cats 1082 BM and 1220 BM to clone unintegrated circular viral DNA (which Bam HI will cut once and therefore linearize). To obtain clones with intact proviruses, DNA from cats 1045 BM and 1161 SI was first cut with Eco RI (which does not cleave within the FeLV-FAIDS genome), and fractionated on a sucrose gradient. Fractions containing DNA of sufficient length to potentially contain full-length proviruses, but within the capacity of the bacteriophage vector [λgtWESλB; P. Leder, D. Tiemeier, L. Enquist, *Science* **196**, 175 (1977)] were pooled. The libraries were prepared and screened with the exU3 probe. Proviruses were subcloned into the plasmid vector pUC18 for subsequent analysis. These procedures, the origin of the RD(FeLV)-2 cell line used as hybridization standard (lane marked RD), and the generation of restriction maps and blot hybridization were generated as previously described [J. I. Mullins, J. W. Casey, M. O. Nicolson, N. Davidson, *Nucleic Acids Res.* **8**, 3287 (1980); J. I. Mullins, J. W. Casey, M. O. Nicolson, K. B. Burck, N. Davidson, *J. Virol.* **38**, 688 (1981)]. The most intense band in the RD lane is 3.6 kb in length and corresponds to approximately 22 copy per cell intensity, whereas the remaining bands correspond to single copy per cell intensity.

20. Plasmids containing the genome of 61E and 61C were digested with Eco RI and Bam HI. A fragment representing the 3′ portion of each virus was gel-purified in low melting agarose, treated with the Klenow fragment of DNA polymerase I to fill in recessed ends, and subcloned into Sma I–digested pUC19. These subclones were subsequently digested with Eco RI and Xho I and ligated with a gel-purified Eco RI–Xho I fragment representing the 5′ portion of either 61E or 61C. The structure of the plasmids containing full-length chimeric viruses was verified by restriction enzyme digestion.
21. Deletion subclones were generated by digestion of the Bam HI–Eco RI subclone of 61C (Fig. 4) with exonuclease Bal 31 [M. Poncz, M. Solowiejczyk, E. Schwartz, S. Surrey, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4298 (1982)], ligated into M13mp18, and sequenced by the Sanger dideoxy method as described [F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977)].
22. We thank V. Stallard for technical assistance. Supported by grants from the American Foundation for AIDS Research (J.O.), by NIH CA43216 (E.A.H. and J.I.M.), and by the Massachusetts AIDS Research Council and NIH CA01058 (J.I.M.). J.O. was supported in part by a fellowship from the Interdisciplinary Programs in Health under EPA assistance agreement CR812699, and P.R.D. by a postdoctoral fellowship from NIH (CA07966). DNA sequence analysis was performed using Intelligenetics program made available through BioNet computer resource.

20 November 1987; accepted 15 January 1988

Site-Directed Mutagenesis of Two Trans-Regulatory Genes (*tat*-III, *trs*) of HIV-1

M. Reza Sadaie,* Thomas Benter, Flossie Wong-Staal

Point mutations were introduced into the overlapping trans-regulatory genes (*tat*-III and *trs*) of human immunodeficiency virus type 1 (HIV-1), and the mutants were evaluated for virus expression. The results showed that *tat*-III has a positive trans-acting role and is required for transcriptional activation. A chain terminating mutation early in the *trs* gene resulted in an increase in transcription of viral messenger RNA as measured by nuclear transcription experiments, but only one major species of viral messenger RNA (1.8 kilobases) was detected, and little or no viral structural proteins were made. Thus, the *trs* gene product is essential for expression of virus structural proteins but, at the same time, may have a negative trans-regulatory role in transcription. Cotransfection of the point mutant proviruses defective in *tat* or *trs* with each other or with a complementary DNA clone containing *tat* and *trs* sequences restored the normal transcription pattern and subsequent virus production.

The prototype human immunodeficiency virus (HIV-1) contains, in addition to the structural genes *gag*, *pol*, and *env*, at least five accessory genes termed *sor*, *R*, *tat*-III, *trs*, and 3′-*orf* (*1*). The *tat* and *trs* genes are of particular interest since both are essential for virus expression (*2–5*). However, the precise functions of these genes in the virus life cycle and pathogenesis are not well understood. The sequences responsive to *tat* are located downstream from the initiation site for transcription, and several studies based on the use of deletion mutants suggest that *tat* increases the efficiency of messenger RNA (mRNA) utilization (post-transcriptional activation) (*5, 6*) or the level of steady-state mRNA either by direct transcriptional activation or mRNA stabilization (*7*). Parallel studies showed that nuclear extracts from HIV-infected cells contain transcriptional activator or activators specific for the HIV-1 long terminal repeat (LTR) (*8*). The *trs* gene (also referred to as *art*) may also act at more than one level, as an antirepressor at the translational level (*4*), and influence the relative abundance of viral mRNAs of large and small molecular weight (*5*). Because the *tat* and *trs* genes overlap, large deletions usually affect both genes and may further affect potential target sequences or secondary structures of the transcribed mRNA (*2–5*). We therefore used oligonucleotide-directed mutagenesis to introduce independently single translational stop codons in the 5′ portion of each gene. Analysis of these mutants indicates that both *tat* and *trs* regulate virus production at transcriptional and post-transcriptional levels.

Using a biologically active HIV-1 clone, HXB2 (*2*), we constructed two mutants with independent changes in the *tat* and *trs* genes (see Fig. 1) that we isolated by means of the double-primer extension method (*9*). This procedure included a GC primer (15 bases in length) previously designed for pGC1 vector (*10*) and a mutated oligonucleotide (21 bases). One mutant contained a termination codon (TGA) in place of the methionine initiator codon (ATG) in the *tat* gene [mutant 80:*tat*(−)]. The other contained a stop codon (TGA) in place of the glycine residue 6 (GGA) in the *trs* gene and simultaneously a leucine (CTG) codon in place of arginine (CGG) at residue 52 in the *tat* reading frame [mutant 107:*trs*(−)]. We then transfected the mutants into COS-1 cells (an African Green monkey kidney-derived fibroblastic cell line), which are highly permissive for virus production but are refractory to subsequent reinfection (*5*). The efficiency of transfection of mutant and wild-type clones appeared comparable as monitored by Southern hybridization of cellular DNA harvested 48 hours after transfection. The use of COS-1 cells allowed us to compare, qualitatively and quantitatively, the mutants for RNA and protein production. Mutant viruses derived from these cells can also be assayed for their infectivity in permissive lymphoid cell lines by cocultivation or by cell-free transmission.

Initially, we screened the mutants for *tat* activity using a transient chloramphenicol acetyltransferase (CAT) assay in which the bacterial acetyltransferase gene was used as an indicator (*11*). Cotransfection of the mutant genomes and an LTR-CAT plasmid into COS-1 cells was mediated by calcium phosphate precipitation. The activity of *tat* was measured as percentage conversion of ^{14}C-labeled chloramphenicol to its acetylated metabolites (Fig. 2). The plasmid HXB2 was used as control for wild-type activity, and the complementary DNA construct C15-CAT, which contains the HIV-1 3′ LTR upstream of CAT (*12*), served as a negative control. In mutant 80 the CAT activity was completely abrogated. However, in mutant 107, the level of CAT activity was only mildly reduced relative to that of the wild type. No significant difference was detected between the LTR-CAT alone and cotransfected LTR-CAT and the *tat*(−) clone over the linear range of the time

Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

*To whom correspondence should be addressed.

course. The *trs*(−) clone showed slightly lowered values as compared to the wild type, that is, approximately 20% reduction during 60 minutes of incubation time (Fig. 2). However, both mutants showed greatly suppressed reverse transcriptase (RT) activity (Table 1). These data indicate that both *tat* and *trs* are essential for virus production.

We then examined viral mRNA expression by the mutant clones, using total cellular RNA prepared from the cell lysates 48 hours after transfection. Mutant 80:*tat*(−) expressed low or undetectable levels of viral mRNA, whereas mutant 107:*trs*(−) clearly expressed augmented levels of the 1.8-kb mRNA species but little or no higher molecular weight species (Fig. 3). This result is similar to an independent finding based on a frame shift *trs* construct (*5*). Variability in the quality and amounts of the RNA was controlled by rehybridization of the same Northern blot with a β-actin insert (Fig. 3A, lower panel). Densitometric tracings of the RNA gels revealed a greater than tenfold reduction of steady-state viral mRNA for 80:*tat*(−) and a fivefold increase for 107:*trs*(−) as compared to wild type. Similar results were obtained by slot blot experiments.

To determine whether the effects of *tat* and *trs* on mRNA levels are transcriptional or post-transcriptional (for example, mRNA turnover), we performed nuclear transcription experiments using isolated nuclei prepared at 24 and 48 hours after transfection. These experiments addressed specifically the elongation of preinitiated heterogeneous viral RNA rather than the initiation of transcription. We hybridized ^{32}P-labeled RNA synthesized by the cell-free nuclei to HIV-1 sequences (BH10 insert; *13*) using the slot blot procedure. The amount of newly transcribed viral mRNA was quantitated by densitrometric tracing. Compared to the wild type, the de novo RNA synthesis was approximately one-fifth as much in the *tat*(−) mutant and increased three- to fourfold in the *trs*(−) mutant (Fig. 4A). This, together with the Northern blot results, suggests that *tat* and *trs* gene products function as positive and negative regulators in transcription, respectively, specifically in the initiation or the chain elongation step or in both.

To test the possibility that *trs* may regulate transcription from different promoters, we carried out S1 nuclease protection analysis using two antisense oligonucleotide fragments, a 40-base fragment spanning the U3/R border (nucleotide positions −10 to +30) and an additional 60-base fragment derived from U3 (nucleotide positions −117 to −58). The protected doublet bands of 32 and 30 nucleotides from the first probe represent the normal initiation site of the HIV-1 (Fig. 4B) consistent with previous findings (*14*). The complete protection of the 60-nucleotide fragment also indicated the lack of an additional initiation site in that region (Fig. 4B). Hence, the promoter site appears to be the same for the transcription of viral RNAs for the *trs*(−) mutant and for the wild type.

We then performed radioimmunoprecipitation studies of the metabolically labeled cell lysates prepared after the DNA transfection into COS-1 cells. The same batch of cells was harvested for RNA isolation as described above and for immunoprecipitation with pooled serum samples from two seropositive patients (*15*). As shown in Fig. 5, a characteristic HIV-1 protein pattern

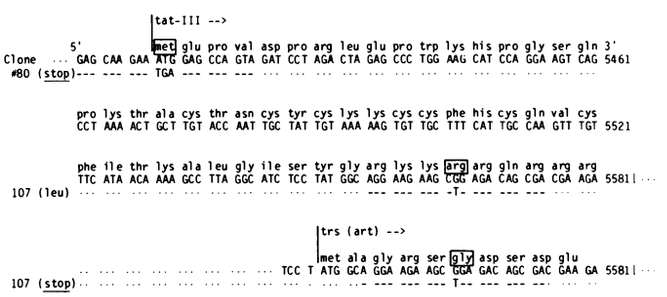

Fig. 1. Sequences of *tat*-III and *trs* mutants. Parts of the overlapping *tat* and *trs* sequences in the HIV-1 pHXB2gpt clone are shown with predicted amino acid sequences starting at the ATG initiator sites bordered with one Met. Two independent mutants are shown aligned with the *tat* sequence of the pHXB2gpt clone. The figure is patterned such that the *trs* sequence is aligned with the *tat* sequence in a different reading frame. Beneath is shown the aligned *trs* mutant, overlapping with the *tat* mutant, with independent identities as indicated with the same numbering system on the left. Oligonucleotides that were used in mutagenesis (*24*) are underlined with dashes, and the nucleotide positions where there are mutations relative to the wild-type pHXB2gpt provirus are also indicated. Aligned dots indicate identical sequences relative to the wild type, verified by DNA sequencing. The boxed amino acids indicate targeted residues with the predicted transversions shown within the parentheses on the left.

Table 1. Comparison of biological properties of wild-type, *tat*(−), and *trs*(−) HIV-1 mutants. Common features are the failure of the mutants to synthesize normal levels of the viral mRNAs, structural proteins, and virus particles in COS-1 cells. Electron microscopy (EM) analysis was repeated three times with similar results. 1×, onefold; NT, not tested. Variability in the mean values for reverse transcriptase (RT) activity (*23*) was less than 20%.

Clone	Genotypic feature	RNA			Protein	Virus production	
		Northern	Slot blot	Nuclear run-on	^{35}S-labeled immunoprecipitates	RT activity [^{3}H]TMP cpm/ml (×10^{-3})	EM
HXB2:WT	*tat*$^+$ *trs*$^+$	All species	1×	1×	+	55.2	+
80	*tat*$^-$ *trs*$^+$	Very low 1.8 kb	<0.1×	0.2×	−	8.7	−
107	*tat*$^+$ *trs*$^-$	Predominantly 1.8 kb	3–5×	3–4×	−	7.3	−
80+107		All species	2×	NT	+	30.1	+
80+pCV1		All species	NT	NT	+	30.0	+
107+pCV1		All species	NT	NT	+ (low)*	13.2	+ (low)*

*Small amounts only.

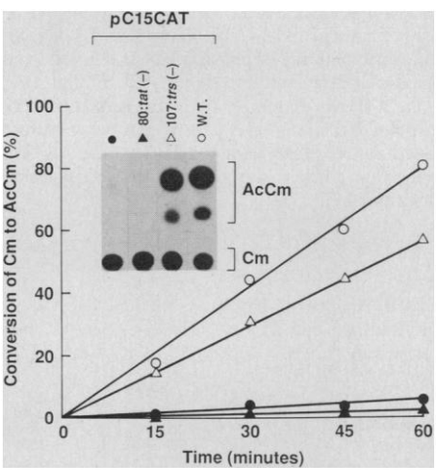

Fig. 2. Effect of *tat* and *trs* point mutations on the LTR-directed CAT expression. The CAT activities assayed at 48 hours after transfecting COS-1 cells with 3 to 10 μg of pC15 (LTR)-CAT alone or together with 10 μg of each HIV-1 proviral DNA designated 80:*tat*(−), 107:*trs*(−), and wild-type pHXB2gpt (W.T.) are compared. Cells at passage 7 to 10 were thawed out routinely 6 to 8 days before transfections and were transfected at a density of 1 × 10^6 cells per 10-cm culture dish. The kinetics of CAT activity for each specimen, as a function of the reaction incubation time, was measured in 10-μl aliquots of the transfected cell lysates. Results represent the mean values obtained from triplicate culture dishes from the same batch of cells in four separate transfection experiments. The inset panel represents autoradiographic images of the chloramphenicol (Cm) and acetylated metabolites (AcCm) at the time point of 30 minutes.

appeared with the wild-type genome (lane 1). In contrast, viral protein synthesis was reduced when the cells were transfected with the clones 80:*tat*(−) and 107:*trs*(−) (lanes 2 and 3). Faint bands with similar electrophoretic mobilities to p53 and gp160/120 bands detected in these specimens were also present in appropriate negative controls (compare lanes 2 through 6). The inability of both *tat*(−) and *trs*(−) mutants to express the full protein profiles is consistent with the lack of virus production by these mutants as determined by RT assay and electron microscopy (Table 1).

The defective clones 80:*tat*(−) and 107:*trs*(−) together or individually in combination with pCV1, a cDNA clone containing *tat*, *trs*, and 3′-*orf* coding sequences (*12*), were introduced into cells and samples were taken, simultaneously for RNA, protein, RT measurement, and electron microscopy 48 hours after transfection. Cotransfection of the mutants 80:*tat*(−) and 107:*trs*(−) restored the normal synthesis of the viral transcripts (Fig. 3B), core proteins (not shown), and virus particles (Table 1). Normal synthesis was also restored after cotransfection of the 80:*tat*(−) and the pCV1 clone. In contrast, the combinations of the 107:*trs*(−) and pCV1 partially restored the normal pattern of the RNA and extremely low levels of virus were produced (Fig. 3B and Table 1). The inability of pCV-1 to fully restore viral expression of 107:*trs*(−) could be due to the lack of optimal consensus sequence around the *trs* gene initiation codon (*16*) in this clone.

Virus particles produced by cotransfection of pCV1 and the *tat*/*trs* mutants are not infectious when cocultivated with a target T-cell line (H9), suggesting that true transcomplementation had occurred. In contrast, cotransfection of 80:*tat*(−) and 107:*trs*(−) produced infectious particles, probably through recombination.

Our analysis of both steady-state viral mRNA and nascently transcribed viral mRNA clearly demonstrates that *tat*-III plays a major role in transcriptional activation, since its abrogation resulted in a great reduction of both. Furthermore, *tat*-III also has a role in post-transcriptional activation (*4, 5*). We previously reported that a mutant deleted in the splice acceptor site of *tat* expressed greatly reduced *tat* activity. Cells transfected with this genome expressed correspondingly lower levels of viral proteins but entirely normal patterns and levels of viral mRNA (*5*). Similarly, a mutant with a single amino acid change in *tat* with reduced trans-activation activity was much more compromised in protein expression than in viral mRNA expression (*17*). Therefore, while *tat* may enhance both transcriptional and post-transcriptional events, the threshold for optimal transcription is much lower than for optimal message utilization. This differential dependence on the level of *tat* activity may account for variations reported for the relative contribution of *tat* to transcriptional versus post-transcriptional activations. This threshold difference may be attributed to the cellular distribution of *tat*. Felber *et al.* (*18*) showed that the transactivator protein of human T-cell leukemia/lymphoma virus (HTLV-I) was restricted to the nucleus in transiently transfected, low expressing cells, but spilled over to the cytoplasm in a stable high expressing cell line. Recently, Hauber *et al.* (*19*) found *tat* to be predominantly of nuclear localization in transiently transfected COS cells. If the analogy holds, *tat* may first be found in the nucleus where it exerts its transcriptional effect, and then in the cytoplasm where it may activate a step or steps leading to protein production.

There is no direct evidence that the *tat* or *trs* protein binds either DNA or RNA. However, the predicted amino acid sequence of *tat* contains two highly conserved domains rich in cysteine residues and arginine/lysine residues, respectively, that could potentially interact with nucleic acids. The responsive sequences for *tat*, referred to as TAR, have been localized to the R region downstream from the initiation site (*20*). Although it has not been shown that the same target sequences are responsible for both the transcriptional and post-transcriptional effects, the location of TAR near transcription initiation in the DNA and the

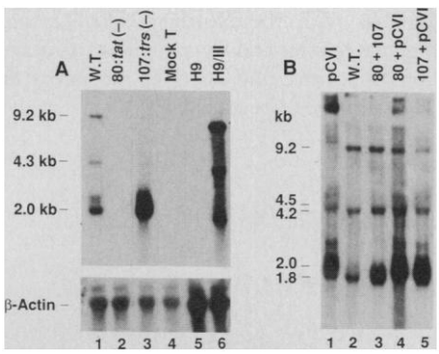

Fig. 3. Northern blot analysis of wild-type and *tat*/*trs* mutants. Eight micrograms of total cellular RNA from various COS-1 transfectants, H9, and H9/HIV-1–infected lymphocytes (H9/III) were loaded per sample lane in a 1% agarose gel and processed for Northern analysis. (**A**, upper panel) Lanes 1 and 6 represent the characteristic genomic 9.2-kb and subgenomic 4.3-, 2.2-, and 1.8- to 2-kb transcripts in both RNA samples from the wild-type (pHXB2) transfected COS-1 cells and H9/III lymphocytes probed with the homologous nick-translated LTR probe [Bgl II to Bgl II 3′ LTR fragment, B12 (*12*)]. Lanes 2 and 3 demonstrate the patterns of proviral RNAs derived from the *tat* and *trs* mutants designated 80 and 107. Lane 2 shows extremely faint bands of the proviral transcripts, which are not visible in this exposure. Lanes 4 and 5 refer to RNAs from mock-transfected COS-1 cells and uninfected H9 lymphocytes which failed to hybridize with the B12 probe. (A, lower panel) Lanes 1 to 6 were reprobed with a nick-translated chicken skeletal muscle β-actin insert. (**B**) The results of the cotransfection (see text for descriptions).

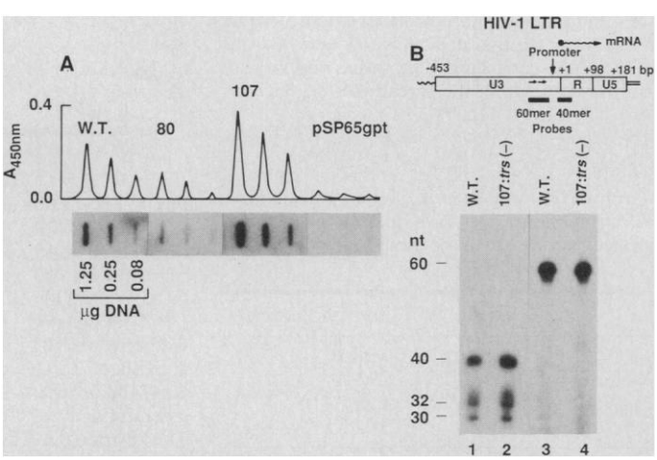

Fig. 4. Analysis of RNA by "run-on" and S1 nuclease. (**A**) COS-1 cells transfected 24 hours previously with wild-type pHXB2gpt (W.T.), (80)*tat*(−), and (107)*trs*(−) HIV-1 mutants were harvested, and nuclei were prepared for "run-on" transcription assay (*25*). Nuclei were allowed to elongate RNA chains in vitro, and acid-precipitable ^{32}P-labeled nuclear RNA was isolated and hybridized with serial dilution of denatured HIV-1 BH10 insert as the probe by slot blot hybridization (Schleicher and Schuell). Curves on the top are the densitometric tracings (Shimadzu apparatus) aligned with the respective hybridized bands. pSP65gpt is the plasmid vector with no proviral DNA (*2*). (**B**) Aliquots of the same RNAs from the wild-type and *trs*(−) transfectants described in the legend to Fig. 3A were analyzed by S1 nuclease protection according to standard techniques. The protected bands of 40, 32, and 30 bp (lanes 1 and 2) and 60 bp (lanes 3 and 4) were generated with the 40- and 60-nucleotide probes, respectively. The top portion of (B) shows the location of the probes in the 5′ LTR region. Negative controls (transfer RNA, RNA from mock-treated cells, and pSP65gpt transfectants) showed no protected bands (data not shown).

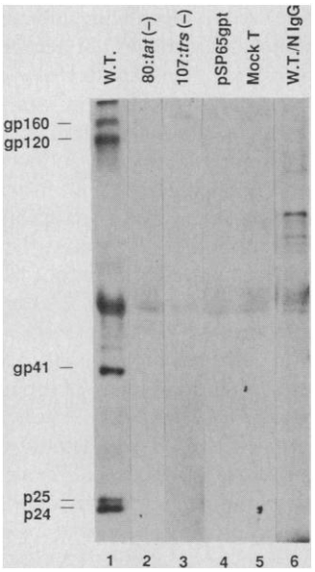

Fig. 5. Analysis of viral structural proteins of wild-type and *tat*/*trs* mutants. COS-1 transfectants, were pulse-labeled with [^{35}S]methionine and [^{35}S]cysteine (NEN) and incubated for 4 hours prior to harvesting at 48 hours after transfection. Each radioactive material (0.5 mCi) was added to every 10-cm culture dish without changing the cell media. Cell lysates from identical dishes were prepared and treated with 5 μl of a pooled antiserum to HIV-1. Immunoprecipitation was carried out as described (*26*). Protein contents of the immunoprecipitates were separated on 10% SDS-polyacrylamide gels and visualized by fluorography with NEN Enhancer solution and Kodak XAR-5 film. See text and legends to Figs. 1 to 4 for key.

cap site in the viral mRNA makes such a dual mechanism feasible.

The *trs* gene appears to be equally versatile in view of its roles as both a positive and negative regulator. In previous studies deletion or insertion mutants in the *trs* gene failed to express virus and exhibited an aberrant pattern of viral mRNA; the predominant species were 1.8 to 2.0 kb, with very little of the higher molecular weight species (9.2-kb *gag-pol* mRNA and 4.2-kb *env* mRNA) detectable (*5*). The lower molecular weight mRNA codes for at least a functional *tat* protein, as shown by the CAT assays. Here, we confirmed these results using point mutants and further showed that *trs* also down-regulates transcription of viral mRNA. Therefore, like *tat*, *trs* also functions both at the transcriptional and post-transcriptional levels. Again, it is not known whether the target sequences for both effects are the same. The predicted amino acid sequence of *trs* contains a highly hydrophilic and basic stretch of lysine and arginine residues (*4*), which may be responsible for the nuclear localization of this protein.

The effects of both genes are mediated through protein products rather than mRNA, since introduction of a translational stop codon, as a result of one or two base changes, should not significantly alter the mRNA structures. Although both *tat* and *trs* are critical for virus expression, they utilize quite different regulatory mechanisms. While *tat* is a positive trans-activator of both transcription and protein synthesis, and enhances expression of all viral proteins, the *trs* gene is a trans-regulator of structural proteins only. Its overall negative effect on viral transcription would actually down-regulate the expression of some of the regulatory proteins (*tat*, 3′-*orf*, and *trs* itself) as well as maintain a moderate level of virus production. At least one other viral gene may also act as a negative regulator, namely the 3′-*orf* gene, since deletion of the 3′-*orf* gene resulted in a more actively replicating virus (*21*). One possible explanation for the evolution of two negative regulatory genes for virus expression pertains to the life cycle of HIV and its cytopathology. The following scenario can be envisioned after infection. A basal level of viral transcription in an activated T4-positive cell first allows the synthesis of *tat*, which would escalate the levels of viral transcription and expression of the other regulatory proteins. However, no viral structural proteins would be expressed until a threshold level of *trs* is accumulated. The expression of *trs* and 3′-*orf* would in turn decrease the level of virus production. These complex control mechanisms afford the virus a means of rapidly responding to external signals to reproduce itself, but in moderation. Since cytopathology is directly correlated with expression of high levels of specific viral structural proteins such as the envelope proteins (in addition to other parameters like T4 concentration of the target cell), the negative feedbacks of *trs* and 3′-*orf* would diminish the pathogenicity of the virus, which may be an evolutionary advantage. These negative feedback controls would also explain the long and variable latency for disease induction even though virus replication is demonstrable throughout the course of infection. Host and environmental factors may tip the balance to overexpression, and this may be one of the first events that lead to clinical deterioration. There are recent demonstrations that antigen/mitogen or trans-activating proteins of DNA viruses can increase transcription from the HIV LTR (*22*). Therefore, chronic antigen overload and infection with various viruses can override the negative feedbacks of 3′-*orf* and *trs* and contribute to disease progression.

REFERENCES AND NOTES

1. F. Wong-Staal, in *Human Immunogenetics: An Advanced Text*, S. Litwin, Ed. (Dekker, New York, in press).
2. A. G. Fisher *et al.*, *Nature (London)* **320**, 367 (1986).
3. A. Dayton, J. G. Sodroski, C. A. Rosen, W. C. Goh, W. A. Haseltine, *Cell* **44**, 941 (1986).
4. J. G. Sodroski *et al.*, *Nature (London)* **321**, 412 (1986).
5. M. B. Feinberg, R. F. Jarrett, A. Aldovini, R. C. Gallo, F. Wong-Staal, *Cell* **46**, 807 (1986).
6. C. A. Rosen *et al.*, *Nature (London)* **319**, 555 (1986).
7. C. M. Wright, B. K. Felber, H. Paskalis, G. N. Pavlakis, *Science* **234**, 988 (1986); B. R. Cullen, *Cell* **46**, 937 (1986); M. A. Muesing, D. H. Smith, D. J. Capon, *ibid.* **48**, 691 (1987).
8. T. Okamoto and F. Wong-Staal, *Cell* **47**, 29 (1986).
9. M. J. Zoller and M. Smith, *DNA* **3**, 479 (1984).
10. R. M. Myers, L. S. Lerman, T. Maniatis, *Science* **229**, 242 (1985).
11. C. M. Gorman, L. F. Moffat, B. H. Howard, *Mol. Cell. Biol.* **2**, 1044 (1982).
12. S. K. Arya, C. Guo, S. F. Josephs, F. Wong-Staal, *Science* **229**, 69 (1985).
13. L. Ratner *et al.*, *Nature (London)* **313**, 277 (1985).
14. B. Starcich *et al.*, *Science* **227**, 538 (1985); M. A. Muesing *et al.*, *Nature (London)* **313**, 450 (1985).
15. G. Franchini, M. Robert-Guroff, A. Aldovini, N. C. Kan, F. Wong-Staal, *Blood* **69**, 437 (1987).
16. S. K. Arya, personal communication.
17. M. R. Sadaie *et al.*, in preparation.
18. B. K. Felber, H. Paskalis, C. Kleinman-Ewing, F. Wong-Staal, G. N. Pavlakis, *Science* **229**, 675 (1985).
19. J. Hauber, A. Perkins, E. P. Heimer, B. R. Cullen, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 6364 (1987).
20. C. A. Rosen, J. G. Sodroski, W. A. Haseltine, *Cell* **41**, 813 (1985).
21. P. A. Luciw, C. Cheng-Mayer, J. A. Levy, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 1434 (1987).
22. H. E. Gendelman *et al.*, *ibid.* **83**, 9759 (1986); G. Nabel and D. Baltimore, *Nature (London)* **326**, 711 (1987).
23. M. Popovic, M. G. Sarngadharan, E. Read, R. C. Gallo, *Science* **224**, 497 (1984).
24. Synthetic oligonucleotides and site-directed mutagenesis–oligonucleotides (Fig. 1) were obtained from J. Derge (National Cancer Institute, Frederick, MD). Standard cloning and subcloning techniques were used throughout the experiments. Utilizing two unique Sal I and Bam HI restriction sites, we excised the 2.6-kb fragment of pHXB2gpt and inserted it into the pGC1 vector in opposite orientation to generate minus strand DNA. Single-stranded recombinant pGC1 was recovered from LE392 cells with helper phage M13 (*10*). Mutant and primer oligonucleotides were annealed to single-stranded recombinant pGC1 in a 40-μl volume, and a primer extension reaction was performed (*9, 10*). Double-stranded DNA products were used to transform *Escherichia coli* HB101 cells. Approximately 100 transformants were processed for colony hybridization with homologous mutant oligonucleotide labeled with ^{32}P at the 5′ end. Hybridized colonies were washed with 6× standard saline citrate at discriminating temperatures. After a second round of colony isolation and screening, DNAs from positive colonies were purified and subjected to DNA sequencing [R. J. Zagursky, K. Baumuster, N. Lomax, M. Berman, *Gene Anal. Tech.* **2**, 89 (1985)]. After verification of the point mutation, the Sal I–Bam HI fragment was excised and reinserted into pHXB2ΔSal I–Bam HI to construct mutant provirus.
25. G. S. McKnight and R. D. Palmiter, *J. Biol. Chem.* **254**, 9050 (1979); M. Groudine, M. Peretz, H. Weintraub, *Mol. Cell. Biol.* **1**, 281 (1981).
26. M. B. Mathews, M. Reichlin, G. R. V. Hughes, R. M. Bernstein, *J. Exp. Med.* **160**, 420 (1984).

27. We thank R. C. Gallo for helpful discussions and support, S. F. Josephs, R. E. Willis, and S. K. Arya for technical advice, L. Jagedzinski and L. Fang for experimental help, and M. Gonda for providing electron micrographs. T.B. was sponsored by a fellowship grant from Deutsche Forschungsgemeinschaft.

18 June 1987; accepted 15 December 1987