Biochemistry. In the article "Tat transactivation of the human immunodeficiency virus type 1 promoter is influenced by basal promoter activity and the simian virus 40 origin of DNA replication" by Mark Kessler and Michael B. Mathews, which appeared in number 22, November 15, 1991, of *Proc. Natl. Acad. Sci. USA* (**88**, 10018–10022), the authors wish to make the following corrections. Further investigation has failed to confirm the conclusion that the position of the simian virus 40 (SV40) origin relative to the human immunodeficiency virus long terminal repeat (HIV LTR) determines its transcriptional effect. Most probably, the constructs designated pH1 and pBAH1CAT, shown in Fig. 1 as having an SV40 origin of DNA replication sited upstream of the HIV LTR, in fact lacked an SV40 replication origin. Therefore, the comparison drawn in the paper is between plasmids containing an SV40 origin located downstream of the HIV LTR chloramphenicol acetyltransferase (CAT) cassette and plasmids with no viral origin. The *Abstract*, as amended, should read:

ABSTRACT We examined the activation of transcription from the human immunodeficiency virus type 1 (HIV-1) promoter by the viral Tat protein in a transient expression system. Plasmids contained a HIV-reporter gene cassette and a simian virus 40 origin of DNA replication. Run-on assays of transcription complex distribution and analysis of cytoplasmic RNA accumulation confirmed that Tat is able to activate transcription by two mechanisms: by increasing the rate of transcriptional initiation and the efficiency of transcriptional elongation. **The degree to which Tat stimulated initiation is determined by the basal level of HIV-directed transcription, which is influenced by the presence of the simian virus 40 replication origin. Tat functions primarily to increase the efficiency of elongation when the origin is present and the basal level of transcription is high. On the other hand, Tat functions primarily to increase the rate of initiation when the origin is absent and the basal level of transcription is 10-fold lower.** These studies suggest that the site of integration of the virus into the cellular genome may significantly affect the level of expression from the HIV promoter and consequently the pathobiology of the virus.

Immunology. In the article "Lymphohemopoietic reconstitution using wheat germ agglutinin-positive hemopoietic stem cell transplantation within but not across the major histocompatibility antigen barriers" by Nagwa S. El Badri and Robert A. Good, which appeared in number 14, July 15, 1993, of *Proc. Natl. Acad. Sci. USA* (**90**, 6681–6685), the authors request that the following correction be noted. On p. 6681 in the right column, the sentence beginning on the fourth line from the bottom of the page should read as follows: "Equal volumes of a uniform suspension of tosyl-activated magnetic beads (Dynal) were added to WGA (Sigma) protein solution [150 μg of protein per 1 ml of 0.5 M borate buffer (pH 9.5)] to achieve a protein/bead ratio of 75 μg/15 mg."

Biochemistry. In the article "Cloning and expression of the human vasoactive intestinal peptide receptor" by Sunil P. Sreedharan, Alain Robichon, Karen E. Peterson, and Edward J. Goetzl, which appeared in number 11, June 1, 1991, of *Proc. Natl. Acad. Sci. USA* (**88**, 4986–4990), the structural characterization of a human lymphocyte receptor, termed GPRN1, was reported. COS cells transfected with the GPRN1 construct exhibited binding to, and intracellular signaling mediated by, vasoactive intestinal peptide (VIP). Recently, we have cloned a cDNA, termed HVR, encoding a high-affinity neuroendocrine VIP receptor of human HT29 colonic adenocarcinoma cells and human lung and kidney tissues, that is a member of the secretin/parathyroid hormone family of guanine nucleotide-binding regulatory protein (G protein)-coupled receptors (1). The HVR-encoded protein is highly homologous to the previously reported rat lung VIP receptor (2). Further evaluation of the functional characteristics of GPRN1 has indicated that transfectants expressing GPRN1 do not bind VIP with the high affinity and specificity observed for HVR-expressing transfectants. GPRN1 has, therefore, been redesignated with GenBank (accession no. M64749) as the human homologue of the canine RDC1 orphan receptor (3).

1. Sreedharan, S. P., Patel, D. R., Huang, J.-X. & Goetzl, E. J. (1993) *Biochem. Biophys. Res. Commun.* **193**, 546–553.
2. Ishihara, T., Shigemoto, R., Mori, K., Takahashi, K. & Nagata, S. (1992) *Neuron* **8**, 811–819.
3. Libert, F., Parmentier, M., Lefort, A., Dinsart, C., Van Sande, J., Maenhaut, C., Simons, M., Dumont, J. E. & Vassart, G. (1989) *Science* **244**, 569–572.

Review. In the article "Alzheimer disease and the prion disorders amyloid β-protein and prion protein amyloidoses," by Donald L. Price, David R. Borchelt, and Sangram S. Sisodia, which appeared in number 14, July 15, 1993, of *Proc. Natl. Acad. Sci. USA* (**90**, 6381–6384), the following corrections should be noted. A colon was deleted from the title. The correct title should read "Alzheimer disease and the prion disorders: Amyloid β-protein and prion protein amyloidoses." In addition, two of the references contained errors. The correct citations are shown below.

2. McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D. & Stadlan, E. M. (1984) *Neurology* **34**, 939–944.
99. Lamb, B. T., Sisodia, S. S., Lawler, A. M., Slunt, H. H., Kitt, C. A., Kearns, W. G., Pearson, P. L., Price, D. L. & Gearhart, J. D., *Nature Genet.*, in press.

Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 10018–10022, November 1991
Biochemistry

Tat transactivation of the human immunodeficiency virus type 1 promoter is influenced by basal promoter activity and the simian virus 40 origin of DNA replication

(replication origin/transcription/transactivator)

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Communicated by James D. Watson, July 15, 1991 (received for review May 28, 1991)

ABSTRACT **We examined the activation of transcription from the human immunodeficiency virus type 1 (HIV-1) promoter by the viral Tat protein in a transient expression system. Plasmids contained a HIV-reporter gene cassette and a simian virus 40 origin of DNA replication. Run-on assays of transcription complex distribution and analysis of cytoplasmic RNA accumulation confirmed that Tat is able to activate transcription by two mechanisms: by increasing the rate of transcriptional initiation and the efficiency of transcriptional elongation. The degree to which Tat stimulated initiation is determined by the basal level of HIV-directed transcription, which is influenced by the position of the simian virus 40 replication origin. Tat functions primarily to increase the efficiency of elongation when the origin is located downstream from the HIV-reporter cassette and the basal level of transcription is high. On the other hand, Tat functions primarily to increase the rate of initiation when the origin is upstream from the cassette and the basal level of transcription is 10-fold lower. These studies suggest that the site of integration of the virus into the cellular genome may significantly affect the level of expression from the HIV promoter and consequently the pathobiology of the virus.**

The human immunodeficiency virus type 1 (HIV-1) Tat protein is a potent transactivator of HIV-1 gene expression and plays an essential role in the infectious process of the virus (1, 2), but its mechanism of action has not been fully resolved. It has been proposed that Tat functions at a number of different levels, including transcriptional initiation, elongation of nascent RNA chains, posttranscriptional processing, and protein synthesis (reviewed in ref. 3). The target for Tat-mediated transactivation is a sequence within the R region of the long terminal repeat (LTR) designated the trans-acting responsive element (TAR), minimally, residues +14 to +44 relative to the transcriptional initiation site (4–8). The TAR element must be situated near the transcription start site in the correct orientation (7). Tat interacts with RNA transcribed from the TAR region, which forms a bulged stem–loop structure (9), and recent work suggests that TAR functions largely, if not exclusively, to draw Tat to the vicinity of the transcription start site (10–12).

Studies of Tat's effect on transcription have typically employed model systems in which LTR-directed transcription of a reporter gene, generally the chloramphenicol acetyltransferase (CAT) gene, is compared in the presence and absence of Tat. Run-on transcription analysis revealed that Tat modulates reporter gene expression by increasing the rate of transcription from the HIV LTR. This conclusion is drawn from transient expression assays, in which the reporter gene was transfected into cells as plasmid DNA (4, 13–16), and from infection assays, in which the reporter gene was delivered via an adenovirus vector (17, 18). Further analysis of transcription complex distribution in a transient expression system suggested that Tat modulates LTR-directed transcription by relieving a block to transcriptional elongation in the TAR region (14). In this study, the transcription complexes were determined to be distributed equally between the promoter proximal region [339 nucleotides (nt)] and the adjacent region (520 nt) in the presence of Tat. In the absence of Tat, however, the density of transcription complexes was unchanged in the promoter proximal region, but no complexes were detected in the promoter distal region. A similar study conducted in our laboratory using the adenovirus system reached somewhat different conclusions (19). When transcription complex distribution was examined over short intervals within the promoter proximal region, most complexes were observed within the immediately promoter-proximal region (23 or 83 nt) when transcription was carried out in the absence of Tat. Tat affected transcription in two ways: it increased the efficiency of transcriptional elongation by suppressing polarity, and it also increased the rate of transcriptional initiation at the HIV promoter.

To resolve the differences between these two studies we examined transcription from the HIV-1 LTR in the transient expression system. We found that the basal transcription rate is the determinative feature and that this is influenced by the structure of the template, in particular by the location of the simian virus 40 (SV40) replication origin. Placement of the origin region downstream from the HIV LTR-CAT cassette permitted 10-fold more transcription from the LTR than when the origin was placed upstream from the LTR. When the origin was in the upstream location, Tat increased both the rate of initiation and the efficiency of elongation; on the other hand, when the origin was downstream Tat primarily functioned to increase the efficiency of transcriptional elongation. We infer that Tat can stimulate both processes but the increase in initiation is apparent only when the basal level of LTR-directed transcription is low.

MATERIALS AND METHODS

Plasmid and Probe Construction. The structures of the LTR-CAT plasmids are diagramed in Fig. 1. Plasmid pH1 contains the *Xho* I–*Bam*HI fragment of pU3RIII (20) flanked by *Xba* I linkers and cloned into pUC19. The SV40 origin of replication (SV40 fragment nt 5171–128) was inserted between the polylinker *Hin*dIII and *Sph* I restriction sites situated upstream of the LTR. Plasmids pH2 and pH2.1 are identical to pH1 except that the origin fragment, flanked with *Bam*HI

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Abbreviations: HIV-1, human immunodeficiency virus type 1; SV40, simian virus 40; LTR, long terminal repeat; CAT, chloramphenicol acetyltransferase; TAR, trans-acting responsive element; nt, nucleotide(s).

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linkers, was inserted in either orientation within the *Bam*HI site downstream of the LTR-CAT cassette. To construct pBAH1CAT, the *Hin*dIII fragment of pH1 (containing the origin region and HIV LTR) was cloned into the *Hin*dIII site of pBACAT (21). Plasmid pSt5 was derived from pHIVCAT (ref. 14; a gift of B. M. Peterlin) by insertion of the SV40 origin, flanked by *Bam*HI linkers, into the *Bam*HI site downstream from the LTR-CAT cassette. pSt4 was derived from pSt5 by deleting the sequences +83 to +220 relative to the transcription start site. pSt5.1 was prepared by replacing the *Kpn* I–*Bgl* II fragment (containing the LTR region from −450 to +21) of pSt5 with that of pH1. DNA probes I, II, and III and the RNA probe used in the RNase protection analysis are as described by Laspia *et al.* (19). Probe 2 was prepared by subcloning the *Hin*dIII fragment (+83 to +194) of pSt5 into M13mp18. The Tat expression vector pRSVtat (pBC12/RSV/t23; ref. 22) was provided by B. R. Cullen. Plasmids pU2/-247/RA.2, encoding a β-globin transcript, and pU2/RA.2/142, used to generate an antisense probe (23), were provided by S. M. Lobo.

Transient Assays. DNA transfections (1.5 μg of reporter gene and 1.13 μg of pRSVtat or carrier DNA per 10^6 COS-1 cells) were carried out using the DEAE-dextran method (24). Run-on transcription assays were conducted at 72 hr post-transfection using 2.5×10^7 cells per assay. Cell permeabilization by digitonin and run-on transcription assays followed the procedure of Ucker and Yamamoto (25). Reaction mixtures contained 1 μM UTP and 330 μCi of [α-^{32}P]UTP (1 Ci = 37 GBq). The RNA was purified and hybridized to slot blots according to the protocol of Greenberg (26). Cytoplasmic RNA was prepared at 48 hr posttransfection (27). RNase protection assays were carried out according to Melton *et al.* (28) with 2 μg of RNA per assay. The quantity of plasmid DNA in transfected cells was determined by extraction according to Hirt (29); this was followed by alkali treatment (1-hr incubation at 68°C in 0.3 M NaOH), serial dilution, and slot blotting onto nitrocellulose filters. The filter was probed with sense-strand RNA probe corresponding to sequences +1 to +83. Hybridization was quantified with the AMBIS Beta Scan system (AMBIS Systems, San Diego). For CAT enzyme assays, cell extracts were prepared at 48 hr post-transfection and assayed as described (17).

RESULTS

Differential Effects of Tat on Transcriptional Initiation and Elongation. Kao *et al.* (14) suggested that Tat functions as an antiterminator without increasing the amount of transcription initiated from the HIV LTR, whereas Laspia *et al.* (19) concluded that Tat modulates transcriptional initiation as well as elongation. In principle, the discrepancy between these two sets of results might reflect any of the several differences that exist between the model systems used in these two studies. Laspia *et al.* (19) introduced the HIV LTR-reporter gene cassette into HeLa cells and HeLa/Tat cells (human cells that stably express the Tat protein) by infection with an adenovirus vector (17). The LTR was derived from the U3RIII clone (20) of the HXB2 virus isolate. Cells were maintained under nonreplicating conditions and run-on analysis of transcription was conducted in isolated nuclei. In contrast, Kao *et al.* (14) used recombinant plasmids containing the reporter gene under the direction of the LTR from the SFII (ARV II) virus isolate. The plasmids carried an SV40 origin allowing them to replicate when introduced by transfection into COS cells (monkey cells stably expressing SV40 large tumor antigen). Tat was supplied by cotransfection of a Tat expression vector and run-on analysis was conducted in cells permeabilized by treatment with digitonin.

We set out to discover whether the discrepant results stemmed from inherent methodological differences between these two systems or had a more interesting underlying cause. As a first step, plasmid pH1, containing the pU3RIII-CAT cassette cloned into pUC19 with a SV40 origin region (Fig. 1), was transfected into COS cells in the presence or absence of pRSVtat. Nascent RNA transcripts were labeled and hybridized to nitrocellulose filters containing single-stranded DNA probes corresponding to the sense and antisense strands of the first 83 nt of the HIV leader and the next 151 nt of the CAT transcription unit (fragments I and II, respectively; Fig. 1*B*). Hybridization to β-actin was also monitored to ensure that RNA recoveries were equivalent.

Fig. 2 shows the patterns of hybridization obtained with RNA labeled for various periods of time in run-on transcription assays. In the absence of Tat, hybridization to fragments I and II was observed after 3 min of labeling. Taking into account the greater length and uridine content of fragment II (Fig. 1*B*), the data indicate that following 3 min and 6 min of labeling the majority of transcriptional complexes were in fragment I. By 12 min of transcription the hybridization signal in fragment II exceeded that in fragment I, consistent with movement of complexes from fragment I into fragment II. In the presence of Tat, RNA hybridizing to fragments I and II was detected after only 1.5 min of labeling. Again, hybridization to fragment I predominated with short labeling periods but was exceeded by hybridization to fragment II with longer labeling times, emphasizing that the shortest practical labeling times should be used to give an accurate representation of polymerase density. The uniform increase in hybridization to fragments I and II indicates that Tat has a greater effect on transcriptional initiation than on elongation, especially with short labeling periods. Inclusion of sarkosyl (30) or α-amanitin demonstrated that most, if not all, of the labeling is due to elongation of previously initiated complexes containing RNA polymerase II (not shown).

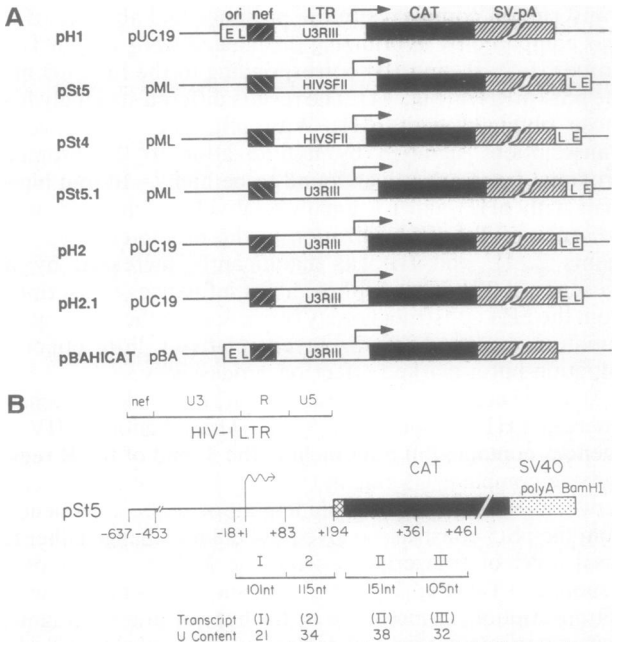

FIG. 1. Map of HIV CAT reporter constructs. (*A*) Schematic diagram of HIV CAT constructs showing the location of the SV40 origin (ori), HIV-1 *nef* gene sequence (striped box), LTR (open box), CAT coding sequences (black box), and SV40 splicing and poly(A) signals (hatched box). The orientation of the origin region, relative to the direction of early (E) and late (L) transcription, is noted as is the source of the viral sequences. The bacterial vector used to construct each plasmid is also noted. (The *nef* region marked in the diagram designates the portion of the *nef* gene lying upstream of the LTR.) (*B*) Schematic diagram depicting the structure of the LTR-CAT cassette in pSt5. The regions corresponding to probes I, 2, II, and III used in the run-on assays are also shown.

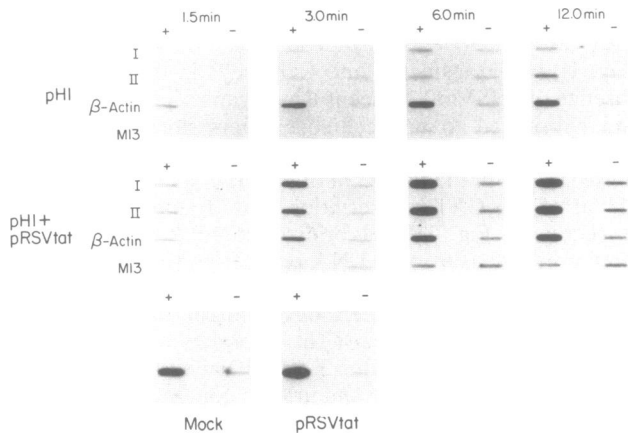

FIG. 2. Run-on transcription analysis of pH1 in the presence and absence of Tat. COS-1 cells were mock transfected or transfected with pH1, pRSVtat, or pH1 and pRSVtat, and run-on transcription reactions were carried out for 1.5, 3, 6, and 12 min. Labeled RNA was isolated and hybridized to blots containing 5 μg of sense (+) or antisense (−) DNA probe corresponding to nt −18 to +83 (I), +83 to +229 (II), β-actin, M13mp18, and M13mp19. The mock and pRSVtat assays represent 12-min run-on reactions.

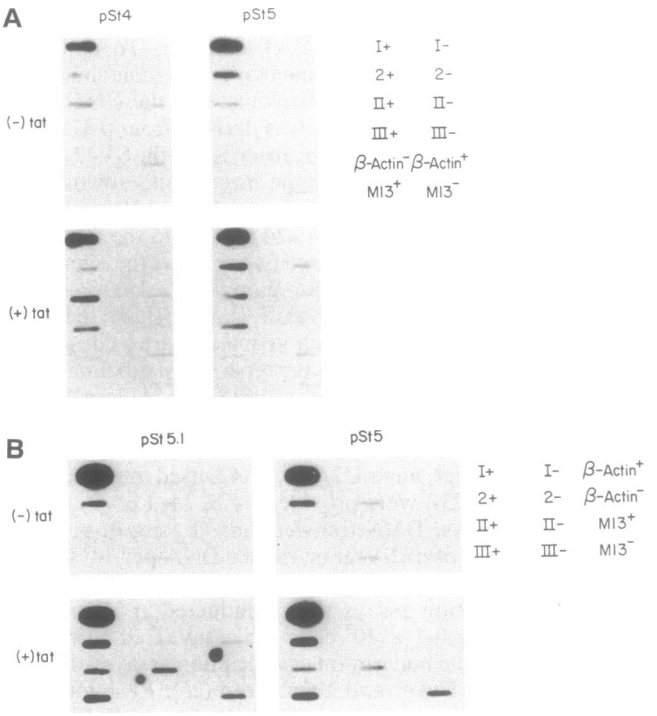

FIG. 3. Influence of LTR sequences from two viral isolates on transcription. (*A*) COS-1 cells were transfected with pSt5 or pSt4 in the presence or absence of pRSVtat, and run-on transcripts were hybridized as in Fig. 2. The regions corresponding to probes I, 2, II, and III and their U contents are noted in Fig. 1. The sequences contained within the region of probe 2 and the polylinker (hatched box) are absent from pSt4. (*B*) Run-on transcription analysis of pSt5 and pSt5.1. COS-1 cells were transfected with pSt5 and pSt5.1 in the presence and absence of pRSVtat, and run-on assays were carried out as described above.

Although the preceding analysis was conducted under conditions similar to those used by Kao *et al.* (14), the pattern of transcription complex distribution more closely resembled that expected from the data of Laspia *et al.* (19). To discover whether the results were influenced by the structure of the plasmid containing the HIV-CAT fusion, we next conducted run-on transcription reactions with plasmid pSt5 (Fig. 1), in which the HIV SFII-LTR is driving CAT gene expression as in the experiments of Kao *et al.* (14). The distribution of transcription complexes in the presence and absence of Tat was compared by hybridizing the labeled RNA to four DNA probes (I, 2, II, and III) corresponding to the first 465 nt of the pSt5 mRNA (Fig. 3*A*). The results differed strikingly from those obtained with pH1. With pSt5 the basal level of transcription, signified by hybridization to the promoter proximal fragment I, was found to be high (≈10-fold higher than with pH1) and to remain relatively unchanged in the presence of Tat. Hybridization to the promoter distal fragments 2, II, and III was significantly increased by the presence of Tat. Thus, although Tat influences transcription from the pH1 construct largely by increasing the frequency of initiation events, pSt5 displayed relatively little effect on initiation but a marked effect on processivity.

The pSt5 construct encodes 215 nt of HIV leader sequences, whereas pH1 encodes only 83 nt. The additional HIV sequences contained in pSt5 include the 3′ end of the R region and the complete U5 region (Fig. 1). Deletion of the region between +83 and +211 (including all of probe 2 sequences) from the pSt5 construct to give pSt4 did not change either the basal level of transcription from the HIV promoter or its response to Tat (Fig. 3*A*). The basal and Tat-stimulated levels of transcription, as measured by the hybridization to fragment I, were very similar for both templates, suggesting that Tat has little or no effect on transcriptional initiation. Hybridization to the promoter distal regions (fragments II and III) was significantly increased with both templates, implying that the primary effect of Tat on transcription with these constructs is at the level of elongation.

The U3 regions of pH1 and pSt5 are about 10% divergent in sequence (31). Since this region contains the HIV promoter, we considered the possibility that the transcriptional differences between pH1 and pSt5 were due to this sequence variation. The U3 region of pSt5 was therefore replaced with that of pH1 to give plasmid pSt5.1 (Fig. 1), and the transcriptional activities of pSt5.1 and pSt5 were compared. Similar patterns of run-on transcription were obtained with these plasmids in the presence and absence of Tat (Fig. 3*B*). We conclude that the different transcriptional activities of pH1 and pSt5 are not due to differences in their LTRs, either upstream or downstream of the transcription start site.

The SV40 Origin Region Influences Transcription from the HIV LTR. Since the different transcriptional activities of pH1 and pSt5 are not due to differences in HIV sequences contained within the promoter or leader regions, the discrepancy must be due to HIV sequences upstream of the U3 region (both constructs were prepared from the 3′ LTR and contain part of the *nef* gene) or to sequences contained within their respective vectors. The two plasmids differ in the vector used in their construction and in the position and orientation of the SV40 origin region relative to the reporter gene (Fig. 1). Three new constructs were prepared to determine which of these differences could account for the different transcriptional activities of pH1 and pSt5 (Fig. 1). The first construct, pH2, is identical to pH1 with the exception that the SV40 origin has been moved to the downstream location that it occupies in pSt5 and with the same orientation. In the second construct, pH2.1, the origin region was left in the same location as in pH2 but was inverted to match its orientation in pH1. The third construct, pBAH1CAT, is identical to pH1 with respect to the position and orientation of the SV40 origin and HIV sequences, but the vector is the pBR-based plasmid pBA (21). When the transcriptional activities and the responses of each of these constructs to Tat were measured by the run-on assay, we found that pBAH1CAT behaved like pH1 and that pH2 and pH2.1 behaved like pSt5 (data not shown). Therefore, the different transcriptional activities of

pH1 and pSt5 are due to the position, but not orientation, of the SV40 origin region relative to the HIV LTR.

RNase protection analysis of cytoplasmic HIV LTR-directed RNA confirmed these conclusions. With an antisense probe extending from +83 to −117, two classes of protected fragments were anticipated: a series of short fragments, 55–59 nt in length, presumably resulting from premature termination of transcription, and a longer fragment (83 nt) representing full-length mRNA (14, 19, 32). In the absence of Tat, pH1 and pBAH1CAT gave rise to very little RNA (Fig. 4*A*, lanes 1 and 5). The protected species corresponding to the long and short RNA populations were observed in roughly equal abundance. In the presence of Tat little change was observed in the quantity of short RNAs, but the abundance of long transcripts was greatly increased (lanes 2 and 6). By contrast, pH2 and pSt4 generated large amounts of short transcripts in the absence of Tat (lanes 3 and 7). Full-length RNA was observed, but as a minor population of the protected RNA. In the presence of Tat, these two constructs gave rise to large amounts of the full-length RNA (lanes 4 and 8). Similar results were obtained with pSt5 (data not shown), confirming the observations of Kao *et al.* (14). Although the quantity of short transcripts was reduced, the reduction did not appear to be sufficient to account for the increase in full-length RNA and was not observed consistently (see Fig. 4*C*). RNA transcribed from pH2.1 in the presence and absence of Tat (Fig. 4*C*, lanes 1 and 2) was similar to that transcribed from pSt4 (lanes 3 and 4). Thus, the positioning of the SV40 origin upstream of the LTR gives rise to a lower basal level of HIV-promoted transcripts and allows for a large effect of Tat on cytoplasmic RNA accumulation. Corresponding results were obtained by measurement of CAT enzyme activity (Table 1).

We also considered the possibility that the different quantities of cytoplasmic RNA observed following transfection of these plasmids were the result of differences in the efficiency of transfection or replication of the plasmid DNA. To control for transfection efficiency and RNA recovery, a plasmid containing β-globin sequences under the direction of the U2 snRNA promoter was included in each transfection. As shown in Fig. 4*B*, the differences in transfection efficiency, reflected in differences in the amount of protected β-globin probe, cannot account for the observed differences in HIV LTR-directed transcription. To measure the relative quantity of plasmid present at the time of RNA extraction, DNA was isolated from the transfected cells and quantified by hybridization. From the data of Table 1 it is apparent that there were variations in the amount of DNA, but the differences (≈2-fold overall) were too small to account for the differences observed at the level of cytoplasmic RNA.

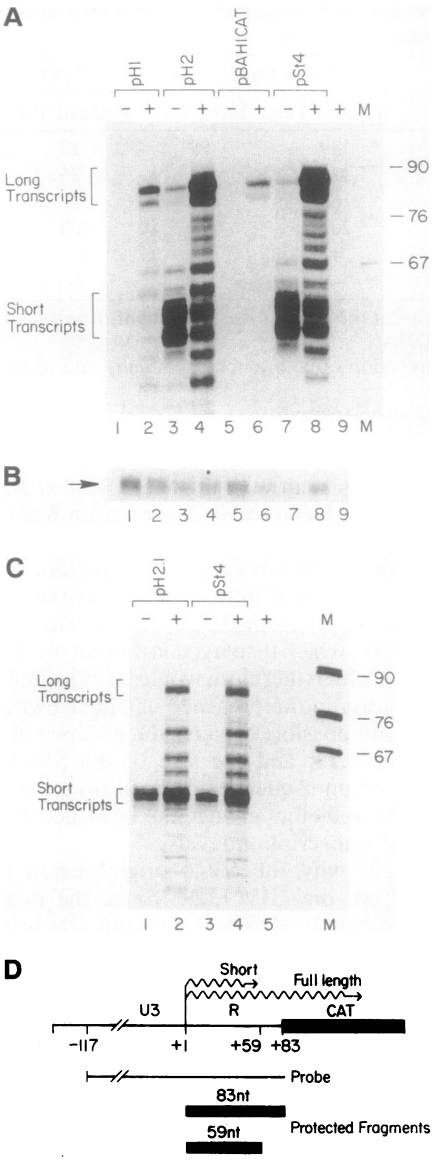

FIG. 4. Effect of vector and SV40 origin sequences on HIV LTR-directed RNA expression. (*A*) RNase protection analysis of cytoplasmic HIVCAT RNA following transfection of COS-1 cells with pH1 (lanes 1 and 2), pH2 (lanes 3 and 4), pBAH1CAT (lanes 5 and 6), or pSt4 (lanes 7 and 8) or without an LTR-CAT plasmid (lane 9) in the presence (lanes 2, 4, 6, 8, and 9) or absence (lanes 1, 3, 5, and 7) of pRSVtat. Lane M contains pBR322/*Hpa* II markers. Locations of the long and short transcripts are marked. (*B*) RNase protection analysis of β-globin transcripts present in the cytoplasmic RNA preparations used in *A*. Plasmid pU2/-247/RA.2 was cotransfected with the HIVCAT reporter constructs and RNase protection analysis was carried out using a riboprobe transcribed from *Eco*RI-digested pU2/RA.2/142. A 142-nt fragment is protected. (*C*) RNase protection analysis of cytoplasmic HIVCAT RNA following transfection of COS-1 cells with pH2.1 (lanes 1 and 2) or pSt4 (lanes 3 and 4) or without a LTR-CAT plasmid (lane 5) in the presence (lanes 2, 4, and 5) or absence (lanes 1 and 3) of pRSVtat. Lane M contains pBR322/*Hpa* II markers. Locations of the long and short transcripts are noted. (*D*) Schematic diagram of the mapping strategy depicting the probe, RNA transcripts, and protected fragments.

DISCUSSION

In a transient expression system, the HIV-1 transactivator protein Tat is capable of activating transcription from the HIV-1 LTR by increasing the rate of transcriptional initiation and by increasing the efficiency of transcriptional elongation. The relative extent to which it performs each of these functions is determined by the basal level of LTR-directed transcription. If the basal level of transcription is high, Tat functions primarily to increase the elongation rate, whereas if the basal level is low Tat primarily increases the rate of initiation. This finding is in accord with previous studies in which we utilized recombinant adenovirus containing the HIV LTR-CAT cassette to study Tat-mediated activation of HIV transcription. Tat increased the rate of transcriptional initiation and stabilized elongation in HeLa cells infected with the virus but only stabilized elongation when transcription was carried out in the presence of the general transcriptional activator E1A (19, 33).

These observations may help to reconcile the results of Kao *et al.* (14), who concluded that Tat acts as an antiterminator to increase transcriptional elongation, with results from our laboratory indicating that Tat also acts to increase the rate of initiation (19). The most likely explanation would be that Kao *et al.* (14) studied Tat transactivation in the context of a high basal level of HIV-directed transcription, whereas Laspia *et al.* (19) examined transactivation under conditions where the promoter exhibited a low basal level of

Table 1. Quantitation of plasmid DNA and HIV-directed CAT activity in transfected cells

	DNA level*		CAT activity†	
	Without Tat	With Tat	Without Tat	With Tat
pH1	149	99	12	2300
pBAH1CAT	130	74	26	1120
pH2	166	160	312	6000
pSt4	202	106	120	6720
pSt4‡	—	2	—	—
None§	—	5	—	—

*Values represent the standardized cpm hybridizing to two dilutions of blotted DNA.
†Percent conversion of chloramphenicol substrate to its acetylated forms.
‡Control, not alkali-treated.
§DNA extracted from cells transfected with pRSVtat only.

transcription. Consistent with this view, Kao *et al.* (14) used a plasmid with the SV40 origin downstream from the HIV-reporter gene cassette for their analysis of RNA accumulation. Unfortunately for this simple explanation, the plasmid used in their run-on transcription analysis contained the SV40 origin region upstream from the HIV LTR. This construct is analogous to pH1, which displayed low basal level of activity in our study and which therefore would be expected to exhibit increased initiation in the presence of Tat. However, direct comparison is not possible because the distance of the origin region from the LTR and the size of the SV40 fragment containing the origin sequences differed from pH1, and it is possible that these factors change the influence of the origin region on basal transcription levels.

It is not clear why the SV40 origin region influences transcription from the HIV LTR. Since the origin region contains, in addition to sequences directing DNA replication, promoter elements involved in the regulation of early and late viral gene expression, its effect could be due to occlusion of the HIV promoter by transcription from the origin region. This explanation seems unlikely, however, since RNA transcripts containing sequences 5′ to the LTR start site were detected only as a minor population with plasmids expressing elevated basal levels of transcription, pH2 and pSt4, and not with plasmids expressing low basal levels (result not shown). An alternative explanation is that the replication process influences transcription from the HIV promoter, but to different degrees depending on the distance of the origin of replication from the promoter. In several instances, replication has been shown to activate transcription (34–38) and the HIV LTR may belong to this class of promoters (N. Proudfoot and J. Monks, personal communication).

The transition from viral latency to growth, as well as from noncytopathic to cytopathic infection, appears to require the coordinated and sequential expression of the viral regulatory and structural proteins (39–41). Viral transcriptional rates directly influence this pathway (40). Activation of the HIV promoter as a result of DNA replication may explain, at least in part, why nonresting T cells are permissive for HIV-1 infection, whereas G_0 cells are not (42). Interestingly, analysis of retroviral replication in resting and actively growing cells revealed the proviral DNA to be integrated only in cellular DNA replicated during infection (43). Whether cellular origin regions are preferred sites for viral integration is unknown. However, if the effect of the SV40 origin region on HIV gene expression is typical, viral integration near a cellular origin may contribute significantly to the pathobiology of the virus.

We thank M. Laspia and A. Rice for discussions, N. Hernandez and W. Herr for critical review of the manuscript, and R. Packer for cell work. These studies were supported by National Institutes of Health Grants CA13106 and AI27270 and by National Institutes of Health Fellowship AI07877 to M.K.

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