Differences in the Basal Activity of the Long Terminal Repeat Determine Different Replicative Capacities of Two Closely Related Human Immunodeficiency Virus Type 1 Isolates

EFIM I. GOLUB, GONGRONG LI, AND DAVID J. VOLSKY*

Molecular Virology Laboratory, St. Luke's/Roosevelt Hospital Center and College of Physicians and Surgeons, Columbia University, 432 West 58th Street, New York, New York 10019

Received 6 February 1990/Accepted 23 April 1990

Two human immunodeficiency virus type 1 (HIV-1) variants derived from a single parental isolate were found to differ substantially in their ability to replicate in CD4-positive cells. Using transient chloramphenicol acetyltransferase expression assays, we show that the long terminal repeat (LTR) of the better-replicating virus has significantly higher capacity than that of the companion virus to direct gene expression in T cells. Sequence data and site-specific mutagenesis experiments demonstrate that the higher LTR activity of the better-replicating HIV-1 is due to a combined effect of two mutations: (i) a point mutation in position –94 (relative to the transcriptional start site), which is located between the two subunits of the HIV-1 enhancer, and (ii) a duplication of 24 base pairs in positions –128 to –151, which was not previously known to be involved in any regulatory function. The presence of these mutations increases the basal level of the LTR-driven gene expression and does not influence the degree of induction caused by the viral *tat* gene product or by cell activation. Reciprocal exchange of LTRs between the respective viral DNAs results in a change of a recombinant virus replication pattern consistent with the activity of the particular LTR. These experiments suggest that the HIV-1 LTR is one of the sites which determines the functional heterogeneity of HIV-1.

Human immunodeficiency virus type 1 (HIV-1), the primary etiologic agent of acquired immune deficiency syndrome (AIDS) (5, 12, 24), comprises many molecularly and functionally divergent isolates. Functional variations include differences in serotype, host cell range, cytopathicity, and replicative potential (9, 25, 37). It has been suggested that progression of HIV disease correlates with the emergence of new HIV-1 variants in an individual (3, 7); for example, HIV-1 isolates from AIDS patients are generally more virulent and exhibit wider host cell range than viruses from asymptomatic individuals (3, 7, 37).

The extensive heterogeneity of HIV-1 has been attributed to the high error rate of HIV-1 reverse transcriptase (30, 38). Using 1 to 5 years as the time of divergence among sequential HIV-1 isolates from patients (16), the rate of evolution of HIV-1 was estimated to be 10^{-3} nucleotide substitutions per site per year for the *env* gene and 10^{-4} substitutions per site per year for the *gag* gene (16, 38). Although these mutations are seen throughout the HIV-1 genome (2), only some are likely to provide the mutant virus with selective advantage and thus facilitate the spread of a particular variant. Frequently observed mutations in the variable regions of the HIV-1 *env* gene (2, 36) could produce variants resistant to the immune response provoked by the original (nonmutated) viral strain. Mutations in the HIV-1 *nef* gene may be responsible for the emergence of HIV-1 strains with altered virulence (26). Selective advantage may also result from mutations in other regulatory genes of the virus, *tat* and *rev*, or in noncoding regulatory sites present in the long terminal repeat (LTR).

Recently, we have described several distinct HIV-1 variants obtained by molecular cloning from a clinical HIV-1 isolate (32, 33). Like other multiple HIV-1 variants from one patient (15, 16), these viruses are closely related to one

another but differ profoundly in their biologic properties (32, 33). In particular, a variant designated N1T-E infects cells slowly and without cytopathic effects but replicates efficiently in chronically infected cells. Another variant, designated N1T-A, causes rapid cytopathic infection but replicates less efficiently than N1T-E. The difference in the replication levels of N1T-E and N1T-A viruses prompted us to compare the respective activities of N1T-E and N1T-A LTR elements. The LTR contains an HIV-1 promoter and several regulatory elements which control proviral DNA expression (39), and it is likely that some mutations in the LTR may have a profound effect on the activity of the element and the resultant virus replication. Our data show that the N1T-E virus LTR is at least three times more active than the N1T-A LTR in directing both viral and heterologous gene expression. The increase in the N1T-E LTR activity is due to two specific mutations in the LTR region. Hence, the observed differences in the biologic properties of N1T-E and N1T-A viruses are, at least in part, caused by mutations in their respective LTRs

MATERIALS AND METHODS

Cell lines and viruses. CD4-positive T-cell lines used in these studies were obtained as follows: CEM cells (10) were received from the American Type Culture Collection (Rockville, Md.); CR-10, the HIV-1 lysis-resistant subclone of CEM, was established in our laboratory (6); and Jurkat cells were received from W. Green. Suspension cell lines were maintained in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 5% fetal bovine serum, penicillin G (100 U/ml), and streptomycin (100 µg/ml). The N1T isolate of HIV-1 (6) was propagated in CEM or CR-10 cells; the N1T-E clone of HIV-1 N1T virus was maintained in CEM cells, and clone N1T-A was maintained in chronically infected CR-10/N1T-A cells. Virus production

* Corresponding author.

FIG. 1. Schematic representation of HIV-1 LTR and infectious DNA recombinants. (A) N1T-A and N1T-E virus LTR constructs. Symbols: ■, enhancer repeats; ▨, sequences duplicated in N1T-E. The point mutation in position -94 is marked (*). (B) Positions of the restriction sites used for construction of the recombinant molecular clones of the N1T-A and N1T-E viruses (pEG823 and pEG824, respectively) containing reciprocally exchanged LTRs.

was monitored by measuring the levels of HIV-1 p24 antigen in culture supernatants.

HIV-1 clones and plasmids. Molecular clones of HIV-1 (strain N1T) were isolated from the high-molecular-weight genomic DNA from the N1T-producing cells as described previously (32). A detailed characterization of these clones and progeny viruses has been published (32). Plasmid pU3R-III, which contains a bacterial chloramphenicol acetyltransferase (*cat*) gene under the control of an HIV-1 (clone C15) LTR, was obtained from W. Haseltine (35). Plasmids pEG601 and pEG602 were derived from pU3R-III by replacing the *XhoI-HindIII* fragment (spanning nucleotides -639 to +80) with respective fragments from N1T-A and N1T-E clones. Plasmids pEG610 and pEG611 were constructed from pEG601 and pEG602, respectively, by deleting a 483-base-pair (bp) *XhoI-AvaI* fragment. Plasmids pEG610 and pEG611 carry HIV-1 LTR sequences spanning from -157 to +80 and from -181 to +80, respectively. pEG614 was derived from pEG602 by substituting cytidine for thymidine in position -94. The substitution was made by the site-specific mutagenesis procedure of Kunkel (22). pEG617 is similar to pEG602, except that the duplication in positions -128 to -151 has been removed by digestion with *ScaI*. A schematic drawing of these constructs is shown in Fig. 1A. Plasmid pEG621 was obtained by the substitution of the 720-bp *XhoI-HindIII* fragment of pU3R-III by the 230-bp *AvaI-HindIII* fragment of the pLTR-1/*cat* plasmid. The last plasmid carries the *cat* gene controlled by the HIV-1/SF-2 (formerly called ARV-2) LTR (23). Recombinant molecular clones of N1T-E and N1T-A viruses containing reciprocally exchanged LTRs (plasmids pEG823 and pEG824) were obtained by exchanging *BssHII-XhoI* fragments of the pN1T-A and pN1T-E. Plasmid pEG823 consists of the N1T-A LTR and N1T-E virus "body"; plasmid pEG824 carries the N1T-E virus LTR and N1T-A virus body (for a schematic representation, see Fig. 1B).

DNA sequencing. The *XhoI-HindIII* fragments from N1T-A and N1T-E provirus clones were cloned into M13mp18 and M13mp19 vectors and sequenced in both directions by

the dideoxyribonucleotide chain termination procedure (34). Reactions were catalyzed with modified T7 DNA polymerase (Sequenase; United States Biochemical Corp., Cleveland, Ohio) according to the instructions of the manufacturer.

CAT assay. Cell extracts were assayed for chloramphenicol acetyltransferase (CAT) activity by thin-layer chromatography as described elsewhere (4). Spots on the chromatography plates corresponding to different forms of [¹⁴C]chloramphenicol were removed and quantitated in a scintillation counter. CAT enzyme activity was linear in each assay. CAT activity values were normalized against the amount of protein contained in the extracts.

Other analytical procedures, chemicals, and radiochemicals. Unless specified otherwise, DNA transfections (DEAE-dextran-dimethyl sulfoxide procedure), plasmid propagation and DNA isolation, and other molecular-biologic procedures were performed according to standard methods (4). HIV-1 infection was monitored as described previously (6, 32) by detecting the presence of HIV antigens in infected cells by the indirect immunofluorescence method and by measuring the level of reverse transcriptase activity or HIV-1 p24 core protein (Coulter HIV Ag assay; Coulter Immunology, Hi-aleah, Fla.) in culture supernatants. Enzymes for recombinant DNA experiments were purchased from New England BioLabs, Inc. (Beverly, Mass.), and Bethesda Research Laboratories (Gaithersburg, Md.), and fluorescein isothiocyanate-conjugated anti-human immunoglobulin G was purchased from Tago Immunochemicals (Burlingame, Calif.). All radiochemicals were obtained from DuPont, NEN Research Products (Boston, Mass.), and other chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo.).

RESULTS

N1T-E virus LTR is more active than N1T-A virus LTR. Previous studies demonstrated that N1T-E virus has a higher replicative capacity in chronically infected cells than N1T-A virus (32, 33). The HIV-1 replication rate is determined by

FIG. 2. Transcriptional activity of N1T-E and N1T-A virus LTRs. CR10/N1T-A cells (10^7) chronically infected with HIV-1 were transfected with the designated amounts of plasmid pEG601 or pEG602 and evaluated for CAT activity as described in the text.

the rate of provirus DNA transcription, and this in turn is controlled by the viral LTR (39). To compare the function of N1T-A and N1T-E virus LTRs, we constructed a series of indicator plasmids containing the *cat* gene under the control of an appropriate viral LTR (Fig. 1A). The initial constructs (plasmids pEG601 and pEG602) contained the entire *XhoI-HindIII* fragment from the respective HIV genome (N1T-A in pEG601 or N1T-E in pEG602) and thus were identical in composition to the previously described pU3R-III plasmid (35). Transfections of pEG602 plasmid into HIV-positive cells resulted in consistently higher CAT activity compared with pEG601-transfected cells (Fig. 2; Table 1). On average (with data from 11 independent transfection experiments), pEG602-transfected cells synthesized 4.5 times more CAT enzyme than did the same cells transfected with pEG601. The CAT activity directed by plasmid pU3R-III (35) or pEG621, in which CAT expression is controlled by the LTR from HIV-1/SF-2 (23), was similar to that observed with the pEG601 plasmid (data not shown). Transfections with pEG602 resulted in higher CAT enzyme activity, compared with pEG601- or pU3R-III-transfected cells, regardless of

whether the cells carried N1T-A, N1T-E, or N1T virus (data not shown). These results suggest that the intrinsic transcriptional activity of the N1T-E virus LTR is significantly stronger than the respective function of the N1T-A or HIV-1/III-B LTR.

Variant nucleotide sequences responsible for the increase in the N1T-E virus LTR activity are located between positions -156 and +80. Nucleotide sequence analysis revealed that the *XhoI-HindIII* fragments of the N1T-E and N1T-A viruses differ by mutations in seven sites (Fig. 3). Five of these mutations are located within the *XhoI-AvaI* fragment (positions -640 to -156). We deleted this fragment from plasmids pEG601 and pEG602 and obtained plasmids pEG610 and pEG611, respectively. The level of CAT activity directed by plasmid pEG611 was significantly higher than that of the comparable N1T-A virus-derived plasmid pEG610 (Table 1). Thus, the changes which increase the transcriptional activity of the N1T-E virus LTR are localized in the fragment between positions -156 and +80.

Mutations which affect N1T-E LTR activity. The -156/+80 fragment of the N1T-E LTR differs from the corresponding fragment of the N1T-A virus by two mutations: (i) a point mutation in position -94, which affects the four-nucleotide spacer between two 10-bp direct repeats which are part of the HIV-1 enhancer (31), and (ii) a duplication of 24 bp in positions -151 to -128, which affects a locus that has not been implicated in any regulatory function (Fig. 3). To elucidate the contribution of these mutations to the increased activity of the N1T-E LTR, plasmids were constructed in which either the point mutation (pEG614-1) or the duplication (pEG617-1) was eliminated (Fig. 1A). As shown in Table 1, removal of either one of the mutations resulted in a partial decrease of CAT activity directed by the N1T-E LTR, but not to the level observed with the N1T-A LTR. Thus, each mutation causes only some of the total increase in the HIV-1 LTR-directed CAT activity, and the high-level activity observed with the N1T-E virus LTR is a result of combined action of the two mutations.

Mutations in the N1T-E LTR result in an increase in the basal level of activity. Different viral and cellular factors can considerably modify the transcriptional activity of the HIV-1 LTR (18, 19, 29, 40). To determine whether the LTR mutations described here influence the basal activity of the HIV-1 LTR, we analyzed transient CAT activity in uninfected Jurkat cells that were transfected with pEG601 or pEG602 in the presence or absence of T-cell activators phorbol 12-myristate 13-acetate and phytohemagglutinin (Fig. 4). As in the case of HIV-1-infected cells, uninfected Jurkat cells transfected with pEG602 synthesized considerably more CAT enzyme than pEG601 transfectants did (Fig. 4). Cell stimulation with phorbol 12-myristate 13-acetate and phytohemagglutinin resulted in CAT activity increases of two- to five-fold above those seen in unstimulated Jurkat cells transfected in parallel. The induction index (CAT activity in stimulated Jurkat cells divided by that in unstimulated Jurkat cells) was similar in pEG602- and pEG601-transfected cells (Fig. 4). Thus, mutations in the N1T-E LTR increase the basal level of the activity of the element, rather than making it more sensitive to the known viral or cellular transcriptional activators.

Reciprocal exchange of LTRs between N1T-E and N1T-A DNA alters the replicative phenotype of recombinant genomes and progeny viruses. To examine the direct effect of variant LTRs on HIV-1 genome expression, we prepared recombinant viral genomes in which the N1T-E or N1T-A DNA body (minus the LTR) was recombined with the LTR of the other

TABLE 1. Effect of mutations in LTRs on CAT activity^a

Plasmid	Relative CAT activity						Average
	Expt no.						
	1	2	3	4	5	6	
pEG601	1.0	1.0	1.0	1.0	1.0	1.0	1.0
pEG602	2.5	3.0	2.5	4.5	3.4	3.4	3.1
pEG610	1.4	1.4					1.4
pEG611	3.9	5.9					4.9
pEG614-1			1.3	2.0	0.8	1.5	1.4
pEG614-2						1.2	1.2
pEG614-3			1.2	2.5		1.0	1.9
pEG617-1			1.3	3.2	1.5	1.5	1.9
pEG617-2				2.2		1.1	1.6
pEG617-3				1.8		0.7	1.2

^a CR10/N1T-A cells (10^7) were transfected with 100 ng of plasmid DNA. Plasmid composition is shown diagrammatically in Fig. 1A. The numbers following plasmid designations (i.e., -1, -2, and -3) indicate independently derived plasmid subclones.

<u>XhoI/AvaI</u>						
-643	<u>CTCGAGACCT</u>	AGAAAAACAT	GGAGCAATCA	CAAGTAGCAA	TACAGCAGCT	AACAATGCTG-
	-----	-----	-----	-----T	-----	-----
-583	CTTGTGCCTG	GCTAGAAGCA	CAAGAGGAGG	AAGAGGTGGG	TTTTCCAGTC	ACACCTCAGG
	-----	-----	-----	-----	-----	-----
-523	TATCTTTAAG	ACCAATGACT	TACAAGGCAG	CTGTAGATCT	TAGCCACTTT	TAAAAAGAAA
	--C-----	-----	-----	-----	-----	-----
-463	AGGGGGGACT	GGAAGGGCTA	ATTCACCTCC	AACGAAGACA	AGATATCCTT	GATCTGTGGA
	-----	-----	-----	-----	-----	-----
-403	TCTACCACAC	ACAAGGCTAC	TTCCCTGATT	GGCAGAACTA	CACACCAGGG	CCAGGGATCA
	-----	-----	-----	-----	-----	-----
-343	GATATCCACT	GACCTTTGGA	TGGTGCTACA	AGCTAGTACC	AGTTGAGCAA	GAGAAGGTAG
	-----	-----	-----	-----	-----	-----
-283	AAGAAGCCAA	TGAAGGAGAG	AACACCGGCT	TGTTACGCC	TGTGAGCCTG	CATGGGATGG
	-----	-----	---T---	---A---	-----	-----
-223	ATGACCCGGA	GAGAGAAGTA	TTAGAGTGGA	GGTTTGACAG	CCGCCTAGCA	TTTCATCAGC
	--A-----	-----	-----	-----	-----	-----
-163	<u>TGGCCGAGA</u>	GC.....	TGCA	TCCGGAGTAC	TTCAAGAACT	
	-----	--TGCATCCG	GAGTACTTCA	AGAACT---	-----	-----
-127	GCTGACATCG	AGCTTGCTAC	AAGGGACTTT	CGCTGGGGA	CTTCCAGGG	AGGCGTGGCC
	-----	-----	-----T-----	-----	-----	-----
-67	TGGGCGGGAC	TGGGGAGTGG	CGAGCCCTCA	GATGCTGCAT	ATAAGCAGCT	GCTTTTGGCC
	-----	-----	-----	-----	-----	-----
+1						
-7	TGTACTGGGT	CTCTGTGGTT	AGACCAGATC	TGAGCCTGGG	AGCTCTCTGG	CTAACTAGGG
	-----	-----	-----	-----	-----	-----
			<u>HindIII</u>			
54	AACCCACTGC	TTAAGCCTCA	ATAAAGCTT			
	-----	-----	-----			

FIG. 3. Nucleotide sequences of the HIV-1 LTR fragments present in plasmids pEG601 and pEG602. The -643 to +83 nucleotide sequence of the N1T-A virus is given in full, and differences that exist in the N1T-E virus are shown below the N1T-A sequence. Direct repeats, which are part of the HIV-1 core enhancer, and the duplication found in the N1T-E LTR are underlined.

viral variant (Fig. 1B). The parental and recombinant clones were compared for their ability to replicate in short-term transfection assays in Jurkat and CEM cells (Table 2). In addition, molecularly cloned progeny viruses with reciprocally exchanged LTRs were evaluated in an infection assay (Fig. 5). As expected on the basis of previous work (32, 33), N1T-E virus DNA produced higher levels of progeny virus than N1T-A DNA did. Reciprocal exchange of LTRs reversed the ratio, and thus plasmid pEG824 (N1T-A proviral DNA with N1T-E LTR) produced more progeny than did its counterpart plasmid pEG823 (N1T-E proviral DNA with N1T-A LTR). Similar results were obtained when CAT activity in the same cells was determined as an independent measure of transfected DNA expression (Table 2).

Infection experiments with recombinant progeny viruses revealed that the reciprocal exchange of the LTRs had an expected effect on the kinetics of virus infection and replication (Fig. 5) but no effect on the cytopathic properties of the respective viruses (data not shown). The EG824 virus showed faster initial kinetics of infection than the parental N1T-A virus, consistent with higher levels of HIV-1 replication in EG824-infected cells (Fig. 5). The N1T-A LTR in the EG823 virus had little effect on the already slow infection and replication kinetics of the parental N1T-E virus (Fig. 5).

DISCUSSION

This paper presents a structural and functional analysis of the LTR elements from two closely related HIV-1 clones with high and low replication capacities. Our data show that the two LTRs differ in their activities in correlation with the replication capacity of the parental viruses. This conclusion is supported by two lines of evidence: (i) independent of either viral transactivator Tat protein or the state of cellular activation, gene expression directed by the LTR from a better-replicating N1T-E virus is significantly enhanced compared with that directed by the LTR from a less efficiently replicating N1T-A virus (Fig. 2 and 4; Table 1); and (ii) reciprocal exchange of LTRs between the N1T-E and N1T-A viral DNAs reversed the replication ratio (as measured by HIV-1 p24 antigen production per unit of transfected DNA) between the respective proviral DNAs (Table 2). Furthermore, the replication kinetics of recombinant progeny viruses have been altered to some extent, consistent with the activity of exchanged LTRs (Fig. 5). By itself, the finding of functional differences between the two HIV-1 LTRs was not entirely unexpected. The LTR of murine leukemia virus is perhaps the most important region in the virus for replication, tissue tropism, and tissue specificity for

FIG. 4. Induction of CAT activity in Jurkat cells. The figure shows a typical autoradiograph from the thin-layer chromatography of conversion of [^{14}C]chloramphenicol (Cm) to its acetylated form (AcCm) after incubation for 20 h with extracts from cells transfected by pEG601 (lanes 2 to 5) or pEG602 (lanes 6 to 9). Lanes 2, 3, 6, and 7 show the results obtained by transfection with 0.5 μg of DNA per 5×10^6 cells, while lanes 4, 5, 8, and 9 are from transfectants with 2 μg of DNA per 5×10^6 cells. Lane 1 represents mock transfection.

pathogenicity (8, 17). Many regions of the HIV-1 genome, including the *env*, *nef*, *vif*, and *tat* genes, are prone to mutation and functional diversification, thus contributing to the observed heterogeneity among HIV-1 isolates (2, 15, 16, 32, 36–38). However, the HIV-1 LTR has not been implicated in this context. Our results suggest that it should be included among the functionally variable genomic elements of HIV.

The analysis presented in this work shows that the higher activity of the N1T-E LTR is due to a combined effect of two mutations. It is unclear from the present data how these mutations may contribute to the observed enhancement of the LTR activity. The point mutation in position -94 affects a four-nucleotide spacer between two identical direct re-

FIG. 5. Infection kinetics of recombinant N1T viruses with reciprocal exchange of LTRs. CEM cells were transfected with appropriate parental and recombinant viral DNA, in duplicate, as described in footnote *a* of Table 2. Two days later, transfected cells were washed and cocultivated with highly HIV-1-susceptible C-8166 cells. The ratio of infected to uninfected cells was 1:10. At the designated time points, samples were removed and evaluated for HIV-1 infection by immunofluorescence staining on acetone-fixed cells (31). At the peak of infection, cell-free culture supernatants were also tested for the levels of HIV-1 p24 antigens; the results are shown in parentheses as picograms of HIV-1 p24 antigens per milliliter of supernatant. All the numbers shown in the figure represent averages of measurements from duplicate test samples.

peats, GGGACTTTCC, which contain binding sequence for the human immunoglobulin enhancer-binding protein NF- κ B (29) and are part of the HIV-1 core enhancer (13, 19, 29). Studies with purified NF- κ B protein have shown that it activates in vitro transcription of the HIV-1 promoter by selective binding to the distal (upstream) repeat sequence (20). This noncooperative binding (and biologic effects) of NF- κ B (14) has been attributed to a steric hindrance resulting from the proximity of the two sites (20). It is possible that the C-to-T mutation in position -94 in the N1T-E LTR changes the configuration of the region to increase NF- κ B binding to the downstream repeat. Interaction with NF- κ B-like proteins, which require the proximal repeat for binding (41), could also be altered. In any event, only the portion of the transcriptional factor(s) binding which is required for basal promoter activity appears to be altered by the -94 mutation, since both the mutated and nonmutated LTRs are induced to the same extent (Fig. 4). In contrast to the -94 mutation, the 24-bp duplication at the -128 to -151 site in the N1T-E LTR is in a region which has not been directly implicated in any regulatory function. However, one recent study has shown that the LTR/CAT plasmid carrying a -119/+80 fragment exhibits a lower level of inducible transcriptional activity than an analogous plasmid with a -139/+80 fragment (19). Conceivably, the -119/-139 fragment, which includes part of the duplication sequence in the N1T-E LTR (spanning positions -128 to -151) influences transcriptional activity of the HIV-1 LTR. Consistent with these observations, in preliminary experiments we have noted that a reduction of the duplicated region in the N1T-E LTR to 12 nucleotides reduced the LTR activity to an

TABLE 2. Biological properties of LTR recombinants between N1T-A and N1T-E viruses

HIV-1 DNA	Measurement of plasmid activity after transfection ^a	
	Relative production of HIV-1 p24 core antigen	Relative CAT activity
pN1T-A1	1.0	1.0
pN1T-E1	3.3 $\pm$ 1.3 (7)	2.1 $\pm$ 0.7 (15)
pEG823	1.0	1.0
pEG824	2.2 $\pm$ 0.6 (4)	1.8 $\pm$ 0.8 (9)

^a Jurkat or CEM cells (5×10^6) were transfected with appropriate proviral DNA (0.5 to 1.0 μg) alone or with the pEG601 plasmid (100 ng). Two days after transfection, culture supernatants were concentrated 10-fold and assayed for the presence of HIV-1 p24 antigens, and cells were assayed for CAT enzyme activity. The results are shown as the mean $\pm$ the standard deviation of *n* transfections, with *n* in parentheses. Plasmid pEG823 carries the N1T-A LTR and N1T-E virus body; plasmid pEG824 carries the N1T-E LTR and N1T-A body (Fig. 1B).

intermediate value between those of the N1T-E and N1T-A LTRs (data not shown).

It is of interest that the more transcriptionally active N1T-E LTR resides in a virus that exhibits slow kinetics of infection and causes minimal cytopathic effects (32, 33), features which ostensibly point to a defect in virus replication (3, 9). This apparent paradox is resolved if one considers that viral replication (during primary infection) correlates with the kinetics of infection (3, 9, 27) and that the latter are under the control of other viral elements besides the LTR. In the case of N1T-E, we have recently mapped the slow infection kinetics and the noncytopathic phenotype of this virus (27, 32) to a defect in the *vif* gene (K. Sakai, X. Ma, I. Gordienko, and D. J. Volsky, 6th Int. Conf. AIDS, 1990, abstr. no. 239). Thus, the N1T-E virus exhibits high basal replicative activity because of the "fast" LTR (this work), whereas it has slow infection and replication kinetics because of a defective *vif* gene.

In summary, our findings point to the HIV-1 LTR as a site which may contribute to the variability in the replicative capacity of HIV strains. The difference in the basal and inducible activity of the N1T-E LTR versus that of the N1T-A or the human T-cell lymphotropic virus type III-B LTR was small compared to the stimulation caused by the *Tat* transactivator protein (35) or by cellular activation (11, 19, 29). However, recent evidence suggests that cell activation is not always a requirement for HIV-1 replication (1, 21), and some agents such as herpes simplex virus type 1 or adenovirus may activate HIV-1 via pathways which are distinct from both the *tat*- and NF- κ B-dependent mechanisms (28). Higher basal LTR activity levels may thus provide an advantage for N1T-E-like viruses in the absence of immune stimulation or in the presence of viral cofactors.

ACKNOWLEDGMENTS

We thank B. Volsky for technical help in parts of this project, K. Sakai for helpful suggestions, W. Haseltine for the pU3R-III plasmid, and V. D'Alto for typing the manuscript.

This work was supported by Public Health Service grants CA37465-04, AI-25902, and AI-27397 from the National Institutes of Health.

LITERATURE CITED

- Adachi, A., H. E. Gendelman, S. Koenig, T. Folks, R. Willey, A. Rabson, and M. A. Martin. 1986. Production of acquired immunodeficiency syndrome-associated retrovirus in human and non-human cells transfected with an infectious molecular clone. *J. Virol.* 59:284-291.
- Alizon, M., S. Wain-Hobson, L. Montagnier, and P. Sonigo. 1986. Genetic variability of the AIDS virus: nucleotide sequence analysis of two isolates from African patients. *Cell* 46:63-74.
- Asjo, B., L. Morfeldt-Manson, J. Albert, G. Biberfeld, A. Karlsson, K. Lidman, and E. M. Fenyo. 1986. Replicative capacity of human immunodeficiency virus from patients with varying severity of HIV infection. *Lancet* ii:660-662.
- Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.). 1987. Current protocols in molecular biology. John Wiley & Sons, Inc., New York.
- Barre-Sinoussi, F., J.-C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Axler-Blin, F. Vezinet-Brun, C. Rouzioux, W. Rozenbaum, and L. Montagnier. 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 220:868-871.
- Casareale, D., M. Stevenson, K. Sakai, and D. J. Volsky. 1987. A human T-cell line resistant to cytopathic effects of the human immunodeficiency virus (HIV). *Virology* 156:40-49.
- Cheng-Mayer, C., D. Seto, M. Tateno, and J. A. Levy. 1988. Biological features of HIV-1 that correlate with virulence in the host. *Science* 240:80-82.
- DesGroseillers, L. D., E. Rassart, Y. Robitaille, and P. Jolicœur. 1985. Retrovirus-induced spongiform encephalopathy: the 3'-end long terminal repeat-containing viral sequences influence the incidence of the disease and the specificity of the neurological syndrome. *Proc. Natl. Acad. Sci. USA* 82:8818-8822.
- Evans, L. E., T. M. McHugh, D. P. Stites, and J. A. Levy. 1987. Differential ability of human immunodeficiency virus isolates to productively infect human cells. *J. Immunol.* 138:3415-3418.
- Foley, G. E., H. Lazarus, J. Dredd, S. Faber, B. Uzman, B. A. Boone, and R. E. McCarthy. 1965. Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukemia. *Cancer* 18:522-529.
- Folks, T. M., J. Justement, A. Kinter, C. A. Dinarello, and A. S. Fauci. 1987. Cytokine-induced expression of HIV-1 in a chronically infected promonocyte cell line. *Science* 238:800-802.
- Gallo, R. C., S. Z. Salahuddin, M. Popovic, G. M. Shearer, M. Kaplan, B. F. Haynes, T. J. Palker, R. Redfield, J. Oleske, B. Safai, G. White, P. Foster, and P. D. Markham. 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science* 224:500-503.
- Garcia, J. A., F. K. Wu, R. Mitsuyasu, and R. B. Gaynor. 1987. Interactions of cellular proteins involved in the transcriptional regulation of the human immunodeficiency virus. *EMBO J.* 6:3761-3770.
- Gaynor, R. B., M. D. Kuwabara, F. K. Wu, J. A. Garcia, D. Harrih, M. Briskin, R. Wall, and D. S. Sigman. 1988. Repeated B motifs in the human immunodeficiency virus type 1 long terminal repeat enhancer region do not exhibit cooperative factor binding. *Proc. Natl. Acad. Sci. USA* 85:9406-9410.
- Hahn, B., M. A. Gonda, G. M. Shaw, M. Popovic, J. A. Hoxie, R. C. Gallo, and F. Wong-Staal. 1985. Genomic diversity of the acquired immune deficiency syndrome virus HTLV-III: different viruses exhibit greatest divergence in their envelope genes. *Proc. Natl. Acad. Sci. USA* 82:4813-4817.
- Hahn, B., G. M. Shaw, M. E. Taylor, R. R. Redfield, P. D. Markham, S. Z. Salahuddin, F. Wong-Staal, R. C. Gallo, E. S. Parks, and W. P. Parks. 1986. Genetic variation in HTLV-III/LAV over time in patients with AIDS or at risk for AIDS. *Science* 232:1548-1553.
- Holland, C. A., J. Wozney, P. A. Chatis, N. Hopkins, and J. W. Hartley. 1985. Construction of recombinants between molecular clones of murine retrovirus MCF 247 and Akv: determinant of an *in vitro* host range property that maps in the long terminal repeat. *J. Virol.* 53:152-157.
- Jones, K. A., J. T. Kadonaga, P. A. Luciw, and R. Tjian. 1986. Activation of the AIDS retrovirus promoter by the cellular transcription factor, Sp1. *Science* 232:755-759.
- Kaufman, J. D., G. Valandra, G. Roderiquez, G. Bushar, C. Giri, and M. A. Norcross. 1987. Phorbol ester enhances human immunodeficiency virus-promoted gene expression and acts on a repeated 10-base-pair functional enhancer element. *Mol. Cell. Biol.* 7:3759-3766.
- Kawakami, K., C. Scheidereit, and R. G. Roeder. 1988. Identification and purification of a human immunoglobulin-enhancer-binding protein (NF- κ B) that activates transcription from a human immunodeficiency virus type 1 promoter *in vitro*. *Proc. Natl. Acad. Sci. USA* 85:4700-4704.
- Koyanagi, Y., W. A. O'Brien, J. Q. Zhao, D. W. Golde, J. C. Gasson, and I. S. Y. Chen. 1988. Cytokines alter production of HIV-1 from primary mononuclear phagocytes. *Science* 241:1673-1675.
- Kunkel, T. A. 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Proc. Natl. Acad. Sci. USA* 82:488-492.
- Levy, J. A., C. Cheng-Mayer, D. Dina, and P. A. Luciw. 1986. AIDS retrovirus (ARV-2) clone replicates in transfected human and animal fibroblasts. *Science* 232:998-1001.
- Levy, J. A., A. D. Hoffman, S. M. Kramer, J. A. Landis, J. M. Shimabukuro, and L. S. Oshiro. 1984. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS.

- Science **225**:840–842.
25. Looney, D. J., A. G. Fisher, S. D. Putney, J. R. Rusche, R. R. Redfield, D. S. Burke, R. C. Gallo, and F. Wong-Staal. 1988. Type restricted neutralization of molecular clones of human immunodeficiency virus. *Science* **241**:357–359.
 26. Luciw, P. A., C. Cheng-Mayer, and J. Levy. 1987. Mutational analysis of the human immunodeficiency virus: the orf-B region down-regulates virus replication. *Proc. Natl. Acad. Sci. USA* **84**:1434–1438.
 27. Ma, X., K. Sakai, F. Sinangil, E. Golub, and D. J. Volsky. 1990. Interaction of a noncytopathic human immunodeficiency virus type 1 (HIV-1) with target cells: efficient virus entry followed by delayed expression of its RNA and protein. *Virology* **176**:184–194.
 28. Nabel, G., and D. Baltimore. 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature (London)* **326**:711–713.
 29. Nabel, G. J., S. A. Rice, D. M. Knipe, and D. Baltimore. 1988. Alternative mechanisms for activation of human immunodeficiency virus enhancer in T cells. *Science* **239**:1299–1302.
 30. Preston, B. D., B. J. Poiesz, and L. A. Loeb. 1988. Fidelity of HIV-1 reverse transcriptase. *Science* **242**:1168–1171.
 31. Rosen, C. A., J. G. Sodroski, and W. A. Haseltine. 1985. The location of cis-acting regulatory sequences in the human T cell lymphotropic virus type III (HTLV-III/LAV) long terminal repeat. *Cell* **41**:813–823.
 32. Sakai, K., S. Dewhurst, X. Ma, and D. J. Volsky. 1988. Differences in cytopathogenicity and host cell range among infectious molecular clones of human immunodeficiency virus type 1 simultaneously isolated from an individual. *J. Virol.* **62**:4078–4085.
 33. Sakai, K., X. Ma, and D. J. Volsky. 1988. Low-cytopathic infectious clone of human immunodeficiency virus type 1 (HIV-1). *FEBS Lett.* **238**:257–261.
 34. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463–5467.
 35. Sodroski, J., C. Rosen, F. Wong-Staal, S. Z. Salahuddin, M. Popovic, S. Arya, R. C. Gallo, and W. A. Haseltine. 1985. Transacting transcriptional regulation of human T-cell leukemia virus type III long terminal repeat. *Science* **227**:171–173.
 36. Starcich, B., B. Hahn, G. Shaw, P. McNeely, S. Modrow, H. Wolf, W. P. Parks, S. F. Josephs, R. C. Gallo, and F. Wong-Staal. 1986. Identification and characterization of conserved and variable regions in the envelope gene of HTLV-III/LAV, the retrovirus of AIDS. *Cell* **45**:637–648.
 37. Tersmette, M., R. E. Y. de Goede, B. J. M. Al, I. N. Winkel, R. A. Gruters, H. T. Cuypers, H. G. Huisman, and F. Miedema. 1988. Differential syncytium-inducing capacity of human immunodeficiency virus isolates: frequent detection of syncytium-inducing isolates in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. *J. Virol.* **62**:2026–2032.
 38. Wong-Staal, F. 1988. Variation of the HIV genome: implications for the pathogenesis and prevention of AIDS, p. 147–157. *In* E. Domingo, J. J. Holland, and P. Ahlquist (ed.), *RNA genetics*, vol. III. Variability of RNA genomes. CRC Press, Inc., Boca Raton, Fla.
 39. Wong-Staal, F., and R. C. Gallo. 1985. Human T-lymphotropic retroviruses. *Nature (London)* **317**:345–403.
 40. Wu, F., J. Garcia, R. Mitsuyasu, and R. Gaynor. 1988. Alterations in binding characteristics of the human immunodeficiency virus enhancer factor. *J. Virol.* **62**:218–225.
 41. Wu, F. K., J. A. Garcia, D. Harrich, and R. B. Gaynor. 1988. Purification of the human immunodeficiency virus type 1 enhancer and TAR binding proteins EBP-1 and UBP-1. *EMBO J.* **7**:2117–2129.