Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 6111–6115, August 1989
Biochemistry

Rev-induced modulation of Nef protein underlies temporal regulation of human immunodeficiency virus replication

NAFEES AHMAD, RATAN K. MAITRA, AND SUNDARARAJAN VENKATESAN*

Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

Communicated by Bernard Moss, May 25, 1989

ABSTRACT **The replication of human immunodeficiency virus type 1 (HIV-1) requires the concerted action of two virus-encoded transactivator proteins, Tat and Rev, and is in turn moderated by the viral transcriptional repressor Nef. We show here that the phenotype of a Rev$^-$ HIV-1 provirus was nonreplicating and was distinguished by accumulation of Nef protein and reduced Tat function. Provirus defective in both the *rev* and *nef* genes (Rev$^-$Nef$^-$) was also nonreplicating but had normal Tat function. Trans-complementation of the Rev$^-$ mutant with Rev caused a decrease of both the steady-state level and the rate of synthesis of Nef. This was accompanied by enhanced synthesis of viral structural proteins. Rev induced even greater levels of virus production from the Rev$^-$Nef$^-$ double mutant. In contrast, exogenous Rev did not augment virus production from wild-type provirus. Virus production from Rev$^-$ and Rev$^-$Nef$^-$ mutants induced by Rev was repressed by exogenous Nef. The repression induced by Nef could not be reversed by exogenous Rev. The ability of Rev to modulate Nef expression solely from the provirus, and thereby relieve the Nef-mediated inhibition of transcription from the viral long terminal repeat, reveals a delicate balance of the functions of these two proteins that might underlie the switch between latency and reactivation.**

Intrinsic to the strategy of replication of human immunodeficiency virus type 1 (HIV-1) is the functional expression of certain small viral regulatory proteins. Among these, Tat and Rev are essential for viral replication (1–9). Tat and Rev are encoded by two overlapping open reading frames (ORFs) within two exons of a heterogeneous class of multiply spliced 2.0-kilobase (kb) transcripts (10) that also encodes another protein, Nef, which is a negative regulator (11, 12). The Tat protein is absolutely required for viral replication and the expression of genes linked to the HIV-1 long terminal repeat (LTR). Rev-defective viruses are nonreplicating and fail to synthesize the HIV-1 *gag* and *env* gene products (5, 6, 13). Rev may enable viral replication by facilitating the nuclear transport and stabilization of large HIV-1 RNAs coding for structural proteins (14–17).

It has been difficult to assign the functions of Rev in the HIV-1 life cycle. For instance, the Rev molecular studies used chimeric reporter plasmids containing the putative target HIV-1 sequences for Rev fused to indicator genes (13) or subgenomic HIV-1 plasmids linked to HIV-1 LTR or heterologous promoters (16–18). On the other hand, studies of the role of Rev in viral replication used a standard provirus that had a premature termination codon in the *nef* ORF (5, 19, 20). We recently demonstrated that Nef is a trans-acting transcriptional inhibitor of the HIV-1 LTR (12). Studies of Rev function would therefore be incomplete without addressing the nature of the functional interactions between Rev and Nef. In this report, we show that Rev induced a marked decrease in both the steady-state levels and rates of synthesis of Nef expressed from a Rev$^-$ provirus that was nonreplicating and had repressed Tat function. Rev-induced loss of Nef synthesis paralleled augmented expression of viral proteins, and the magnitude of the Rev effect could be reduced by addition of exogenous Nef.

MATERIALS AND METHODS

Virus and Cell Cultures. CD4$^+$ (Leu-3$^+$) A3.01 T lymphocytes (21) in RPMI 1640 medium with 10% fetal bovine serum were used for infection. For most DNA transfections, SW480 (22) or HeLa S3 monolayers or A3.01 cells were used. A recombinant HIV-1 strain, pNL432 (22), referred to as wild type (wt), served as a standard. Particulate reverse transcriptase (RT) assays were as described (22) and the infectivity titers were determined by end-point dilution.

cDNA Plasmids. Refer to Fig. 1*B*. We constructed an Okayama–Berg cDNA library of mRNAs from A3.01 lymphocytes infected with wt virus. The library was screened to obtain recombinants corresponding to the heterogeneously spliced 1.8- to 2.0-kb mRNA and one of these, pV102 (12), had a 69-base-pair (bp) central exon fused to 1352 bp of 3′-terminal exon with an intact *nef*. The HIV-1 insert of pV102 was recovered by partial cleavage at the flanking *Hin*dIII sites in the 5′ and 3′ LTRs and inserted between the corresponding *Hin*dIII sites of the wt provirus, yielding pHIV Nef. An oligonucleotide with a methionine codon and an *Nco* I site was inserted in-frame (11 codons upstream of the *rev* ORF) at the lone *Bss*HII site of pHIV Nef to yield pHIV RevNef. The *nef* in pHIV RevNef was annulled by filling in the lone *Xho* I site, generating pHIV Rev. The *rev*-containing *Nar* I–*Xho* I fragment of pHIV Rev was blunt-end-ligated between the *Xba* I and *Hpa* I sites of pCMV CAT in place of the chloramphenicol acetyltransferase (CAT) gene, downstream of the cytomegalovirus (CMV) immediate early promoter (9), generating pCMV Rev. Alternatively, the HIV-1 cDNA sequence in pHIV Rev was recovered by cleaving with *Bgl* II in the 5′ and 3′ LTRs and cloned downstream of the Rous sarcoma virus (RSV) LTR in the vector pRSV.5 to yield pRSV Rev.

Proviral Plasmids. The infectious provirus pNL432 (wt) has been described (19). The *Eco*RI–*Bam*HI fragment of wt was mutagenized (23) to introduce a single termination codon after the first two residues in the *rev* ORF. This fragment was then transferred to wt to generate the Rev$^-$ mutant provirus. The wt provirus was also cleaved at the lone *Xho* I site in the *nef* ORF, blunt-ended with T4 DNA polymerase, and self-ligated to yield the Nef$^-$ mutant (12). A Rev$^-$Nef$^-$ double mutant was constructed from the Nef$^-$ provirus by replacing its own wt *rev* ORF with the mutagenized version.

DNA Transfections. Subconfluent monolayers in T25 flasks were transfected with 10 μg of total DNA (adjusted with

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CAT, chloramphenicol acetyltransferase; HIV-1, human immunodeficiency virus type 1; LTR, long terminal repeat; ORF, open reading frame; RT, reverse transcriptase; wt, wild type.

*To whom reprint requests should be addressed.

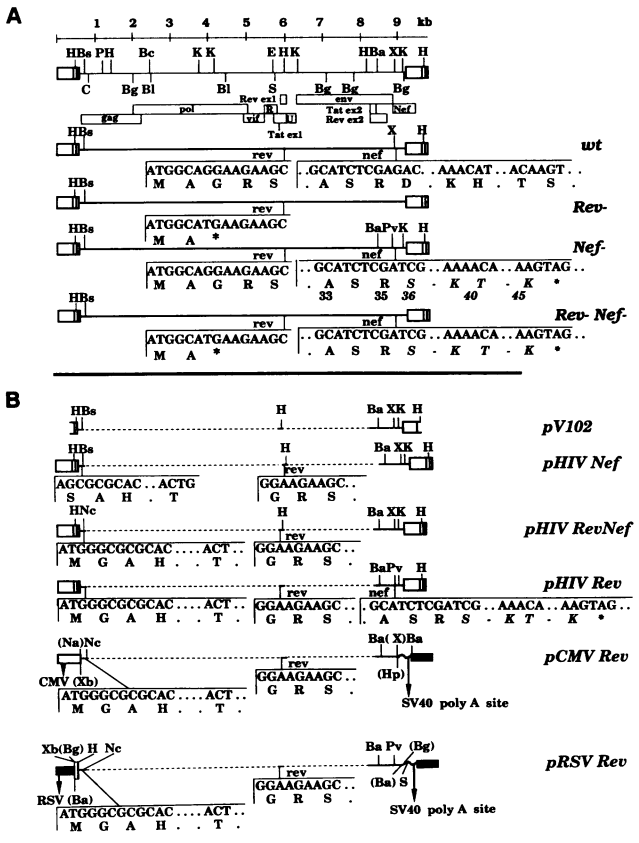

FIG. 1. HIV-1 plasmids. (*A*) The infectious HIV-1 proviral DNA, referred to as wt (pNL432; ref. 22) is drawn to scale with the relevant restriction sites. The various ORFs are identified by rectangles. ex, Exon. The relevant amino acids encoded at the start of the *rev* and *nef* ORFs are shown. The Rev$^-$ provirus has a chain-terminating mutation at codon 3 of *rev*. Nef$^-$ has a frameshift mutation in the *nef* ORF that results in premature termination (12). Rev$^-$Nef$^-$ is the double mutant. (*B*) Nef and Rev expression plasmids. Broken lines correspond to the introns in the mRNA. pV102 is the original Okayama–Berg cDNA clone. pHIV Nef is the HIV-1 LTR-linked Nef expression plasmid. pHIV RevNef is the Rev expression plasmid constructed from pHIV Nef by linker insertion. pHIV Rev is the Nef$^-$ Rev expression plasmid. pCMV Rev and pRSV Rev are plasmids with the *rev* ORF under the control of the cytomegalovirus (CMV) immediate early promoter and the Rous sarcoma virus (RSV) LTR, respectively. SV40, simian virus 40.

carrier DNA) by a modified calcium phosphate procedure (23). For provirus transfections, 5 μg sufficed to saturate the system. In all experiments, an irrelevant indicator plasmid, pSVβgal, containing the *Escherichia coli* β-galactosidase gene under the control of the simian virus 40 (SV40) early promoter (24) was included. All analyses were normalized to constant levels of β-galactosidase expression. For transfection of A3.01 lymphocytes, a modified DEAE-dextran procedure (6) was used. Virus production was monitored by RT assay (22, 23) of the culture fluid every 12 hr. In experiments with multiple cDNA plasmids, optimal conditions for expression were worked out by adjusting the molar ratio of the test and activator plasmids. In general, 5 μg each of the test and transactivator plasmid were adequate in SW480 cells. Cells were generally harvested 30–70 hr after transfection. In virus-production experiments, the culture fluid of the transfectants was filtered through a 0.45-μm Millipore filter and titered by terminal dilution on A3.01 lymphocytes. For cocultivation of transfectants, the culture fluid was removed 48 hr after transfection, and the cells were rinsed three times with medium and overlaid with 10 ml of A3.01 cells at 10^6 per ml.

Immunological Procedures. Viral proteins in the tranfectants were detected by immunoblotting (25) using pooled AIDS patient sera, rabbit antibodies against *E. coli* fusion protein corresponding to the p24 gag protein, and antisera raised against synthetic peptides corresponding to residues 67–77 or 196–206 of the *nef* ORF. Rabbit anitserum against residues 27–51 of the *rev* ORF was supplied by Bryan Cullen (Duke University). Radiolabeled cell extracts were processed for immunoprecipitation (26) and the immunoreactive proteins were resolved by SDS/PAGE and visualized by fluorography.

CAT Assay. Cell lysates of CAT gene transfectants were assayed as described (27). Typically, assays were run for 20 min in duplicate with three different volumes of each lysate, which were adjusted to constant levels of β-galactosidase expression. For each set of plasmids, the values were calculated from six independent transfections. CAT activity, expressed as percent acetylated chloramphenicol, was determined by scintillation counting.

β-Galactosidase Assay. Two different volumes of cell extracts were assayed in duplicate in 96-well microtiter plates with *o*-nitrophenyl β-D-galactoside as substrate (28). Reactions were monitored by A_{405} with an automated ELISA reader.

RESULTS

Rev Induces Enhanced Virus Production from Rev$^-$ and Rev$^-$Nef$^-$ Mutants. The functional role(s) of Rev protein during infection was evaluated in a transient virus-production assay using wt, Rev$^-$, and Rev$^-$Nef$^-$ proviruses (Fig. 1*A*). Rev was supplied in trans by the expression plasmid pHIV Rev or pRSV Rev (Fig. 1*B*) and transient virus production was monitored by RT assay of the culture fluid. All the tranfectants also received a constant amount of pSVβgal, whose expression was used to normalize the individual transfections. SW480 cells tranfected with wt provirus produced infectious HIV-1 particles (Fig. 2*A*, bar 2), whereas Rev$^-$ or Rev$^-$Nef$^-$ mutants were negative (bars 4 and 6). Cotransfection with pHIV Rev or pRSV Rev did not alter virus production by wt transfectants (bar 3) but increased virus production by the Rev$^-$ mutant to >1.5 times the wt level (bar 5). With the Rev$^-$Nef$^-$ double mutant, the magnitude of transactivation was even greater (bar 7). Enhanced mutant virus complementation also occurred in CV-1, COS-1, HeLa, and Vero cells and in A3.01 T lymphocytes. The magnitude of transactivation of mutant provirus was relatively constant over a wide range of pHIV Rev concentration (0.5–20 μg), and even the minimum amount of the Rev plasmid required for the complementation induced enhanced replication. The trans-complemented virus was neither infectious for CD4$^+$ lymphocytes nor cocultivable.

Rev Downmodulates Nef and Enhances Structural Protein Synthesis. To confirm that the enhanced release of RT particles seen in contransfections reflected an increase in virus production, viral protein synthesis in the transfectants was examined by immunoblotting cell extract samples containing equal amounts of β-galactosidase activity. The viral structural proteins were identified by using pooled AIDS patient sera. The wt transfectants expressed all the structural polypeptides, notably the env glycoproteins gp160 and gp120, the gag precursor proteins p55, p46, and p41, and the mature gag proteins p24 and p17 (Fig. 2*B*, lane 2). In contrast, the expression of these structural proteins was markedly reduced or even absent in cells transfected with the Rev$^-$ (lane 4) or Rev$^-$Nef$^-$ (lane 6) mutant. The quantitative defect in structural protein synthesis was further substantiated by immunoblotting with antiserum raised against the p24 subunit of the HIV-1 gag protein (Fig. 2*C*). This defect in structural protein expression in the Rev$^-$ and Rev$^-$Nef$^-$ transfectants

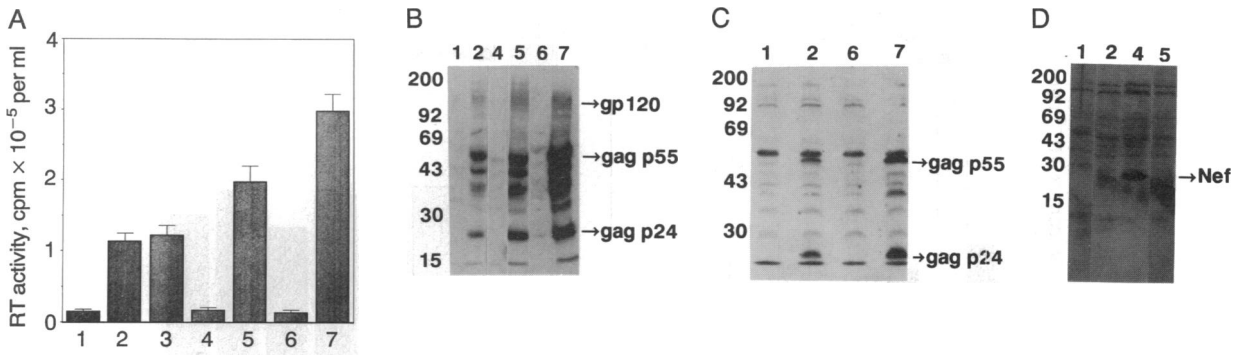

FIG. 2. Effect of Rev on transient HIV-1 expression from wt, Rev⁻, and Ref⁻Nef⁻ provirus after transfection of SW480 cells (19). (*A*) Transient virus production (expressed as RT in cpm of dTMP incorporated per ml) from SW480 cells transfected with calf thymus DNA (bar 1), wt provirus (bar 2), wt plus pHIV Rev (bar 3), Rev⁻ (bar 4), Rev⁻ plus pHIV Rev (bar 5), Rev⁻Nef⁻ (bar 6), and Rev⁻Nef⁻ plus pHIV Rev (bar 7). In all cases, pSVβgal was cotransfected and the RT values were normalized to β-galactosidase expression. Standard errors from five experiments are denoted by error bars. (*B–D*) Immunoblot detection of viral proteins by pooled human AIDS sera (*B*) or rabbit hyperimmune serum raised against *E. coli*-expressed HIV-1 p24 gag protein (29) (*C*) or against Nef oligopeptide (residues 67–77) (*D*). Lane numbers correspond to bars in *A*. Mobilities of protein markers are denoted by their masses in kilodaltons on the left.

was overcome when Rev cDNA was cotransfected. Actually, the steady-state level of viral structural proteins in the Rev⁻/pHIV Rev cotransfectants was about 1.5-fold higher than in wt transfectants (Fig. 2*B*, lane 2 vs. 5), correlating with the enhanced release of the particulate RT. Rev induced an even greater level of viral structural protein synthesis from the Rev⁻Nef⁻ mutant (Fig. 2 *B* and *C*, lane 7). Cotransfection of wt with Rev cDNA did not augment viral protein expression. Although the Rev⁻ transfectants failed to synthesize viral structural proteins, there was a marked accumulation of the 27-kDa *nef* gene product (Fig. 2*D*, lane 4). Complementation of the mutant by Rev markedly reduced Nef accumulation (Fig. 2*D*, lane 5).

Since the steady-state phenotype of the Rev-complemented Rev⁻ mutant was accompanied by loss of Nef and accumulation of structural proteins, we inquired whether Rev coexpression induced a kinetic transition in viral protein synthesis, with the expectation that this analysis might offer some clues as to potential temporal regulation of viral replication. HeLa cells were cotransfected with Rev⁻ and pCMV Rev and the synthesis of viral proteins was monitored by immunoprecipitation after metabolic labeling. Nef synthesis, demonstrable as early as 8 hr (with AIDS patient serum) or 12 hr (with Nef-specific serum) after transfection, started a steady decline coinciding with the onset of Rev expression from the pCMV Rev (Fig. 3, compare *B* and *C*) and was almost undetectable by 24–36 hr. In contrast, the viral env and gag proteins started appearing at 18 hr, about the time Nef was declining, and their synthesis continued up to 36 hr after transfection (Fig. 3*A*). The repressive effect of Rev on Nef synthesis was observed only when Nef expressed from the provirus. Rev expression had virtually no effect on Nef expression from pHIV Nef or pCMV Nef (data not shown).

Nef Represses Rev-Induced Transactivation by Antagonizing Tat Function. If the enhanced replication of Rev-activated Rev⁻ mutant were due to a relative loss of Nef that removed the constraints on Tat function, then exogenous Nef might reduce this transactivation. When pHIV Nef was cotransfected with wt or Rev⁻ provirus and pCMV Rev, there was indeed a dose-dependent repression of virus production from both the standard provirus and Rev-complemented Rev⁻ (Fig. 4*A*). The repression induced by Nef could not be alleviated by exogenous Rev (Fig. 4*B*). This result is consistent with the posttranscriptional mode of Rev function, inasmuch as Rev had virtually no effect on the synthesis of Nef from the cDNA plasmid (data not shown), although it

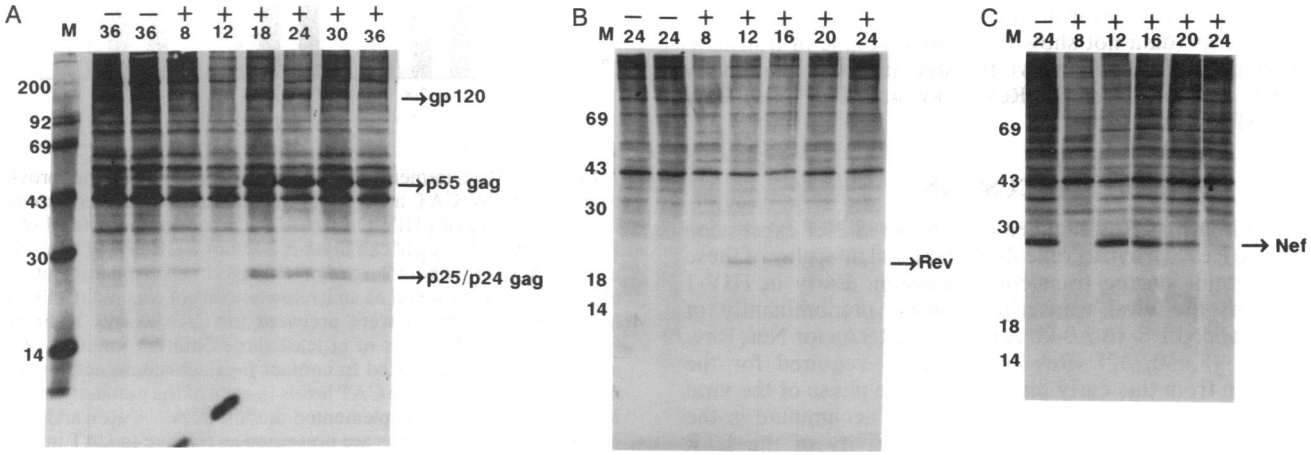

FIG. 3. Kinetic analysis of viral protein synthesis during transactivation of Rev⁻ mutant provirus. Subconfluent HeLa cell monolayers in T75 flasks were transfected with 10 μg of Rev⁻ (−) or with a mixture of 10 μg of Rev⁻ and 5 μg of pCMV Rev (+). At various times after DNA addition (time in hours above each lane), the tranfectants were metabolically labeled for 1 hr with a mixture of [^{35}S]cysteine and [^{35}S]methionine (250 μCi/ml) and [^{3}H]leucine (500 μCi/ml; 1 μCi = 37 kBq). Total cellular lysates were immunoprecipitated with pooled AIDS patient sera (*A*) or with anti-Rev (*B*) or anti-Nef (*C*) antiserum. The precipitated proteins were resolved by SDS/15% PAGE and visualized by fluorography. Viral structural and regulatory proteins are identified by arrows. The band seen in the left three lanes of *A* apparently comigrating with the p25/p24 gag protein was actually Nef that was also recognized by the patient sera. Molecular markers with their respective masses in kilodaltons are shown in lane M.

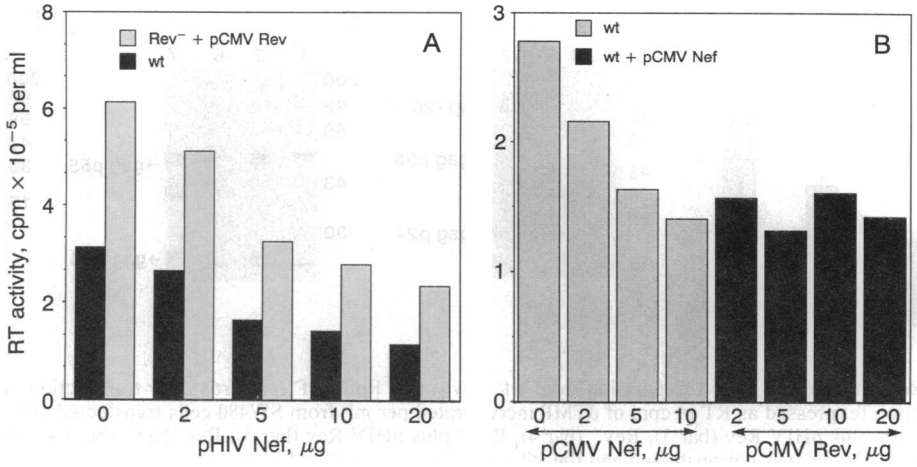

FIG. 4. Effects of Rev and Nef on the expression of wt and Rev⁻ proviruses in SW480 cells. Virus production was monitored by RT assay. (*A*) Nef expression from pHIV Nef inhibits virus production by wt and Rev⁻/pCMV Rev transfectants. To supply Rev in trans, 2 μg of pCMV Rev was used. Nef was supplied by cotransfecting increasing amounts of pHIV Nef and was monitored by immunoblotting. (*B*) Inability of Rev coexpression to reverse the Nef-induced repression of standard provirus. Light bars illustrate repression of wt provirus (5 μg) by various amounts of pCMV Nef. Dark gray bars represent the effects of cotransfection of various amounts of pCMV Rev with 5 μg of wt and 10 μg of pCMV Nef. Standard deviations in four experiments were ≤10%.

turned off Nef expression from the provirus. Unlike what was shown before (30), increasing levels of exogenous Rev had no negative effect on virus production over a broad range (2–20 μg) of Rev cDNA input (31).

Since the phenotype of the Rev⁻ mutant was distinguished by accumulation of Nef protein, a relative functional deficiency of Tat might be expected in the Rev⁻ transfectants. This possibility was examined by comparing the transactivation of the HIV-1 LTR-linked CAT gene, mediated by the wt, Rev⁻, and Rev⁻Nef⁻ proviruses in the presence or absence of exogenous Rev. In each experiment, pSVβgal was included as a control to monitor the transfection efficiency and the CAT assays were normalized to constant β-galactosidase expression. When expressed relative to the CAT activity observed in wt transfections, Rev⁻ cells had about 40% as much transactivation potential (Fig. 5). Interestingly, the CAT levels in the Rev⁻Nef⁻ mutant transfectants were similar to the wt transfectants, suggesting that the repression of the LTR in the Rev⁻ provirus was due to Nef. Upon complementation with Rev, both the mutants acquired essentially similar magnitudes of Tat function that were not significantly different from the levels seen with wt. In other experiments (data not shown), the time course of transactivation of pHIV CAT in wt transfectants was essentially similar to those seen in Rev⁻/Rev and Rev⁻Nef⁻/Rev cotransfectants.

DISCUSSION

Our finding that Rev induces a decrease in Nef expression from the Rev⁻ provirus reveals a functional interplay of these two proteins during transient expression. Early in HIV-1 infection, the viral transcripts consist predominantly of highly spliced 1.8- to 2.0-kb candidate mRNAs for Nef, Rev, and Tat (5, 30, 32). Rev is absolutely required for the transition from this early phase to the late phase of the viral life cycle (5, 6). Therefore, Tat and Nef accumulate in the absence of Rev. The transcriptional activity of the LTR would then be determined by the balanced effects of Nef and Tat. Thus, the reduced transcriptional activity of HIV-1 LTR in the Rev⁻ transfectants reflected the Nef-mediated repression of the LTR. Lack of Nef in the Rev⁻Nef⁻ transfectants correlated with nearly twice as much transactivation potential as in Rev⁻ cells (Fig. 5). During Rev complementation of Rev⁻ and Rev⁻Nef⁻ mutant proviruses, there was a definite lag before the effects of Rev (expressed from the cDNA) became noticeable (Fig. 3). The Rev-induced decrease of Nef in the Rev⁻/Rev cotransfectants would have resulted in a relative increase in Tat function, resulting in enhanced virus production. In the case of the Rev⁻Nef⁻ mutant, the magnitude of Rev complementation was even greater, since the Tat function was unimpeded even from the outset. The wt

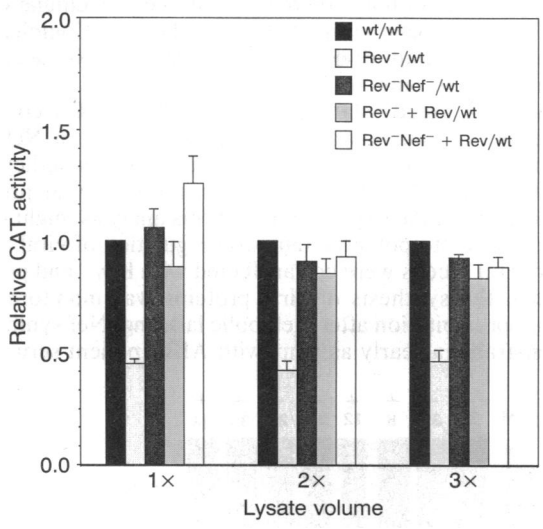

FIG. 5. Measurement of HIV-1 Tat function in the provirus transfectants by CAT assay. HeLa cell monolayers were cotransfected with 5 μg of pHIV CAT (CAT gene under the control of full HIV-1 LTR) and 10 μg of calf thymus DNA or wt, Rev⁻, or Rev⁻Nef⁻ provirus with or without 2 μg of pHIV Rev. In each experiment, 2 μg of pSVβgal was included as an irrelevant control plasmid. Cells were harvested and extracts were prepared for CAT assays 36 hr after transfection. CAT assays of at least three different volumes of cell extracts that were adjusted to contact β-galactosidase activity were incubated for 20 min. The CAT levels in each of the mutant (Rev⁻ and Rev⁻Nef⁻) or trans-complemented mutant (Rev⁻ + Rev and Rev⁻Nef⁻ + Rev) transfections are presented as relative to CAT in the wt transfectants. Each set of five bars shows the following CAT activity ratios, from left to right: wt/wt, Rev⁻/wt, Rev⁻Nef⁻/wt, (Rev⁻ + Rev)/wt, and (Rev⁻Nef⁻ + Rev)/wt. Means and SEM of the relative ratios from six independent experiments are shown. By Student's *t* test, the Rev⁻/wt ratio was found to be statistically distinct from the other ratios. For instance, using the values for the middle set of bars in the histogram, correlation probabilities (*P* values) of <0.003, <0.015, <0.025, and <0.005 were calculated for Rev⁻/wt vs. wt/wt, Rev⁻Nef⁻/wt, Rev⁻ + Rev, and Rev⁻Nef⁻ + Rev/wt, respectively.

provirus could not be overstimulated by Rev since there was no excess accumulation of Tat or Tat and Nef to begin with. Rev^- and Rev^-Nef^- transfectants have temporal blocks in the viral life cycle and have thus enabled us to resolve the subtle interactions among the regulatory proteins that may be operative in natural infection and latency.

The two initial proposals for the mechanism of Rev function—namely, antirepression of transactivation caused by cis elements in the *env* and *pol* coding sequences (6, 13) and transregulation of splicing (5)—could be combined in a role that ameliorates a defect in the nuclear transport (16) or transport and stability (14, 15) of unspliced HIV-1 mRNAs containing a Rev-responsive element. The Rev-mediated increase of unspliced mRNAs at the expense of spliced mRNAs would have an autoregulatory effect on the expression of Tat, Nef, and Rev. Therefore, a relative deficiency in the quantity of Tat might prevail during the phase of optimal gag and env protein synthesis induced by Rev (30, 32). However, a recent report (17) suggested that Rev modulates the synthesis of two forms of Tat, a two-exon form from the spliced mRNA and a one-exon form from unspliced mRNA. Although we were unable to quantify Tat during Rev complementation of the Rev^- or Rev^-Nef^- mutants, Rev induced an increase in Tat function in the Rev^- transfectants. Since Rev expression does not totally abolish small-RNA accumulation (5), it is likely that enough small mRNAs are available for Tat expression. Under these circumstances, differences in the turnover rates of Nef and Tat could result in a relative excess of Tat even when the mRNAs for all the regulatory proteins were reduced. Tat functional levels in the Rev-complemented Rev^-Nef^- transfectants continued unabated since there was no Nef in these cells.

The negative feedback control exerted by Nef HIV-1 LTR transcription (12) would be desirable as a buffer against excessive Tat function early in infection. The decay of Nef following Rev expression would ensure sustained viral activation by unhampered Tat function. HIV-1 Tat can activate a minimally configured LTR lacking the upstream enhancer and Sp1-responsive elements (refs. 3, 12, 33–35; A. Rabson, personal communication) and is quite potent in overriding the Nef effect (12). However, during incipient viral transcription or during viral latency, the LTR would be regulated by numerous homeotic stimuli (mitogens, growth factors, etc.) whose effects might be counterbalanced by Nef under conditions of limited or no Tat expression (12, 36). Rev-induced decrease of the small viral transcripts (5, 14–17, 32) would eventually result in a relative deficiency of all the regulatory proteins, but the apparently low threshold requirements of Rev for transactivation (31) would ensure a smooth transition from the latent phase of infection to the lytic phase. Subtle alterations in the delicate balance of the function of these regulatory proteins by cellular and environmental factors would therefore be expected to modulate viral latency and reactivation.

We thank Dr. Malcolm A. Martin (National Institute of Allergy and Infectious Diseases) for support and enthusiasm for this work. Acknowledgements are also due to Drs. Malcolm Martin and Arnold Rabson for critical review of the manuscript. Ruchir Seehra, a summer student, was helpful in construction of pCMV Rev plasmid. Dr. Bryan Cullen (Duke University) is warmly acknowledged for the generous supply of Rev-specific antiserum. We thank Dr. Eric Long (National Institute of Allergy and Infectious Diseases) for the gift of pRSV.5 plasmid vectors.

1. Arya, S. K., Guo, C., Josephs, S. F. & Wong-Staal, F. (1985) *Science* **229**, 69–73.
2. Sodroski, J. G., Patarca, R., Rosen, C. & Haseltine, W. A. (1985) *Science* **227**, 74–77.
3. Cullen, B. R. (1986) *Cell* **46**, 973–981.
4. Wright, C. M., Felber, B. K., Paskalis, H. & Pavlakis, G. N. (1986) *Science* **234**, 988–992.
5. Feinberg, M. A., Jarrett, R. F., Aldovini, A., Gallo, R. C. & Wong-Staal, F. (1986) *Cell* **46**, 807–817.
6. Sodroski, J. G., Patarca, R., Rosen, C. & Haseltine, W. A. (1986) *Nature (London)* **321**, 412–417.
7. Dayton, A. I., Sodroski, J. G., Rosen, C. A., Goh, W. C. & Haseltine, W. A. (1986) *Cell* **44**, 941–947.
8. Fisher, A. G., Feinberg, M. B., Josephs, S. F., Harper, M. E., Marselle, L. M., Reyes, G., Gonda, M. A., Aldovini, A., Debouk, C., Gallo, R. C. & Wong-Staal, F. (1986) *Nature (London)* **320**, 367–371.
9. Felber, B. K., Cladaras, M., Cladaras, C., Wright, C. M., Tse, A. & Pavlakis, G. N. (1988) in *The Control of Human Retrovirus Gene Expression*, eds. Franza, B. R., Cullen, B. R. & Wong-Staal, F. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 103–112.
10. Muesing, M. A., Smith, D. H., Cabradilla, C. V., Benton, C. V., Lasky, L. A. & Capon, D. J. (1985) *Nature (London)* **313**, 430–438.
11. Luciw, P. A., Cheng-Mayer, C. & Levy, J. A. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1434–1438.
12. Ahmad, N. & Venkatesan, S. (1988) *Science* **241**, 1481–1485.
13. Rosen, C. A., Terwilliger, E. T., Dayton, A., Sodroski, J. G. & Haseltine, W. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2071–2075.
14. Hadzopolou-Cladaras, M., Felber, B. K., Cladaras, C., Athanassopoulos, A., Tse, A. & Pavlakis, G. N. (1989) *J. Virol.* **63**, 1265–1274.
15. Felber, B. K., Hadzopolou-Cladaras, M., Cladaras, C., Copeland, T. & Pavlakis, G. N. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1495–1499.
16. Malim, M. H., Hauber, J., Le, S.-Y., Maizel, J. V. & Cullen, B. R. (1989) *Nature (London)* **338**, 254–257.
17. Malim, M. H., Hauber, J., Fenrick, R. & Cullen, B. R. (1988) *Nature (London)* **335**, 181–183.
18. Knight, D. M., Flomerfelt, F. A. & Ghryab, J. (1987) *Science* **236**, 837–840.
19. Fisher, A. G., Collati, E., Ratner, L., Gallo, R. C. & Wong-Staal, F. (1985) *Nature (London)* **316**, 262–265.
20. Rosen, C. A., Sodroski, J. G., Goh, W. C., Dayton, A. I., Lippe, J. & Haseltine, W. A. (1986) *Nature (London)* **319**, 555–559.
21. Folks, T. M., Powell, D. M., Lightfoote, M. E., Benn, S., Martin, M. A. & Fauci, A. S. (1986) *Science* **231**, 600–602.
22. Adachi, A., Gendelman, H. E., Koenig, S., Folks, T., Willey, R., Rabson, A. & Martin, M. A. (1986) *J. Virol.* **59**, 284–291.
23. Willey, R. L., Smith, D. A., Lasky, L. A., Theodore, T. S., Earl, P. L., Moss, B., Capon, D. J. & Martin, M. A. (1988) *J. Virol.* **62**, 139–147.
24. Hall, C. V., Jacob, P. E., Ringold, G. M. & Lee, F. (1983) *J. Mol. Appl. Genet.* **2**, 101–109.
25. Towbin, M., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4353.
26. Cullen, B. R. (1987) *Methods Enzymol.* **152**, 684–704.
27. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2**, 1044–1051.
28. Miller, J. H. (1974) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 353–355.
29. Mervis, R. J., Ahmad, N., Lillehoj, E. P., Raum, M., Rick. Salazar, F. H., Chan, H. & Venkatesan, S. (1988) *J. Virol.* **62**, 3993–4002.
30. Sadaie, M. R., Benter, T. & Wong-Staal, F. (1988) *Science* **239**, 910–913.
31. Venkatesan, S., Ahmad, N. & Mervis, R. J. (1988) in *The Control of Human Retrovirus Gene Expression* eds. Franza, B. R., Cullen, B. R. & Wong-Staal, F. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 113–118.
32. Wong-Staal, F. & Sadaie, M. R. (1988) in *The Control of Human Retrovirus Gene Expression*, eds. Franza, B. R., Cullen, B. R., Wong-Staal, F. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 1–10.
33. Rosen, C. A., Sodroski, J. G. & Haseltine, W. A. (1985) *Cell* **41**, 813–823.
34. Sodroski, J. G., Patarca, R., Rosen, C. & Haseltine, W. A. (1985) *Science* **227**, 74–77.
35. Muesing, M. A., Smith, D. H. & Capon, D. J. (1987) *Cell* **48**, 691–701.
36. Niederman, T. M. J., Thielan, B. J. & Ratner, L. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1128–1132.