K per ml at 37°C for 1 h and extracted twice with phenol-chloroform and twice with chloroform. The probe obtained was ethanol precipitated, resuspended in TE, and boiled 5 min before use. Filters were washed three times at 22°C with 6× SSC–0.1% SDS and twice for 20 min at 37°C with the same buffer. The autoradiograms were exposed overnight at −70°C.

RESULTS

Viral DNA is carried by mature HIV-1 particles. To verify whether viral DNA is carried by mature viral particles, HIV-1 virions, purified by double banding on sucrose gradients, were analyzed for the presence of viral DNA by PCR amplification (Fig. 1A, B, and C). Viral DNA was found to be associated with the purified virions. This DNA was completely insensitive to DNase treatment of the intact viral particles, as demonstrated by determination of the amount of DNA in the pelleted virus before and after DNase treatment (Fig. 1A), thus suggesting that the DNA is specifically contained within the virion. In contrast, the same DNase treatment completely digested all the extraviral DNA present at low levels in the supernatant fraction (Fig. 1A). To exclude the possibility that the DNA in the viral pellet could be contained in subviral particles or cellular debris (and therefore might not be affected by DNase treatment), HIV virions were first treated with DNase and then were subjected to sucrose density centrifugation. Fractions from the sucrose gradient were analyzed for the presence of viral DNA, RT activity, and viral proteins. The association between complete HIV-1 particles and viral DNA was demonstrated by the almost perfect correlation between the peaks of RT activity, amounts of DNA, and positivity for viral proteins as detected by immunoblot assay (Fig. 1B). These results strongly argue against the presence of DNA as due to nonspecific contamination. Furthermore, the concomitant presence in the same fractions of the major capsid protein p24 and the external envelope protein gp120 with the DNA suggests that this DNA is carried by complete mature viral particles. Treatment of lysed virus from positive fractions with DNase but not RNase before PCR analysis eliminated the positive signal, confirming the DNA nature of the sequences amplified by PCR (Fig. 1C).

The viral DNA carried by HIV-1 is heterogeneous in size. To further analyze the nature of the DNA carried by HIV-1 particles, the gradient fractions were extracted and a quantitative PCR assay, previously applied to quantitate HIV-1 DNA in infected peripheral blood mononuclear cells (33), was employed to amplify several regions of the HIV-1 genome. The primers used to amplify the viral DNA were M667/AA55 (33) (R-U5 in Fig. 2), M667/BB301 (33) (R-PB in Fig. 2), and M667/M661 (33) (R-gag in Fig. 2). M667 is a sense primer in the R region of the long terminal repeat (LTR). AA55 is an antisense primer immediately 5′ to the PB (tRNA primer binding) region. The M667/AA55 primer pair amplifies the minus-strand region initially synthesized by RT. BB301 is complementary to the PB region. Amplification by M667/BB301 can be achieved in the presence of plus-strand DNA which has been synthesized starting at the polypurine tract upstream from the right LTR and, after jumping to the other end of the template, extended up to the PB region (32). The minus strand which is not fully completed is not expected to be amplified because sequences complementary to the PB are RNA, which has been digested in these experiments (Fig. 2). M661 is an antisense primer in the *gag* region. Amplification by M667/M661 reflects the presence of complete minus-strand DNA. These primers were designed to estimate the extent of reverse transcription at three replicative steps: (1) initial minus-strand synthesis, (2) initial plus-strand synthesis up to the tRNA primer binding region, and (3) complete minus-strand synthesis (Fig. 2A). Steps 1, 2, and 3 occur in subsequent order during reverse transcription (32). DNA synthesis is finally completed with the formation of the full-length double-stranded DNA. If the DNA carried by the virus were a full-length double-stranded DNA, the three regions analyzed by quantitative PCR should be amplified to equivalent levels. However, the virus-associated DNA did not appear to be equally represented in the three regions analyzed (Fig. 2B). The majority of the DNA appeared to represent early synthesis of the minus strand in the R-U5 region of the LTR, while a smaller portion of DNA appeared to be a product of complete minus-strand synthesis (R-gag in Fig. 2). The amount of initial plus-strand DNA synthesized up to the tRNA primer binding site (R-PB in Fig. 2) also appeared to be greater than that of the complete minus strand, indicating that the synthesis of plus strand had efficiently started. The progressive decrease of the DNA amounts from step 1 to step 3 indicated that the synthesis by RT decreased with the distance from the origin of retroviral DNA replication.

Previously we determined that immunoaffinity-purified RT could mediate a Mg^{2+} dependent, RNase-insensitive, endogenous polymerase activity in the absence of exogenously added template-primer (13). This was found to be due to the fact that the RT copurified with the virus-associated DNA, as shown in Fig. 2B. We took advantage of these findings in order to further characterize the nature of the endogenous DNA template. To determine which region of the viral DNA

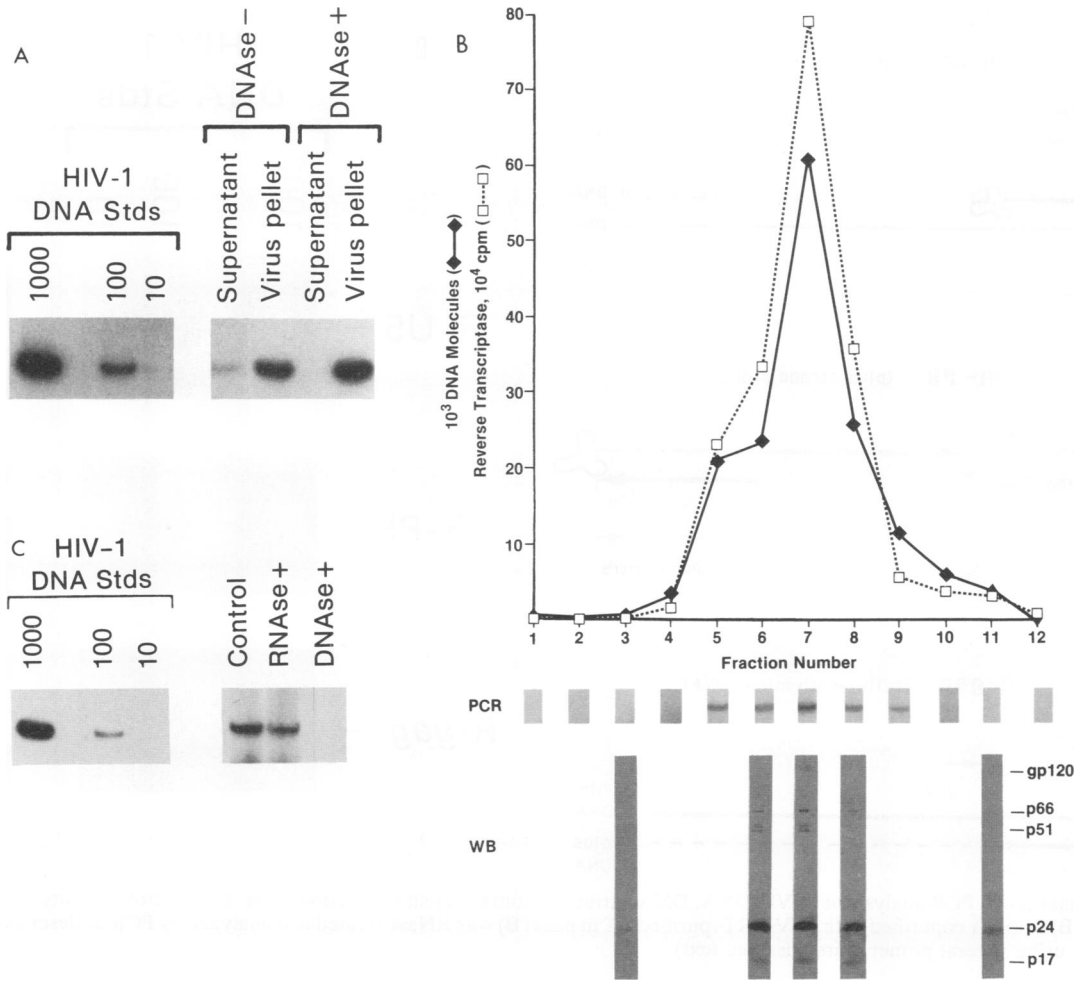

FIG. 1. Presence of viral DNA in HIV-1 virions. (A) Double-banded purified virus (8 ml) was centrifuged, and the supernatant was saved for PCR analysis (supernatant DNAse$^-$). The pellet was resuspended in 8 ml of TN (virus pellet DNAse$^-$) and subjected to DNase treatment. After extensive washing in TNE to eliminate the DNase, the virus was centrifuged and the supernatant was saved for PCR analysis (supernatant DNAse$^+$). The pellet (virus pellet DNAse$^+$) was resuspended in 8 ml of TNE. Ten microliters each from the supernatant DNase$^-$, pellet DNase$^-$, supernatant DNase$^+$, and pellet DNase$^+$ was analyzed by quantitative PCR using primers M667/AA55. (B) The data illustrated in the graph are relative to the first 12 fractions (the remaining fractions were negative for all the assays performed) of HIV-1 sucrose density analysis. The quantitation of the number of HIV-1 DNA molecules was performed after PCR by measuring the intensity of each band (the bands are reported below the graph as PCR) as quantified by absorbed β-emission using a Molecular Dynamics PhosphorImager in comparison with a standard curve of serial dilutions of HXB2(RIP7) plasmid DNA (16). Some representative fractions (fractions 3, 6, 7, 8, and 11) analyzed by immunoblot are designated WB. (C) Virions from fraction 7 (panel B) of sucrose density gradient were lysed, and three aliquots were preincubated without DNase or RNase (control), with DNase-free RNase (RNAse+), and with RNase-free DNase (DNAse+). Quantitative PCR was performed by using primers M667/AA55.

acted as a template for RT, the labelled product of the RT endogenous reaction was hybridized to a Southern blot of cloned HIV-1 DNA [pHXB2(Rip7)] (16) digested with several restriction enzymes. The fragments which most intensely hybridized with the DNA probe generated by the RT endogenous reaction (Fig. 3b) shared a minimum common region located between the *Pvu*II and *Xho*II sites at nucleotides 3925 to 5047 (23). The *Pvu*II-*Xho*II region contains a polypurine tract (nucleotides 4365 to 4380) used as a primer to initiate plus-strand DNA synthesis. A synthetic oligomer specific for this region was shown to hybridize to the same fragments which were most intensely labelled by the RT endogenous probe (Fig. 3c), whereas a probe representing the full-size HIV-1 genome gave a completely different pattern (Fig. 3a). The observation that the viral DNA can act as template-primer for DNA synthesis by RT in vitro provides further evidence that some of the DNA carried by the HIV-1 does not represent a complete provirus. Conceivably, plus-strand DNA was synthesized during this reaction, since the synthesis was not affected by RNase pretreatment, indicating that the template was not RNA. This was confirmed by extracting the viral DNA and performing reconstitution experiments with the use of HIV-1 recombinant RT. Recombinant RT activity was not affected by alkali pretreatment of extracted viral DNA (data not shown).

HIV-1 DNA can be synthesized during virus maturation. The presence of incomplete DNA in mature HIV-1 particles implies that reverse transcription can occur before or during the formation of mature virus particles. To further explore this possibility, we transfected cos-1 cells with a biologically

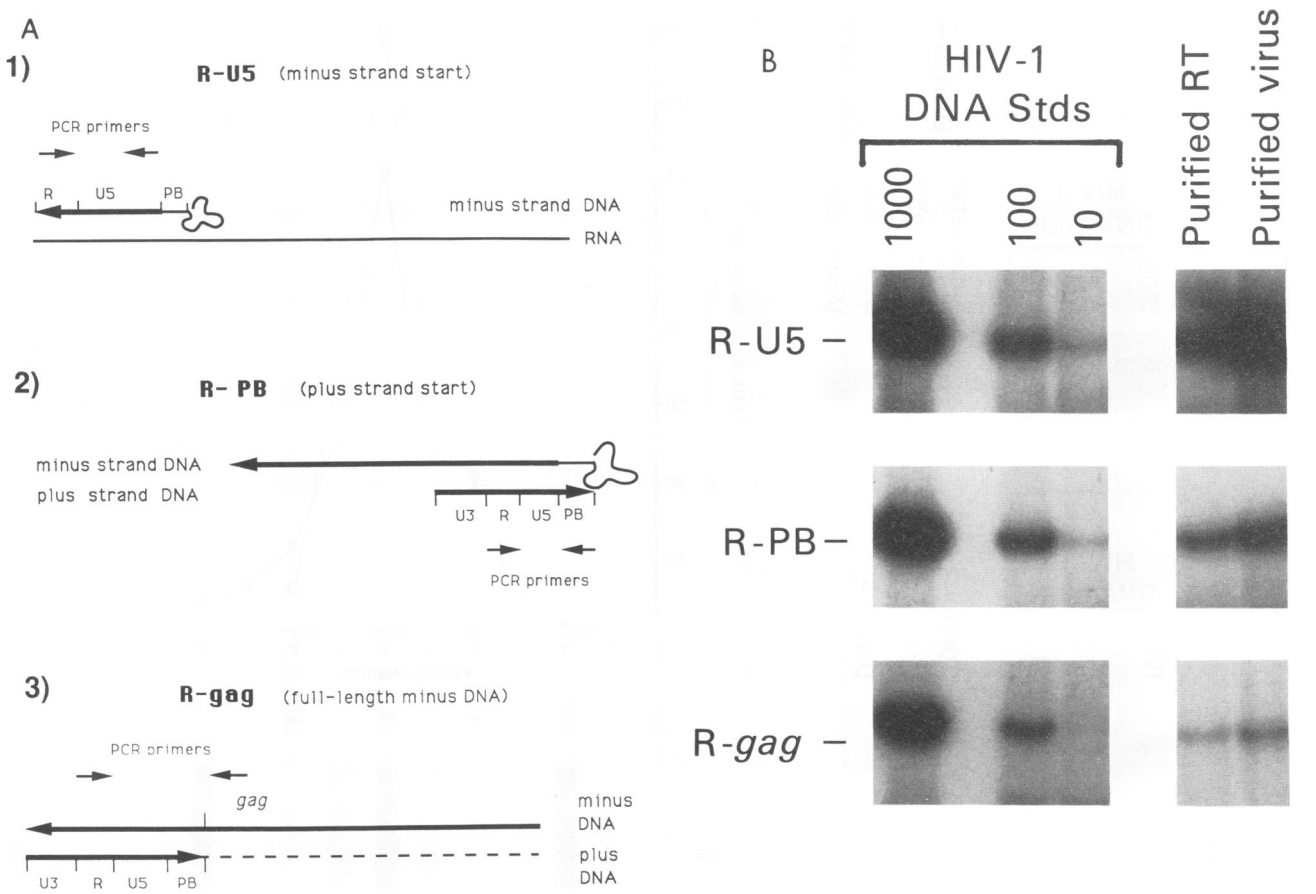

FIG. 2. Quantitative PCR analysis of HIV-1 DNA. DNA extracted from the positive fractions (Fig. 1) of sucrose density gradient (purified virus in panel B) or DNA copurified with HIV-1 RT (purified RT in panel B) was RNase treated and analyzed by PCR as described in Materials and Methods, using several primer pairs (also see text).

active molecular clone of HIV-1 [pHXB2(Rip7)] (16). Since cos-1 cells lack the CD4 receptor, a secondary infection by virus released from the transfected cells should not occur. This was confirmed by using the virus produced after cos-1 transfection to subsequently infect fresh nontransfected cos-1 cells. PCR analysis of these cells did not reveal any viral DNA (data not shown). In such a system, any products of DNA synthesis by reverse transcription would have to be generated independently from infectious events. RT was purified from the virus released into the supernatant and shown to mediate DNA-dependent endogenous activity. These endogenous transcripts were similar to the transcripts obtained from the endogenous synthesis by RT purified from virus produced by H9 chronically infected cells. In both cases, the transcripts hybridized to the same regions of HIV-1 genome (Fig. 4). This indicates that the same incomplete DNA was also present in viruses produced after transfection. Thus, RT activity indeed occurred in the absence of cell infection, as has previously been shown in cells infected with some other retroviruses (24, 31). Moreover, the partially completed product of this reverse transcription was packaged into viral particles.

Preformed viral DNA is carried by infectious virions. The human $CD4^+$ T-cell line CCRF-CEM was exposed to cell-free HIV-1_{IIIB} from chronically infected MOLT-3 cells and subsequently analyzed at different time intervals for the presence of viral DNA by PCR amplification. A sharp positive signal was obtained when the incubation was carried out for only 3 min at 4°C (Fig. 5, lane 1). Since at this temperature only adsorption, and not fusion or entry of the virus, has been demonstrated to occur (10, 19), these results are consistent with the presence of preformed DNA in the viral particle. To rule out the possibility of contamination from extraviral DNA originating from cell lysis, experiments were reproduced after DNase treatment of the intact virus (data not shown). Trypsin treatment after harvest of the CCRF-CEM cells almost completely eliminated the signal (Fig. 5, lane 2) by detaching the adsorbed DNA carrying virus from the cell surface. In contrast, at 37°C, the signal was only partially abolished by trypsin treatment (Fig. 5, lanes 5 and 6). The DNA signal not abolished by trypsin treatment (Fig. 5, lane 6) could result from fusion and entry of the virus carrying preformed DNA but also from synthesis of new DNA after virus entry. However, entry of preformed DNA was also suggested by the fact that no trypsin-detachable DNA remained on the external cell surface after 24 h (Fig. 5, lanes 7 and 8). These results were consistent with the presence of viral DNA within HIV-1 infectious virions, since the DNA entered the target cells as if it were encapsulated by viral particles. The same results were obtained by using other HIV-1 isolates, namely, HIV-1_{RF} (21) and HIV-1_{MN} (26) (data not shown).

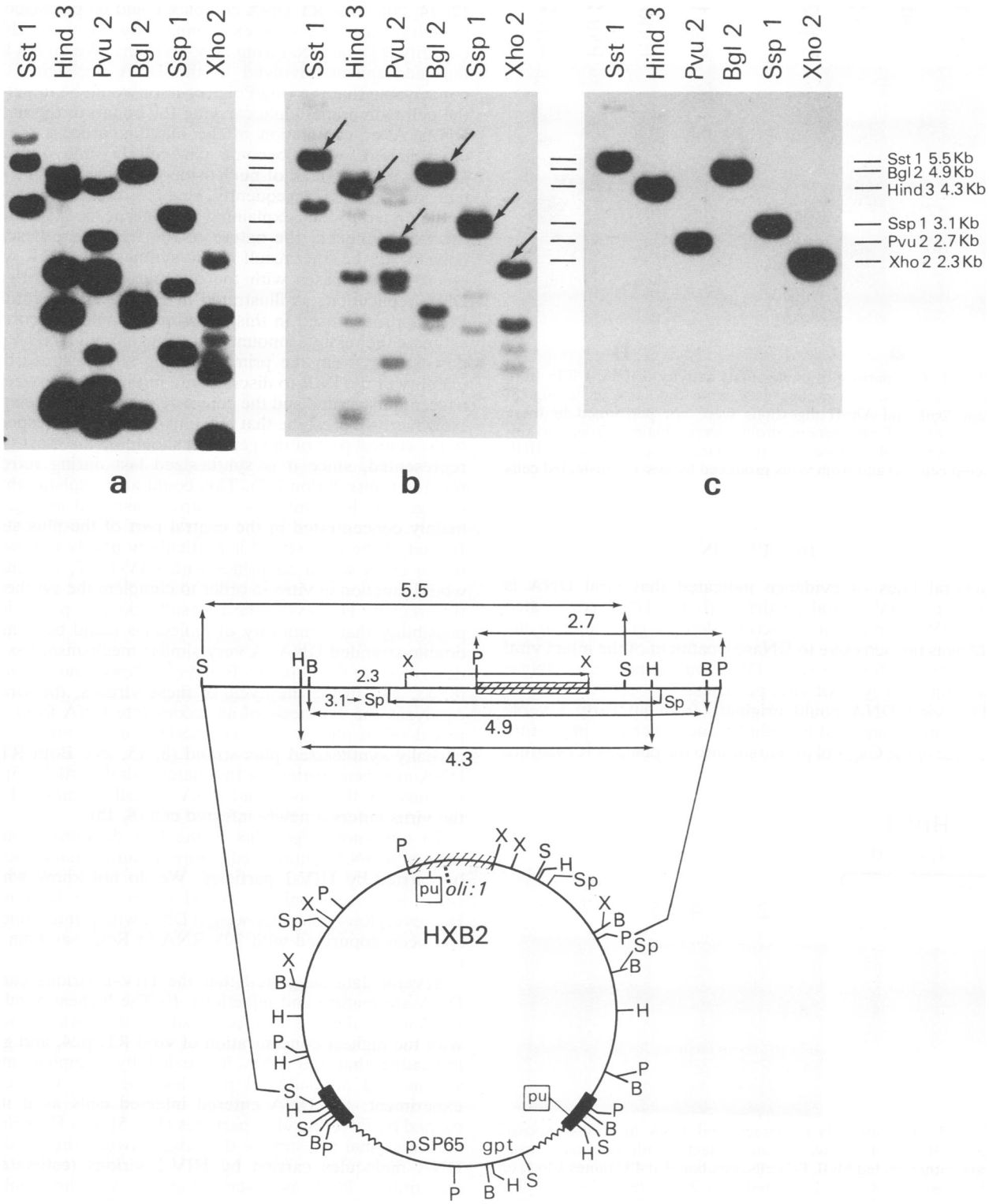

FIG. 3. Localization of the DNA product of HIV-1 endogenous RT activity. Different probes were used to hybridize the Southern blot of pHXB2(RIP7) (16). (a) Full-length HIV-1 probe using standard conditions. (b) Endogenous RT DNA product as a probe (see Materials and Methods). The arrows indicate the relatively more intense bands in each lane as quantified by absorbed β-emission using a Molecular Dynamics PhosphorImager. The value of each band was normalized on the basis of the apparent molecular weight in order to determine the relative intensity of each band. (c) The probe used was the 18mer oli:1 (nucleotides 4525 to 4542) (23). S, *Sst*I; H, *Hin*dIII; P, *Pvu*II; B, *Bgl*II; Sp, *Ssp*I; X, *Xho*II; pu, polypurine tracts. The shaded bar indicates the minimal common region shared by the arrowed bands.

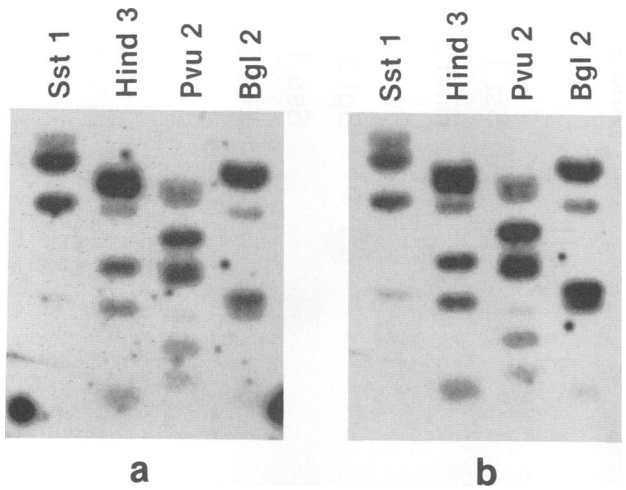

FIG. 4. Comparison of endogenous activity of HIV-1 RTs from different sources. The probes used were the same as in Fig. 3, except *Ssp*I and *Xho*II digestions were not performed in these experiments. RT endogenous probes were obtained from two different sources of purified RT: from virus produced by H9-IIIB-infected cells (a) and from virus produced by cos-1-transfected cells (b).

DISCUSSION

Several lines of evidence indicated that viral DNA is carried by HIV-1 viral particles: (i) the DNA comigrated with HIV-1 virions in a sucrose density gradient; (ii) the DNA was not sensitive to DNase treatment of the intact viral particles; and (iii) the same DNA was sensitive to DNase treatment after lysis of viral particles.

This viral DNA could originate from an early reverse transcription step, and it could be due either to a premature cleavage of the Gag/Pol precursor into the p66/p51 RT mature enzyme or to transcription by the Gag/Pol precursor itself (5, 12, 14, 20). This RT-DNA complex could be packaged into the viral particles, thus explaining why we were able to copurify RT and DNA from HIV-1 virions. We do not know the mechanisms involved in the DNA packaging. It is conceivable that the Gag/Pol precursor would be targeted to the cell membrane while carrying RT bound to the nascent DNA. After completion of the budding process the new environment would become particularly unfavorable for DNA synthesis (lack of nucleoside triphosphates and divalent ions, etc.). Consequently, reverse transcription would randomly stop, thus explaining the heterogeneous nature of the DNA. Indeed, the nature of the DNA here described appears to be the result of a synthesis by RT whose efficiency decreases with the distance from the origin of DNA replication, as illustrated in Fig. 2. The quantitative PCR approach used in this experiment could not correctly estimate the relative amount of the plus-strand DNA regions downstream from the primer binding site, because of the inability of the PCR to discriminate in that area between the nascent plus strand and the complete minus-strand template. However, we presume that this plus-strand DNA (especially in the central part of the genome) should be the most poorly represented, since it is synthesized last during retroviral reverse transcription (32). This could also explain why the endogenous RT synthesis in vitro, illustrated in Fig. 3, is mainly concentrated in the central part of the plus strand. Indeed, if the plus strand is particularly poorly represented in the DNA which copurifies with HIV-1 RT, the enzyme would function in vitro in order to complete the synthesis of this region. However, these results do not preclude the possibility that a minority of molecules could be complete double-stranded DNA. A very similar mechanism has been described for the hepatitis B and cauliflower mosaic viruses (8, 15, 29). In the life cycle of these viruses, the viral RT catalyzes the synthesis of an incomplete DNA form, composed of an almost fully synthesized minus strand and a partially synthesized plus strand (8, 15, 29). Both RT and DNA are then carried by the mature viral particle (3). The synthesis of the plus-strand DNA is finally completed when the virus enters a newly infected cell (8, 15).

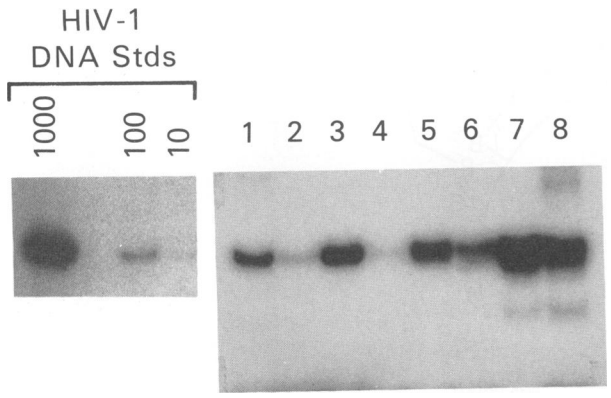

FIG. 5. PCR analysis to detect viral DNA in HIV-1-exposed cells. CCRF-CEM cells were acutely infected with supernatant from persistently infected MOLT-3 cells, incubated at 4°C (lanes 1 to 4) or 37°C (lanes 5 to 8), and harvested after 3 min (lanes 1 and 2), 1 h (lanes 3 to 6), and 24 h (lanes 7 and 8). In lanes 2, 4, 6, and 8, after harvesting, cells were treated with 0.1% trypsin–0.02% EDTA in PBS at 37°C for 5 min and washed two times with cold PBS to remove trypsin. Cell viability was evaluated before and after trypsin treatment and found to be over 95% in both cases. DNA was extracted from 10^6 cells from each sample, and quantitative PCR was performed by using primers M667/AA55. Standard curve of serial dilutions of pHXB2(Rip7) (16) is reported.

To our knowledge, this is the first demonstration that retroviral DNA synthesized before or during maturation can be carried by HIV-1 particles. We do not know whether DNA is present only in HIV-1 or in other lentiviruses. However, low-molecular-weight DNA with primer functions has been copurified with 70S RNA in Rous sarcoma virus (6).

Several data suggested that the HIV-1 virions carrying DNA are mature and infectious. (i) The highest number of DNA molecules in a sucrose density gradient was coincident with the highest concentration of viral RT, p24, and gp120, indicating that the DNA is carried by complete mature virions and not subviral particles. (ii) In a time course experiment, the DNA entered infected cells as if it was carried by infecting viral particles (Fig. 5). (iii) Experiments are on course to estimate the ratio between the number of DNA molecules carried by HIV-1 virions (estimated by quantitative PCR, as described above) and the number of infectious viral particles (calculated as 50% tissue culture infective doses). Preliminary results obtained by infecting different T-cell lines such as H9 (21), MOLT-3 (17), SupT1 (27), or primary peripheral blood mononuclear cells with early prototype HIV-1 strains isolated in our laboratory, including IIIB (21), MN (26), and RF (21), as well as a strain of HIV-1 isolated later which was the prototypic macro-

phagetropic isolate (BaL) (9), indicated that the ratio of the number of DNA molecules and number of infectious viruses was fairly constant from one experiment to the next, being approximately 1:1 ± 10 (11). The consistency of this ratio seemed even more significant since it was maintained in different experiments in which the 50% tissue culture infective doses varied by several orders of magnitude. It has been reported (18) that one 50% tissue culture infective dose approximately corresponds to 200 HIV-1 virions. We do not know whether virion-associated DNA is required for a particle to be infectious. However, the observation that DNA can be synthesized prior to formation of a viral particle could explain the postintegrational effects of AZT (zidovudine) on virus production in chronically infected cells (25).

The biological role of the DNA in HIV-1 virions can only be speculative at this time. We propose that one role might be to help maintain the virus in a latent form more stable than RNA in cells which are not in a metabolic state compatible with viral replication. Indeed, a similar incomplete DNA, which is putatively involved in virus latency in quiescent primary lymphocytes infected with HIV-1, has been recently described (33). Since the same DNA was shown to be completed and integrated after stimulation of T lymphocytes (4, 28, 33), it appears to represent a way to preserve the viral genomic information in a stable form until the cell becomes activated and therefore permissive for the completion of reverse transcription (33) and integration (4, 28). The intermediate DNA described here, since already present in the viral particle, could become immediately available after virus entry. This could represent a further advantage in those instances where an infected cell is not yet in a metabolic state accessible to viral replication or even to reverse transcription.

In conclusion, we propose that HIV-1 has the potential to adopt different strategies of replication, which may include partial reverse transcription prior to entry of the target cell. This potential may be important for viral latency in nondividing lymphocytes, which are known to play a major role in the pathogenesis of AIDS.

ACKNOWLEDGMENTS

We are grateful to Howard Temin for helpful discussions. We thank Matthew Gonda for useful suggestions, Cindy Boyer for excellent technical assistance, Andrea Cara for helpful contributions, and Anna Mazzuca for manuscript preparation. F. Lori is grateful to Umberto Bertazzoni for his encouragement.

REFERENCES

1. **Baltimore, D.** 1970. RNA-dependent DNA polymerase in virions of RNA tumour viruses. Nature (London) **226**:1209–1211.
2. **Barat, C., V. Lullien, O. Schatz, G. Keith, M. T. Nugeyre, F. Gruninger-Leitch, F. Barre-Sinoussi, S. F. LeGrice, and J. L. Darlix.** 1989. HIV-1 reverse transcriptase specifically interacts with the anticodon domain of its cognate primer tRNA. EMBO J. **8**:3279–3285.
3. **Bavand, M. R., and O. Laub.** 1988. Two proteins with reverse transcriptase activities associated with hepatitis B virus-like particles. J. Virol. **62**:626–628.
4. **Bukrinsky, M. I., T. L. Stanwick, M. P. Dempsey, and M. Stevenson.** 1991. Quiescent T lymphocytes as an inducible virus reservoir in HIV-1 infection. Science **254**:423–427.
5. **Crawford, S., and S. P. Goff.** 1985. A deletion mutation in the 5′ part of the *pol* gene of Moloney murine leukemia virus blocks proteolytic processing of the *gag* and *pol* polyproteins. J. Virol. **53**:899–907.
6. **Darlix, J.-L., P. A. Bromley, and P.-F. Spahr.** 1977. New procedure for the direct analysis of in vitro reverse transcription of Rous sarcoma virus RNA. J. Virol. **22**:118–129.
7. **di Marzo Veronese, F., A. L. DeVico, T. D. Copeland, S. Oroszlan, R. C. Gallo, and M. G. Sarngadharan.** 1985. Characterization of gp41 as the transmembrane protein coded by the HTLV-III/LAV envelope gene. Science **229**:1402–1405.
8. **Ganem, D., and H. E. Varmus.** 1987. The molecular biology of the hepatitis B viruses. Annu. Rev. Biochem. **56**:651–693.
9. **Gartner, S., P. Markovits, D. M. Markovitz, M. H. Kaplan, R. C. Gallo, and M. Popovic.** 1986. The role of mononuclear phagocytes in HTLV-III/LAV infection. Science **233**:215–219.
10. **Grewe, C., A. Beck, and H. R. Gelderblom.** 1990. HIV: early virus-cell interactions. J. AIDS **3**:965–974.
11. **Lori, F.** Unpublished observations.
12. **Lori, F., G. Achilli, E. Cattaneo, C. Casoli, and U. Bertazzoni.** 1990. Activity gel analysis of reverse transcriptase of HIV-1 and other retroviruses. Arch. AIDS Res. **4**:1–8.
13. **Lori, F., F. di Marzo Veronese, A. L. De Vico, P. Lusso, M. S. Reitz, Jr., and R. C. Gallo.** 1991. Viral DNA is carried by HIV-1 virions associated with reverse transcriptase, p. 10. Proceedings of the Annual Meeting of the Laboratory of Tumor Cell Biology. National Cancer Institute, Bethesda, Md.
14. **Lori, F., A. I. Scovassi, D. Zella, G. Achilli, E. Cattaneo, C. Casoli, and U. Bertazzoni.** 1988. Enzymatically active forms of reverse transcriptase of the human immunodeficiency virus. AIDS Res. Hum. Retroviruses **4**:393–398.
15. **Mason, W. S., J. M. Taylor, and R. Hull.** 1987. Retroid virus genome replication. Adv. Virus Res. **32**:35–96.
16. **McCune, J. M., L. B. Rabin, M. B. Feinberg, M. Lieberman, J. C. Kosek, G. R. Reyes, and I. L. Weissman.** 1988. Endoproteolytic cleavage of gp160 is required for the activation of human immunodeficiency virus. Cell **53**:55–67.
17. **Minowada, J., T. Onuma, and G. E. Moore.** 1972. Rosette-forming human lymphoid cell lines. I. Establishment and evidence for origin of thymus-derived lymphocytes. J. Natl. Cancer Inst. **49**:891–895.
18. **Pauza, C. D.** 1991. HIV-1 infection of T cells and monocytes via endocytosis, p. 111–144. *In* N. Duzgunes (ed.), Mechanisms and specificity of HIV entry. Advances in experimental medicine and biology, vol. 300. Plenum Press, New York.
19. **Pauza, C. D., and T. M. Price.** 1988. Human immunodeficiency virus infection of T cells and monocytes proceeds via receptor-mediated endocytosis. J. Cell Biol. **107**:959–968.
20. **Peng, C., N. T. Chang, and T. W. Chang.** 1991. Identification and characterization of human immunodeficiency virus type 1 *gag-pol* fusion protein in transfected mammalian cells. J. Virol. **65**:2751–2756.
21. **Popovic, M., M. G. Sarngadharan, E. Read, and R. C. Gallo.** 1984. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science **224**:497–500.
22. **Psallidopoulos, M. C., S. M. Schnittman, L. M. Thompson III, M. Baseler, A. S. Fauci, H. C. Lane, and N. P. Salzman.** 1989. Integrated proviral human immunodeficiency virus type 1 is present in CD4+ peripheral blood lymphocytes in healthy seropositive individuals. J. Virol. **63**:4626–4631.
23. **Ratner, L., W. Haseltine, R. Patarca, K. J. Livak, B. Starcich, S. F. Josephs, E. R. Doran, J. A. Rafalski, E. A. Whitehorn, K. Baumeister, L. Ivanoff, S. R. Petteway, M. L. Pearson, J. A. Lautenberger, T. S. Papas, J. Ghrayeb, N. T. Chang, R. C. Gallo, and F. Wong-Staal.** 1985. Complete nucleotide sequence of the AIDS virus, HTLV-III. Nature (London) **313**:277–284.
24. **Ringold, G. M., K. R. Yamamoto, P. R. Shank, and H. E. Varmus.** 1977. Mouse mammary tumor virus DNA in infected rat cells: characterization of unintegrated forms. Cell **10**:19–26.
25. **Rooke, R., M. Tremblay, and M. A. Wainberg.** 1990. AZT (Zidovudine) may act postintegrationally to inhibit generation of HIV-1 progeny virus in chronically infected cells. Virology **176**:205–215.
26. **Shaw, G. M., B. H. Hahn, S. K. Arya, J. E. Groopman, R. C. Gallo, and F. Wong-Staal.** 1984. Molecular characterization of human T-cell leukemia (lymphotropic) virus type III in the acquired immune deficiency syndrome. Science **226**:1165–1171.
27. **Smith, S. D., M. Shatsky, P. S. Cohen, R. Warnke, M. P. Link, and B. E. Glader.** 1984. Monoclonal antibody and enzymatic

profiles of human malignant T-lymphoid cells and derived cell lines. Cancer Res. **44**:5657–5660.

28. **Stevenson, M., T. L. Stanwick, M. P. Dempsey, and C. A. Lamonica.** 1990. HIV-1 replication is controlled at the level of T cell activation and proviral integration. EMBO J. **9**:1551–1560.
29. **Summers, J., and W. S. Mason.** 1982. Replication of the genome of a hepatitis B-like virus by reverse transcription of an RNA intermediate. Cell **29**:403–415.
30. **Temin, H. M., and S. Mizutani.** 1970. RNA-dependent DNA polymerase in virions of Rous sarcoma virus. Nature (London) **226**:1211–1213.
31. **Varmus, H. E., and P. R. Shank.** 1976. Unintegrated viral DNA is synthesized in the cytoplasm of avian sarcoma virus-transformed duck cells by viral DNA polymerase. J. Virol. **18**:567–573.
32. **Varmus, H. E., and R. Swanstrom.** 1984. Replication of retroviruses, p. 369–512. *In* R. Weiss, N. Teich, H. Varmus, and J. Coffin (ed.), RNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
33. **Zack, J. A., S. J. Arrigo, S. R. Weitsman, A. S. Go, A. Haislip, and I. S. Y. Chen.** 1990. HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. Cell **61**:213–222.