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We compared the efficiency of human immunodeficiency virus (HIV-1) vectors that express a marker gene (chloramphenicol acetyltransferase, CAT) using different promoter elements. In one vector, CAT was expressed under the control of an internal murine leukemia virus (MuLV) long terminal repeat (LTR). In other vectors, CAT production was regulated by the HIV-1 LTR; these vectors also contained the HIV-1 *tat* gene and *pol* sequences reported to exert *cis*-acting positive effects on reverse transcription or gene expression. Vectors employing the Tat-driven HIV-1 LTR exhibited up to 500-fold greater CAT expression in Jurkat lymphocytes or human peripheral blood mononuclear cells compared with vectors using the internal MuLV LTR element as a promoter. This difference was not due to improved packaging of the vector RNA into virions, but to an improved level of gene expression in the target cells. Target cell CAT expression was two- to threefold higher for the vector containing the *pol* sequences and was only slightly less than that seen for a *trans*-complemented *env*-deleted provirus. These results indicate that defective HIV-1 vectors with efficiencies of gene transfer and expression comparable with that of HIV-1 itself are feasible.

INTRODUCTION

Retrovirus-mediated gene transfer has provided an efficient means of transferring genes into several cell types and is the current method of choice for clinical gene therapy (Anderson, 1984; Hock and Miller, 1986; Dzierzak *et al.,* 1988; Anderson, 1992; van Beusechem *et al.,* 1992; Ohashi *et al.,* 1992; Einerhand *et al.,* 1993; Miller *et al.,* 1993; Mulligan, 1993; Leiden *et al.,* 1995; Naldini *et al.,* 1996). The primary advantages of retroviral vectors are the high efficiency of infection, stable proviral integration into the host genome, and high levels of expression in a wide range of cell types (Varmus, 1988). Most currently used retroviral vectors are based on amphotropic murine leukemia viruses (MuLV-A).

It may be possible to take advantage of the unique properties of human immunodeficiency virus (HIV-1) to augment the utility of retroviral vectors. HIV-1 vectors have been shown to be able to infect growth-arrested cells refractory to MuLV-A vectors (Lewis *et al.,* 1992), a property that may be useful for delivering genes to resting target cells. HIV-1 can be efficiently pseudotyped by MuLV-A envelope glycoproteins (Chesebro *et al.,* 1990; Landau *et al.,* 1991), allowing an expansion of the host range of HIV-1 vectors beyond CD4-positive cells. Furthermore, given the ability of the wild-type HIV-1 genome to express multiple gene products via a number of naturally occurring *cis*-acting splice control elements, HIV-1 vectors should allow the simultaneous expression of several genes of interest. Finally, for the gene therapy of HIV-1 infection, HIV-1 vectors can be conveniently made that express products with antiviral activity only upon infection of the cell by HIV-1. Replication-defective HIV-1 vectors have been previously developed that can be propagated when essential viral proteins are provided *in trans,* resulting in a single cycle of replication (Helseth *et al.,* 1990; Page *et al.,* 1990; Poznansky *et al.,* 1991; Buchschacher and Panganiban, 1992; Parolin *et al.,* 1994; He and Landau, 1995). The generation of replication-competent viruses can be minimized by providing the *trans*-acting viral functions on two separate expression plasmids (Poznansky *et al.,* 1991). Currently, the efficiency of gene transfer that has been obtained with HIV-based vectors is significantly lower than that achieved by the existing MuLV-A-based systems. To overcome this problem, HIV-1 vectors must be modified to attain a high efficiency of both transduction and gene expression. Efficient transduction requires the optimal positioning of viral *cis*-acting sequences within the vector and the availability of packaging cell lines capable of producing high titers of replication-defective recombinant viruses. Sustained expression of exogenous genes in target cells depends upon the choice of promoters and the location of the transcriptional units within the vector framework. Accordingly, the expression of a gene of interest can be driven by a heterologous promoter at an internal site

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within the vector or by the retroviral promoter present in the 5′ LTR. The latter strategy can take advantage of alternative splicing mechanisms that complex retroviruses such as HIV-1 adopt to express high levels of regulatory proteins (Haseltine, 1988). One of these proteins, Tat, is essential for HIV-1 replication (Dayton *et al.,* 1986; Fisher *et al.,* 1986). Tat has been shown to activate transcription *in trans* from the HIV-1 LTR, increasing the steady-state levels of all HIV-1 mRNAs up to 1000-fold (Sodroski *et al.,* 1984, 1985; Arya *et al.,* 1985). Here we report the design of new HIV-1 vectors in which the expression of a heterologous gene is controlled by the Tat-driven HIV-1 LTR. The ability of these HIV-1 vectors to be packaged into virions and to mediate stable gene transfer into human T-lymphocyte cell lines and human peripheral blood mononuclear cells (PBMCs) was compared with that of a previously reported HIV-1 vector, which utilizes a heterologous internal promoter.

MATERIALS AND METHODS

Construction of the packaging system and vectors

The main features of the packaging system and vectors are illustrated in Figs. 1A and 1B. HIV-1 related sequences were derived from the HXBc2 provirus (Ratner *et al.,* 1985) with the exception of the *vpu* and *vpr* sequences, which were derived from the pNL4-3 molecular clone (Adachi *et al.,* 1986). The nucleotide sequences in the packaging and vector constructs are numbered according to Ratner *et al.* (1985). The packaging system (Fig. 1A) includes the CMVΔP1ΔenvpAvpu/vpr and the CMVenvΔXho plasmids. The CMVΔP1ΔenvpAvpu/vpr plasmid is a derivative of the CMVΔP1ΔenvpA construct previously described (Parolin *et al.,* 1994). To generate the CMVΔP1ΔenvpAvpu/vpr plasmid, an *Nde*I–*Nde*I fragment (nucleotide 4703 to 5981) of the CMVΔP1ΔenvpA plasmid was replaced by the corresponding sequence from the pNL4-3 molecular clone (Adachi *et al.,* 1986), introducing functional *vpu* and *vpr* genes. The CMVenvΔXho plasmid expresses the HIV-1 *rev* and *env* genes under the control of the cytomegalovirus immediate early promoter. The CMVenvΔXho construct was derived from the CMVenv plasmid. The latter plasmid contains the *Sal*I–*Xba*I fragment (nucleotide 5496-3′ flanking sequences) of the HXBc2 provirus cloned into the *Xho*I and *Xba*I sites of the pcDNAI/Amp plasmid (Invitrogen). The CMVenvΔXho construct was derived by digesting CMVenv with *Xho*I and *Xba*I, blunting the ends with the Klenow fragment of DNA polymerase I and ligation. This procedure removes the 3′ LTR and flanking sequences. Both the CMVΔP1ΔenvpAvpu/vpr and CMVenvΔXho plasmids contain polyadenylation signals derived from SV40.

The marker gene in all the HIV-1 vectors used herein (Fig. 1B) encodes chloramphenicol acetyltransferase (CAT) and was contained on a 0.78-kb *Bam*HI fragment derived from the pCM4 plasmid (Pharmacia). All vector plasmids contain the complete 5′ LTR, flanking leader sequences, *gag* sequences up to the *Sph*I site (nucleotide 989), and an *env* fragment from *Bgl*II (nucleotide 7199) to *Bam*HI (nucleotide 8053), inclusive of the Rev-responsive element (RRE). The v653 RSC vector is a derivative of the v653 RSN vector previously described (Parolin *et al.,* 1994), with the CAT gene located 3′ to the SL3-3 U3 LTR sequences, in place of the *neo* gene. To make the v653 RtatpC vector, an *Msc*I–*Bam*HI fragment (nucleotides 4132 to 8053), containing an *env* deletion between the *Bgl*II sites (at nucleotides 6619 and 7199), was used to replace the *Sph*I–*Bam*HI fragment (nucleotide 989 to 8053) of the HXBc2 provirus. The inserted fragment, which contains sequences from *pol, tat,* and the 5′ end of *env,* was introduced by joining *Bcl*I linkers at the *Msc*I site (nucleotide 4132) of the provirus. The v653 RtatC vector was constructed similarly, except that a *PflM*I–*Bam*HI fragment (nucleotides 4878 to 8053), containing an *env* deletion between the *Bgl*II sites (at nucleotides 6619 and 7199) and blunted at the 5′ end, was used to replace the *Sph*I–*Bam*HI segment (nucleotides 989 to 8053) of the HXBc2 provirus. In the v653 RtatpC and v653 RtatC vectors the CAT gene has been cloned at the *Bam*HI site (nucleotide 8053). In all vectors the sequences 3′ to the CAT gene include HXBc2 sequences from the *Bam*HI site (nucleotide 8053) to the 3′ boundary of the LTR.

Cell culture and transfection

Jurkat cells were grown in RPMI 1640 supplemented with 10% fetal calf serum. To assess promoter function, Jurkat cells ($7-10 \times 10^6$) were transfected by the DEAE-dextran procedure (Queen and Baltimore, 1983) with 10 μg of the v653 RtatpC vector or equivalent molar amounts of the other constructs examined and harvested 3 days later. Equivalent amounts of cell protein extracts were used to measure the level of CAT activity, as previously described (Sodroski *et al.,* 1984).

Generation of recombinant virus and transduction

Recombinant viruses were generated by cotransfection of COS-1 cells with the packaging system plasmids along with the vector, as described previously (Parolin *et al.,* 1994), except for the following modifications: (i) 10 μg of the CMVΔP1ΔenvpAvpu/vpr plasmid, 5 μg of the CMVenvΔXho plasmid, and 7 μg of the vector were used for transfection experiments; (ii) the recombinant virus was harvested 65 hr following transfection without filtration. PBMCs were obtained from healthy donors by FicoII-Hypaque density gradient separation and grown for 48–72 hr with phytohemagglutinin (PHA) (1 μg/ml) (Murex Diagnostics) in RPMI medium supplemented with 10% fetal calf serum. Eighteen hours before the infection, human interleukin 2 (Collaborative Research) was added to the culture (5%). Cell density was maintained at a constant level ($0.5-1.0 \times 10^6$ cell/ml) during all proce-

dures. Equivalent reverse transcriptase (RT) units (from 27,500 to 93,500 cpm in different experiments) of COS-1 supernatants were used to infect 1.5–2.0 $\times$ 10^5 Jurkat cells, in a total volume of 5 ml. Approximately 1 $\times$ 10^7 PBMCs were infected with recombinant virus equivalent to 1 $\times$ 10^5 cpm of RT activity. To assess the transduction ability of the vectors, the cells were collected on different days and lysed. Protein concentrations in the lysates were determined with the Pierce protein determination kit using BSA as a standard. Equivalent amounts of protein were used for determination of CAT activity, as previously described (Sodroski *et al.,* 1984). Only values in the linear range of CAT activity were used to calculate the relative CAT activities shown in Fig. 2.

Determination of vector RNA in virions and in packaging cell lines

Cytoplasmic RNA was harvested from COS-1 cells transfected with the packaging system and with different vectors as reported elsewhere (Parolin *et al.,* 1994). Virion RNA was purified from particles released into the supernatant of the transfected cultures after pelletting through 20% sucrose. RNA samples were adjusted for the amount of $p24^{gag}$ in the virion pellets as described previously (Dorfman *et al.,* 1993). Levels of vector RNA in the samples were quantified by RNase protection analysis using a uniformly labeled RNA probe synthesized from the *Mro*I-linearized pTZ18U/MX plasmid as described previously (Parolin *et al.,* 1994). This plasmid contains 5′ long terminal repeat (LTR) and untranslated sequences (HXBc2 nucleotides 308 to 815), covering the major splice donor site upstream of the *gag* gene, in an antisense orientation to a T7 RNA polymerase promoter (Dorfman *et al.,* 1993, 1994). All experiments were carried out under conditions in which excess probe was present. RNase-resistant fragments were separated on a 4.5% polyacrylamide–8.3 *M* urea gel, dried, and subjected to autoradiography. The relative intensity of bands on the gel was quantitated by densitometric analysis.

RESULTS

Production and testing of vectors

To minimize the generation of replication-competent virus, the packaging component was produced by two different plasmids (Fig. 1), as previously described (Parolin *et al.,* 1994). One plasmid expresses the *gag, pol, tat, vif, vpu,* and *vpr* genes and the other expresses the *env* and *rev* genes. Both plasmids contain the cytomegalovirus immediate early promoter and SV40 polyadenylation signals and lack sequences shown to be important for efficient HIV-1 packaging (Lever *et al.,* 1989; Aldovini and Young, 1990). Compared to the packaging component previously utilized in our laboratory (Parolin *et al.,* 1994), two principal modifications were made: (i) replacement of the 5′ LTR by the cytomegalovirus immediate early promoter in the *env* expressor plasmid, to eliminate the possibility of LTR-mediated homologous recombination between the HIV-1 vector and the packaging components, and (ii) inclusion of the *vpu* and *vpr* genes in the *gag-pol*-expressing plasmid.

Previous studies have identified the sequences that are necessary and sufficient for HIV-1-mediated gene transfer into human lymphocytes (Lever *et al.,* 1989; Aldovini and Young, 1990; Clavel and Orenstein, 1990; Poznansky *et al.,* 1991; Hayashi *et al.,* 1992). These encompass the HIV-1 LTRs and immediate flanking sequences, including the regions implicated in viral RNA packaging located between the 5′ LTR and the *gag* gene initiation codon. At the 3′ end of the genome, a polypurine tract is important for plus-strand DNA synthesis during reverse transcription. Recently, we have observed that inclusion of 653 nucleotides of *gag* sequences along with the Rev-responsive element (RRE) in the HIV-1 vector improved gene transfer efficiency into human lymphocytes (Parolin *et al.,* 1994). All of the vectors tested herein contain these sequences.

This study was designed to determine whether additional *cis*- or *trans*-acting HIV-1 sequences would increase the efficiency of HIV-1 vectors. For comparison with previously characterized HIV-1 vectors, the v653 RSC vector was constructed and included in this study. The v653 RSC vector is identical to the previously designed v653 RSN vector (Parolin *et al.,* 1994), except that the *neo* gene in the latter construct has been replaced by the chloramphenicol acetyltransferase (CAT) gene. The CAT gene was used to investigate promoter strength and transcriptional activity in the target cells. In the v653 RSC vector, the expression of the CAT gene is regulated by an internal promoter, the U3 sequences derived from the SL3-3 ecotropic murine leukemia virus (Celander and Haseltine, 1984; Losardo *et al.,* 1990). Compared with the v653 RSC vector, the v653 RtatC and v653 RtatpC vectors contain, in addition, the *tat* coding sequence and the 5′ end of the *env* gene (nucleotides 4878 to 6619). The v653 RtatC and v653 RtatpC vectors express the CAT gene under the transcriptional control of the HIV-1 LTR. In these constructs, the CAT gene can be expressed from a subgenomic mRNA generated by the same splicing events used for the natural HIV-1 *nef* message.

In addition to the polypurine tract (PPT) located at the border of the 3′ LTR, HIV-1 and other lentiviruses contain another PPT copy, located within the *pol* coding region (Charneau and Clavel, 1991; Charneau *et al.,* 1992). This central PPT is likely to be used as a second origin for plus-strand viral DNA synthesis (Charneau and Clavel, 1991; Charneau *et al.,* 1992). Furthermore, functional enhancer sequences within the *pol* gene have been identified (Verdin *et al.,* 1990; Van Lint *et al.,* 1994). To determine whether the presence of these PPT and enhancer sequences in the vector influences the efficiency of gene transfer, a 746-bp sequence (nucleotides 4132 to 4878), encompassing the PPT sequence as well as part of the

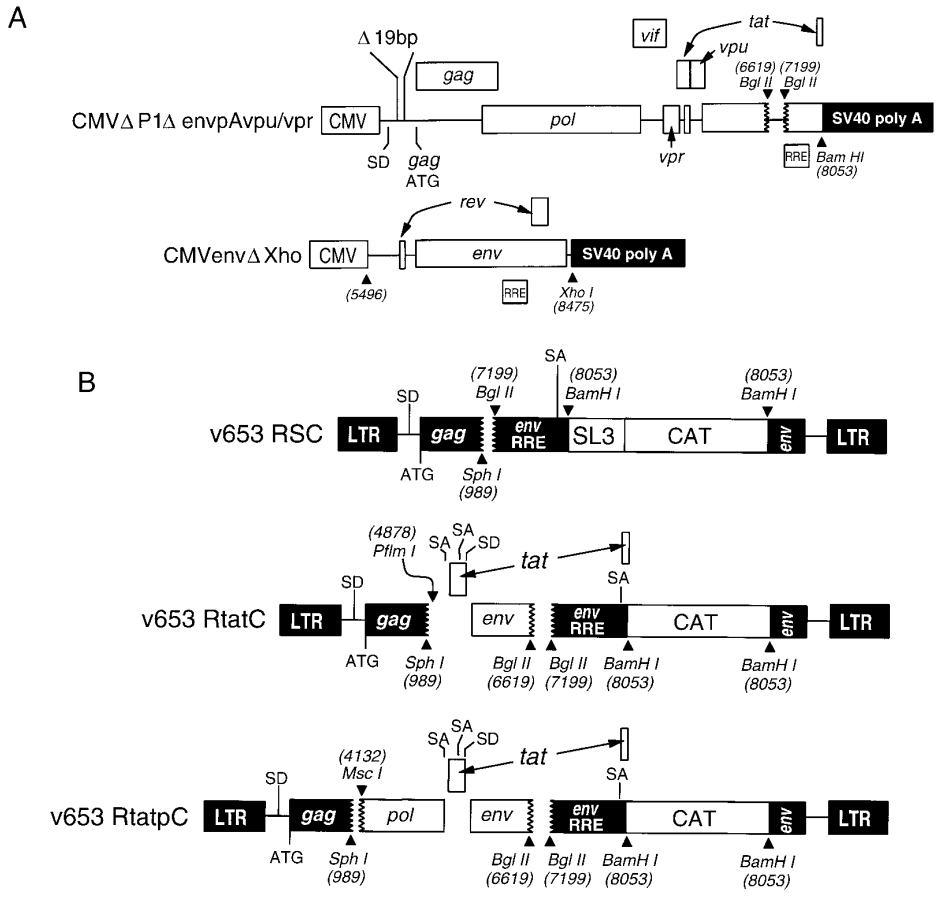

FIG. 1. Structure of the packaging system and vector plasmids. (A) Packaging system. The CMVΔP1ΔenvpAvpu/vpr and CMVenvΔXho plasmids are shown. HIV-1 genes that are functionally active are indicated. (B) HIV-1 vectors. The expression of the marker gene (CAT) is regulated by the SL3-3 U3 LTR sequences (SL3) in the v653 RSC vector and by the HIV-1 LTR in the v653 RtatpC and v653 RtatC vectors. Positions of some restriction endonuclease sites in the parental provirus are also indicated. SD, splice donor; SA, splice acceptor; RRE, Rev-responsive element; SV40 poly(A), polyadenylation signal from SV40; Δ19bp, deletion of sequences important for HIV-1 RNA packaging.

enhancer region, was deleted from the v653 RtatpC vector, generating the v653 RtatC vector. In all vectors the polyadenylation signal for the CAT gene transcript was provided by the 3′ LTR sequences.

Effect of inclusion of *tat*/*pol* sequences on vector-mediated gene transfer

The ability of the newly designed HIV-1 vectors to mediate gene transfer was analyzed by transduction experiments using Jurkat T lymphocytes and PHA-stimulated PBMCs as target cells. COS-1 cells were transfected with each HIV-1 vector along with the two-component packaging system described above. In parallel, COS-1 cells were also transfected with the HXBΔenvCAT construct together with the *env* expressor plasmid. The pHXBΔenvCAT construct contains an HIV-1 provirus with an *env* deletion and carries the CAT gene in place of the *nef* gene (Helseth *et al.*, 1990). Reverse transcriptase activity in the culture supernatants was determined 65 hr following transfection, and equivalent reverse transcriptase units were added to the target cells. To test for expression of the inserted CAT gene, aliquots of transduced cells were collected on different days following infection. Results of CAT assays carried out on these samples are summarized in Fig. 2. Comparable CAT activity was observed in Jurkat cells transduced with either the v653 RtatpC vector or the HXBΔenvCAT construct (Fig. 2A). CAT activity in Jurkat cells transduced with the v653 RtatC vector was lower than that observed in cells transduced with the v653 RtatpC vector, suggesting that the presence of *pol* sequences in the vector influenced either CAT gene transfer or expression. CAT activity in Jurkat cells transduced with the v653 RSC vector was approximately 500-fold lower than that observed for the v653 RtatpC vector. In all experiments, CAT activity in the Jurkat cells peaked between 10 and 25 days of infection. The CAT activity declined thereafter but was still readily detectable at 75 days following infection.

Similar experiments were carried out using PHA-stimulated PBMCs as target cells. As shown in Fig. 2B and Table 1, CAT activity was detected in the target PBMCs transduced with the v653 RtatpC and v653 RtatC vectors, as well as with the pHXBΔenvCAT virus. The relative

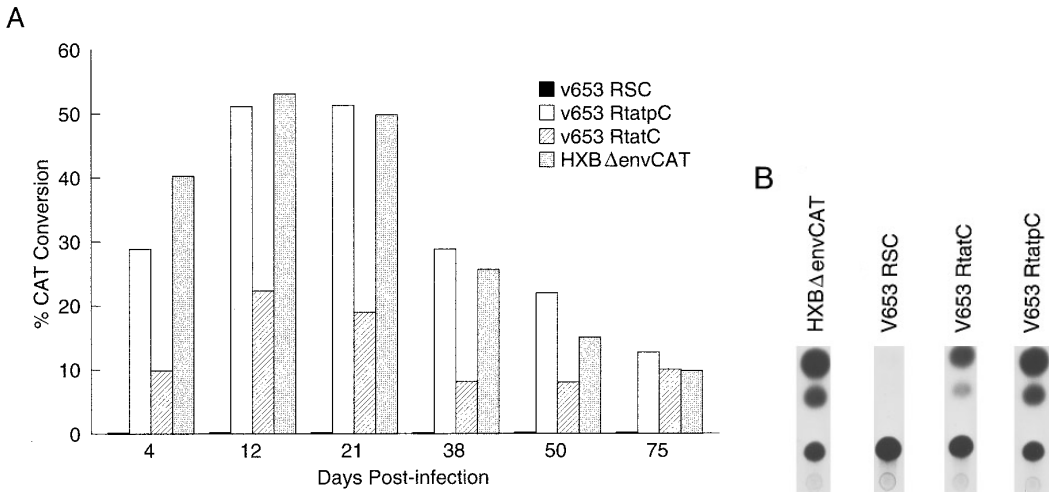

FIG. 2. Transduction efficiency of HIV-1 vectors in Jurkat lymphocytes and peripheral blood mononuclear cells (PBMCs). Supernatants from COS-1 cells, cotransfected with the packaging component along with each of the vectors, were incubated with Jurkat lymphocytes (A) and PBMCs (B) and CAT activity was measured on different days. The percentage conversion of chloramphenicol to its acetylated forms is shown. (A) The mean CAT activity derived from three experiments is presented. (B) An autoradiograph of a representative CAT assay is shown.

efficiencies of the different HIV-1 vectors in PBMC target cells were comparable to those observed in Jurkat lymphocytes. Experiments in which equivalent amounts of the vector and target cell preparations were used indicated that the HIV-1 vectors can transduce human PBMCs with an efficiency that is at least as high as that seen for Jurkat T cell lines (data not shown).

Effect of vector design on RNA packaging

The varying levels of CAT activity associated with the HIV-1 vectors could be due to differences in gene transfer efficiency or in the efficiency of gene expression in the target cells, or both. To determine whether differences in the efficiency of packaging the vector RNA into virions were responsible for the observed differences in vector-mediated gene transfer, the steady-state levels of vector RNA in virions and in the cytoplasm of the transfected COS-1 cells were measured by an RNase protection assay. A probe (probe MX) complementary to the viral long terminal repeats and untranslated leader sequences, encompassing the HIV-1 major splice donor site, was used to distinguish between spliced and unspliced vector RNA (Fig. 3). Protected fragments of the expected size for unspliced (355 nucleotides) and spliced (287 nucleotides) were detected in the cytoplasmic RNA. Unspliced RNA predominated in virion RNA preparations. Only minor differences in the intensity of the specifically protected fragments were observed among the different vectors. These results demonstrate that the packaging efficiencies of the v653 RtatpC, v653 RtatC, and v653 RSC vectors are similar and comparable with that of the previously described v653 RSN vector (Parolin *et al.,* 1994). Thus, differences in packaging efficiency are not likely to account for the observed differences in target cell CAT activity among the various vectors examined.

TABLE 1

CAT Activity in Human PBMC Target Cells[a]

	Day following transduction	
HIV-1 Vector	3	8
v653 RSC	0.20 ± 0.13	0.25 ± 0.07
v653 RtatC	9.0 ± 2.8	28.1 ± 8.7
v653 RtatpC	26.4 ± 2.2	68.5 ± 9.2
HXBΔenvCAT	29.7 ± 7.9	66.3 ± 22.6

[a] The percentage conversion of chloramphenicol to its acetylated forms is shown. The means and standard deviations are derived from two separate experiments.

Effect of vector design on gene expression in the target cells

To evaluate whether the vector design influenced the efficiency of CAT gene expression in the target cells, Jurkat lymphocytes were directly transfected with vector DNA. Three days following transfection, cells were lysed and similar amounts of cell protein extracts were used to measure the level of CAT activity. The results of two representative experiments are reported in Fig. 4. Low CAT activity was obtained with the v653 RSC vector. The CAT activities associated with transfection of the v653 RtatpC and v653 RtatC vectors were approximately 500-fold higher than that of the v653 RSC vector. Since the CAT activities observed for the v653 RtatpC and v653 RtatC vectors were comparable, the presence of the *pol* sequences had little effect on the expression efficiency of the vectors. Compared with cells transfected with the v653 RtatpC plasmid, Jurkat cells transfected with the pHXBΔenvCAT construct exhibited two- to threefold less CAT activity.

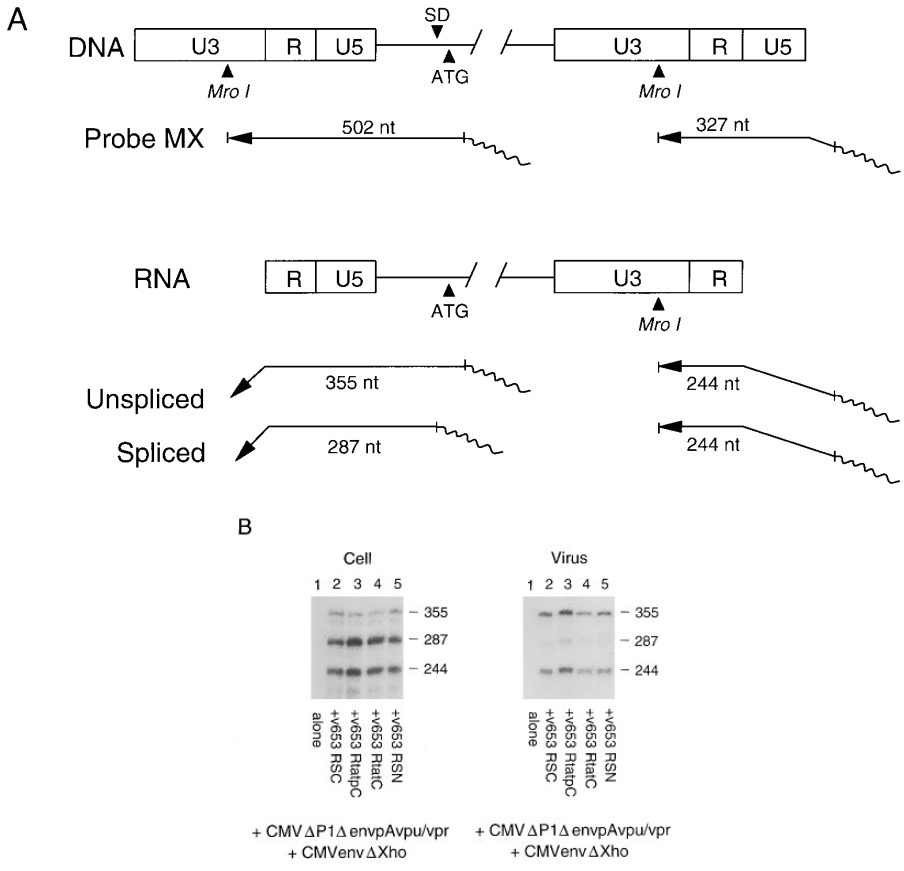

FIG. 3. RNase protection analysis of cytoplasmic and virion RNA. (A) The expected annealing of the MX probe with both vector DNA and RNA is shown. The MX probe is complementary to 355 and 244 nucleotide fragments of unspliced vector RNA derived from the 5′ and 3′ ends, respectively. Annealing of the MX probe with spliced vector RNA is expected to yield a 287-nucleotide fragment from the 5′ end and a 244-nucleotide fragment from the 3′ end. (B) RNase protection analysis of cytoplasmic RNA derived from the packaging cells or of viral RNA. The RNA samples were derived from COS-1 cells transiently transfected with plasmids expressing the packaging system alone (lane 1) or with the packaging system plus v653 RSC (lane 2), v653 RtatpC (lane 3), v653 RtatC (lane 4) or v653 RSN (lane 5).

DISCUSSION

In this study, HIV-1 vectors using the Tat-regulated viral LTR to express a heterologous CAT gene were compared with those using a heterologous internal promoter derived from the SL3-3 murine leukemia virus LTR U3 region. The latter element has been shown to exhibit substantial promoter activity in human and murine B and T lymphocytes (Celander and Haseltine, 1984; Losardo *et al.,* 1990). Although the strength of the SL3-3 LTR promoter is decreased by placement in an internal position within HIV-1 vectors (data not shown), the activity is sufficient to allow selection of cells transduced with HIV-1 vectors expressing neomycin phosphotransferase (Parolin *et al.,* 1994). Compared with this prototypic HIV-1 vector, HIV-1 vectors using the Tat-regulated LTR exhibited up to 500-fold greater CAT expression in Jurkat lymphocytes or human PBMCs. The majority of this improved CAT expression can be explained by the increased efficiency with which the Tat-regulated vectors express CAT in the target cells. The likely explanations for the efficiency of CAT expression in these vectors are the strength of the Tat-driven HIV-1 LTR as a promoter and the placement of the CAT gene. The latter feature allows CAT to be expressed from multiply spliced messenger RNAs that are particularly abundant in cells, like the target cells, that lack expression of the HIV-1 Rev protein. For similar reasons, Tat expression in the target cells should also be favored.

Inclusion of *pol* sequences, which contain the second polypurine tract (PPT) and part of the enhancer region, in the vector resulted in a two- to threefold increase in gene transfer efficiency. Direct transfection experiments indicated that only a small part of this increase appeared to result from a greater efficiency of vector gene expression in the target cells. However, it remains a formal possibility that the enhancer sequences identified in this *pol* segment exhibit greater activity in the context of the integrated vector sequences than in the context of transiently transfected plasmid DNA. The presence of the second PPT in these *pol* sequences, which has been reported to act as an additional site for initiation of HIV-1 plus-strand synthesis (Charneau *et al.,* 1992), probably also contributes to the observed positive effect on gene transfer.

The expression of the CAT gene by the HIV-1 vectors

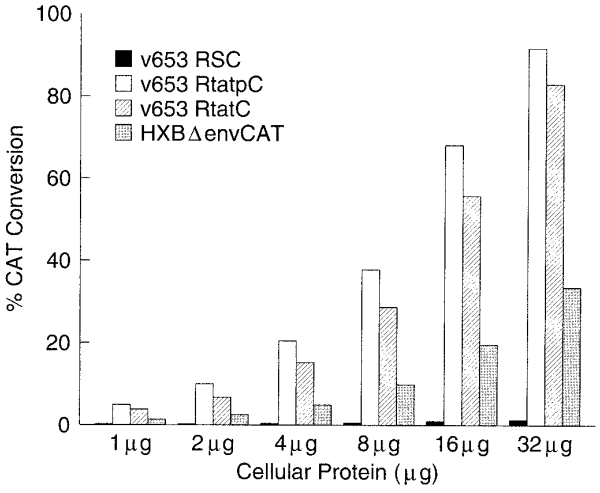

FIG. 4. Transient expression assay for the function of promoter elements. Jurkat cells were transfected with 10 μg of the v653 RtatpC vector or equivalent molar amounts of the other HIV-1 vectors and assayed for CAT activity three days following transfection. The values shown were determined by averaging the results from two separate experiments.

could be detected for at least 75 days following the transduction of Jurkat lymphocytes. The level of CAT expression in the target cell pool exhibited an initial increase followed by a decrease. The exact reasons for this pattern of expression are unclear. The initial rise might reflect a slow increase in steady-state CAT levels in transduced cells resulting from a turnover of the CAT protein that is slightly slower than the rate of production. The decrease in CAT production could have several explanations. Cells exhibiting high steady-state levels of CAT expression may be counterselected. This phenomenon might explain why the differences in CAT expression among the vectors are less apparent after 2 months of culture. Counterselection of Tat-producing cells could also explain the decrease. The HIV-1 Tat protein has recently been reported to increase the susceptibility of Jurkat lymphocytes to apoptosis (Li *et al.,* 1995). This effect, if operating in this case, must be extremely subtle, since several weeks are required to observe the counterselection. Finally, methylation-mediated shutdown of the HIV-1 LTR, as has been observed for other retroviral LTRs (Stewart *et al.,* 1982; Tsukiyama *et al.,* 1989), may contribute to the decrease in CAT expression upon long-term culture.

Among other potential uses, HIV-1 vectors might be utilized for gene therapy-mediated intervention in HIV-1-infected individuals. In this case, the vectors described herein might be modified by deletion of the functional *tat* gene, thus rendering expression of the heterologous gene dependent upon infection of the cell by HIV-1, which would result in the presence of high levels of Tat protein. Such HIV-1-inducible vectors would avoid the potential problem of detrimental effects of expression of foreign proteins on the host cell, such as the elicitation of cytotoxic T cell responses.

The efficiency of gene transfer and expression observed for the v653 RtatpC vector approaches that of the nearly full-length *env*-deleted HIV-1 provirus contained in the HXBΔenvCAT construct. Assuming that the transduction of HXBΔenvCAT sequences is as efficient as that of a complete HIV-1 genome, this result suggests that defective HIV-1 vectors could potentially achieve titers and efficiencies approaching those of HIV-1 itself. The optimization of vector components reported herein should help to achieve that end. The optimization of stable packaging systems will be addressed in future studies.

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