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Feedback Regulation of Human Immunodeficiency Virus Type 1 Expression by the Rev Protein

BARBARA K. FELBER, CONNIE M. DRYSDALE, AND GEORGE N. PAVLAKIS*

National Cancer Institute–Frederick Cancer Research and Development Center, Basic Research Program, Frederick, Maryland 21701-1013

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Rev is an essential regulatory protein of the human immunodeficiency virus type 1 (HIV-1) that affects the transport and half-life of certain viral mRNAs. Rev exerts its function via a unique element, the Rev-responsive element (RRE), located within the *env* region of HIV-1. It has been previously demonstrated that Rev affects the relative levels of RRE-containing and RRE-lacking mRNAs. We have studied the effects of Rev on the expression of the three different groups of small, multiply spliced mRNAs that lack the RRE sequence and encode the regulatory proteins Tat, Rev, and Nef. To monitor the *tat*, *rev*, and *nef* mRNAs we generated specific S1 nuclease mapping probes that distinguish among them. Analysis of all the mRNA species producing Tat, Rev, and Nef revealed that their levels are coordinately regulated by Rev. They are increased in the absence of Rev protein and are down regulated in the presence of Rev. The corresponding proteins were measured by immunoprecipitations, and their levels are in agreement with the RNA levels. These results verify the model proposing that Rev is a general regulator indirectly affecting all the multiply spliced mRNAs to a similar extent. Therefore, Rev down regulates its own expression and the expression of Tat and Nef.

Human immunodeficiency virus type 1 (HIV-1) encodes regulatory proteins that promote the expression of the viral genome. Tat and Rev are essential for virus production (8, 11, 18, 52). A third factor, Nef, is not essential for virus propagation in tissue culture (33). Nef has been proposed to act as a negative regulator (2, 33, 39), but its function remains controversial (23, 30). Tat either directly or indirectly interacts with a *cis*-acting element (TAR) in the R region of the long terminal repeat and increases the steady-state levels of all the viral mRNAs (4, 7, 16, 26, 37, 40, 41, 46, 52, 55). Rev acts via a unique *cis*-acting element named the Rev-responsive element (RRE), which is localized in the *env* region of HIV-1 (9, 10, 21, 22, 35, 47). The presence of Rev promotes the transport of RRE-containing mRNAs from the nucleus to the cytoplasm (10, 14, 22, 35) and results in an increased half-life of these mRNAs (14). Since RRE-containing mRNAs encode Gag, Pol, and Env, this leads to the expression of high levels of these structural proteins and results in the production of virus particles (11, 14, 21, 52).

The genome of HIV-1 is complex and contains nine known open reading frames. The different proteins are expressed by ribosomal frameshifting (for Gag-Pol) and by the production of alternatively spliced mRNAs from the full-length precursor RNA. In addition, many mRNAs encode more than one protein (50; S. Schwartz, B. K. Felber, E. M. Fenyö, and G. N. Pavlakis, submitted for publication). HIV-1 produces more than 20 mRNAs, which can be grouped into three size classes. The full-length mRNA encodes the Gag-Pol polyprotein. The intermediate size class of mRNAs consists of a family of RNAs (3, 21; Schwartz et al., submitted) encoding Env and other proteins such as Vpu, one-exon Tat, and possibly Vif and Vpr. The class of the small (1.7 to 2 kilobases) mRNAs also includes many mRNAs (50) encoding the regulatory proteins Tat, Rev, and Nef, as well as other proteins (5). The full-length and intermediate-size mRNAs contain RRE, whereas the small mRNAs lack RRE.

Northern (RNA) blot analysis has shown that proviruses lacking Rev produce low levels of full-length and intermediate-size mRNAs and high levels of the small mRNAs. The presence of Rev results in increased levels of full-length and intermediate-size mRNAs and decreased levels of the small mRNAs (11, 21). Therefore, this increase in RRE-containing mRNAs occurs at the expense of the small mRNAs that do not contain RRE (14). Since Rev function is mediated by RRE, it has been suggested that the effect of Rev on the level of the small mRNAs is indirect. According to this model, the presence of Rev promotes the transport of RRE-containing RNAs from the nucleus to the cytoplasm; this results in lower levels of nuclear RNA available for splicing and, therefore, reduced levels of the multiply spliced small species. Since all multiply spliced mRNAs encoding Tat, Rev, and Nef are produced from partially overlapping genomic regions and are of similar size, characterization and quantitation of these mRNAs have not been possible by Northern analysis. In the work described in this report, we have used specific probes in S1 nuclease analysis experiments that can distinguish between *tat*, *rev*, and *nef* mRNAs. We have measured the levels of mRNAs produced by the HIV-1 provirus as distinct groups and have verified that all of the small, multiply spliced species are decreased by Rev to a similar extent. This effect of Rev is not observed when the regulatory proteins are expressed from cDNAs lacking introns and RRE. Therefore, regulation of Tat, Rev, and Nef expression by Rev requires the proviral context that allows alternative splicing and the generation of mRNAs that contain or lack the RRE. Our investigation also demonstrated the presence of three different groups of multiply spliced mRNAs that encode Tat, Rev, and Nef in infected and provirus-transfected cells.

MATERIALS AND METHODS

Recombinant plasmids. The proviral HIV-1 clone HXB2 (17) and the *rev* mutant fB (11, 21, 52) have been described previously. The Rev-producing plasmid pMcrev has been described previously as pMctrs (21). pMcrev contains the mouse metallothionein promoter and the intronless *rev* cod-

* Corresponding author.

ing sequence followed by the simian virus 40 polyadenylation site. pL3crev has been described previously as pL3ctrs (14). pL3crev contains the HIV-1 long terminal repeat promoter and TAR region ligated to the intronless *rev* coding sequence followed by the simian virus 40 polyadenylation signal. In the presence of Tat, pL3crev produces higher levels of Rev than pMcrev does.

Transfections. Plasmid DNA was prepared by using the standard Triton X-100 clear lysis procedure and was purified twice through cesium chloride gradients. The DNA was transfected into human HeLa cells by the calcium phosphate coprecipitation technique (20) as previously described (15), except that the cells were not treated with glycerol. The next day the medium was changed twice within 1 h to wash out the remaining precipitate. At 2 days posttransfection, the cells were analyzed for RNA and protein production.

RNA analysis. Total RNA was isolated by the hot phenol procedure (49) and was analyzed by the S1 nuclease protection technique (6, 53) as described previously (15), with uniformly labeled single-stranded probes. For the analysis of the different splice sites, different probes were generated. An *Eco*RI-*Bam*HI fragment of HXB2c (nucleotides [nt] 5289 to 8021) and an *Eco*RI-*Bam*HI cDNA fragment of HXB2c spanning exons 2 to 7 were inserted into the *Eco*RI and *Bam*HI sites of M13tg130 (Amersham Corp.). Synthetic oligonucleotide primers starting at nt 5600, 5615, and 7960 of HXB2c were annealed to the template, elongated by using [^{32}P]dATP, and digested with *Mst*II (M, nt 5501), *Rsa*I (R, nt 5441), *Bst*NI (B, nt 5405), or *Xho*II (X, nt 5388). The probe names consist of the enzyme used in the digestion and the position of the first nucleotide at the 5′ end of the primer. These probes were hybridized to total RNA isolated from transfected or HIV-1-infected cells and, after S1 nuclease digestion and gel electrophoresis, revealed fragments of distinct sizes corresponding to the different exons of HXB2c. The probes contain oligonucleotide tails, which do not hybridize to mRNA; therefore, the size of the full-length protected fragment can be distinguished from that of the undigested probe. The sizes of the protected fragments were determined by comparison with radiolabeled DNA markers (*Msp*I-digested pBR322) run on the same gels.

Immunoprecipitation. At 2 days posttransfection the cells were incubated for 30 min in cysteine-free medium and then labeled with [^{35}S]cysteine (200 μCi/ml) for 3 to 4 h. The cells were washed with phosphate-buffered saline and lysed in 0.5× RIPA buffer as described previously (21). The cell lysates were divided into four aliquots and were immunoprecipitated with nonimmune serum for 1 h and then precipitated with 200 μl of protein A-Sepharose in RIPA buffer for 30 min. All manipulations were performed at 4°C. The supernatants were then incubated overnight with nonimmune rabbit serum, anti-Tat, anti-Rev (14), or anti-Nef (19) rabbit antiserum. The anti-Tat antiserum was raised in rabbits against an amino-terminal peptide of Tat (amino acids 1 to 62). The anti-Rev antiserum was raised against the carboxy-terminal 13 amino acids of Rev. The proteins were precipitated by addition of 200 μl of protein A-Sepharose for 4 h, spun for 5 min, and separated on a denaturing 15% acrylamide gel (32). ^{14}C-labeled Rainbow protein markers (Amersham Corp.) were run as size markers on the same gels.

RESULTS

Detection of mRNA species encoding Rev. Some of the characterized mRNA species identified in HIV-1-infected cells by cDNA cloning (3, 36, 50; Schwartz et al., submitted) are shown in Fig. 1. The full-length RNA (Fig. 1, row a) and all mRNAs that belong to the intermediate size class (for example, Fig. 1, rows b and c) contain the intron defined by the splice sites at positions 5591 and 7925 that is spliced out in all the small multiply spliced mRNAs (Fig. 1, rows d through g). Tat and Nef are encoded by different groups of small mRNAs each containing exon 4 spliced to exon 7 (Fig. 1, row d) or exon 5 spliced to exon 7 (Fig. 1, row e), respectively. Three members of the *tat* group of mRNAs and three members of the *nef* group of mRNAs have been cloned, differing by the presence or absence of two small upstream exons, called 2 and 3 in Fig. 1 (3, 36, 50). A splice acceptor site upstream of the *rev* AUG initiation codon has been reported (48) and was proposed to generate a *rev* mRNA. Cloning and functional analysis of the small mRNAs (50) revealed that there are two types of mRNAs producing Rev, which utilize two different acceptor sites upstream of the *rev* AUG, at positions 5501 and 5507 (50) (Fig. 1 and 2A).

To study the regulation of the small multiply spliced mRNA species in detail, we generated single-stranded, uniformly labeled probes designed to distinguish among the individual groups. We and others have previously shown that the *rev*-deficient provirus mutants overproduce the class of the small multiply spliced mRNAs (11, 21). To determine the levels of the individual groups of the small mRNAs in the absence of Rev, HeLa cells were transfected with the proviral mutant clone fB (*tat*$^+$ *rev*) (11, 21, 52). Total RNA was isolated and analyzed by the S1 nuclease protection assay (Fig. 2B) with probe B7960C (Fig. 2A), which is derived from a HXB2 cDNA. Since all the multiply spliced small mRNAs utilize the splice donor at position 5591 (Fig. 2A), they can be distinguished from the unspliced and intermediate-size mRNAs by this probe. The full-length (U) and the intermediate-size mRNAs containing exon 6 (e6) yielded a band of 173 nt (Fig. 2B). RNAs containing exon 4 spliced to exon 7 (e4e7) and exon 5 spliced to exon 7 (e5e7) yielded bands of 210 nt and 104 nt, respectively (Fig. 2B). In addition, this experiment revealed the presence of two bands of 128 and 122 nt, labeled e4Ae7 and e4Be7, respectively, suggesting the presence of two additional splice sites. The use of additional probes (see below) verified that these splice acceptors (named 4A and 4B) are located at positions 5501 and 5507, respectively, upstream of the *rev* AUG initiation codon (Fig. 2A). The exact structures of mRNAs using these splice sites have been verified by cloning and sequencing of cDNAs prepared from HIV-1-infected cells (50) (Fig. 1, rows f and g). Exons 4A and 4B generate mRNAs that contain *rev* as the first open reading frame, and their coding potential has been verified (50). The intensity of the bands obtained from 4A- and 4B-containing mRNAs also demonstrate that both exons are utilized to a similar extent. S1 nuclease analysis of RNA from HXB2-infected cells, with probes such as B7960C (data not shown) or probe M5615 (see below) (Fig. 3B), also revealed the presence of these exons in HIV-infected lymphocytes. Hybridization to RNA isolated from mock-transfected cells did not result in S1 nuclease-protected bands (data not shown).

These results demonstrate that *tat*, *rev*, and *nef* mRNAs, which are characterized by the presence of exon 4, 4A, or 4B and 5, respectively, can be distinguished by S1 nuclease analysis with RNA from transfected and infected cells. This allowed measurements of their levels in the presence and absence of Rev.

Down regulation of all multiply spliced mRNAs in the presence of Rev. We used the S1 nuclease protection assay to

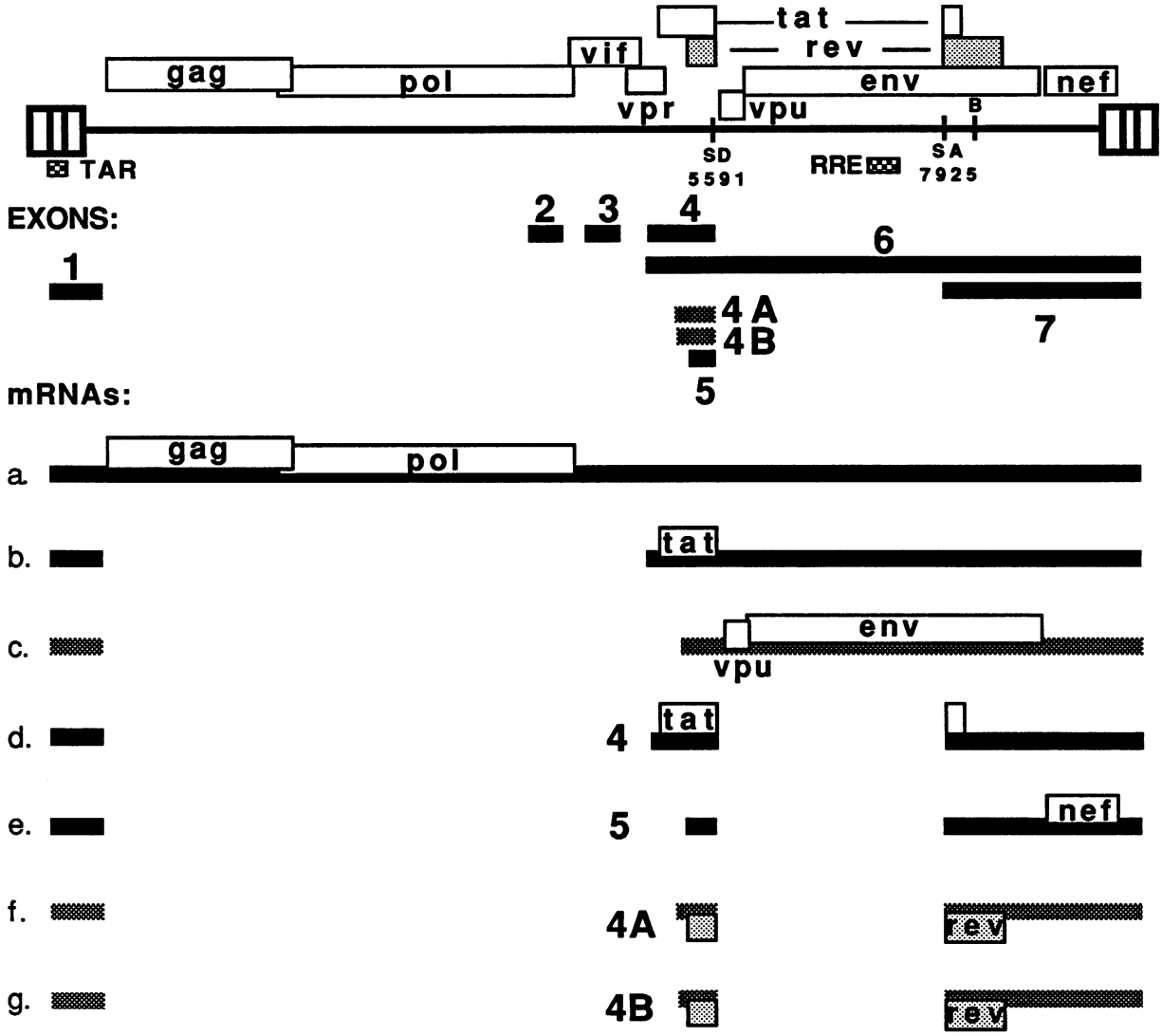

FIG. 1. Representative mRNA species produced by HIV-1. The structure of HIV-1 provirus with the identified open reading frames is shown at the top. The unique *Bam*HI site (B) was used to produce the frameshift mutant fB (*tat*$^+$ *rev*). The locations of TAR (Tat-responsive element) and RRE (Rev-responsive element) are shown. The identified exons are numbered according to the system of Muesing et al. (36) and Schwartz et al. (50). Some of the identified mRNA species are shown (rows a to g). mRNAs previously identified by cDNA cloning are shown (▬). mRNA in row b was previously identified (36) and exclusively produces the one-exon Tat protein of 72 amino acids. The Vpu/Env-producing mRNAs have different structures, as shown in one example (row c) (Schwartz et al., submitted). These mRNAs and exon 4A- or 4B-containing mRNAs (rows f and g), (▩) have been cloned recently from HIV-infected cells (50; Schwartz et al., submitted). This list indicates that exons 4, 4A and 4B, and 5 are diagnostic of mRNA species producing Tat, Rev, and Nef, respectively. The splice sites (SD, SA) at positions 5591 and 7925 used in the production of the multiply spliced species are shown.

compare the levels of the different small mRNAs in the presence and absence of Rev. RNA was prepared from HeLa cells transfected by the proviral clone HXB2 (H) or by the *rev* mutant fB, and the same samples were subjected to S1 nuclease analysis with different probes (Fig. 3A). S1 mapping probes M5615, R5600, and X5615 allowed the distinction of exons 4, 4A, 4B, and 5. The nested arrangement of these probes also verified the correct assignment of the bands to the corresponding exons. Exon 5 yielded an S1-protected band of 69 nt (e5) with all the probes, as expected (Fig. 3A, lanes fB). Probe M5615 yielded a band of 86 nt for exons 4, 4A, and 4B (Fig. 3A, left panel). Probes R5600 and X5615 yielded bands of 91 and 86 nt for exons 4A (e4A) and 4B (e4B), respectively (Fig. 3A, middle and right panels, lane fB). The length of the protected fragment for exon 4 (e4) depends on the probes used. Probe R5600 yielded a band of 149 nt, and probe X5615 yielded a band of 199 nt. The bands corresponding to exons 4, 4A, 4B, and 5 were very weak in cells transfected with the intact provirus HXB2 (Fig. 3A, lanes H) and were much stronger in cells transfected with the *rev* mutant fB (lanes fB). Therefore, the absence of Rev resulted in an increased production of all mRNAs containing exon 4, 4A, 4B, and 5. In contrast, unspliced (U) and exon 6-containing (e6) mRNAs were decreased in the absence of Rev. Cotransfections of the *rev* mutant fB with the Rev-producing plasmid pMcrev or pL3crev restored the mRNA levels to those of HXB2 (Fig. 3B, compare lane fB with lanes HXB2 and fB+rev, respec-

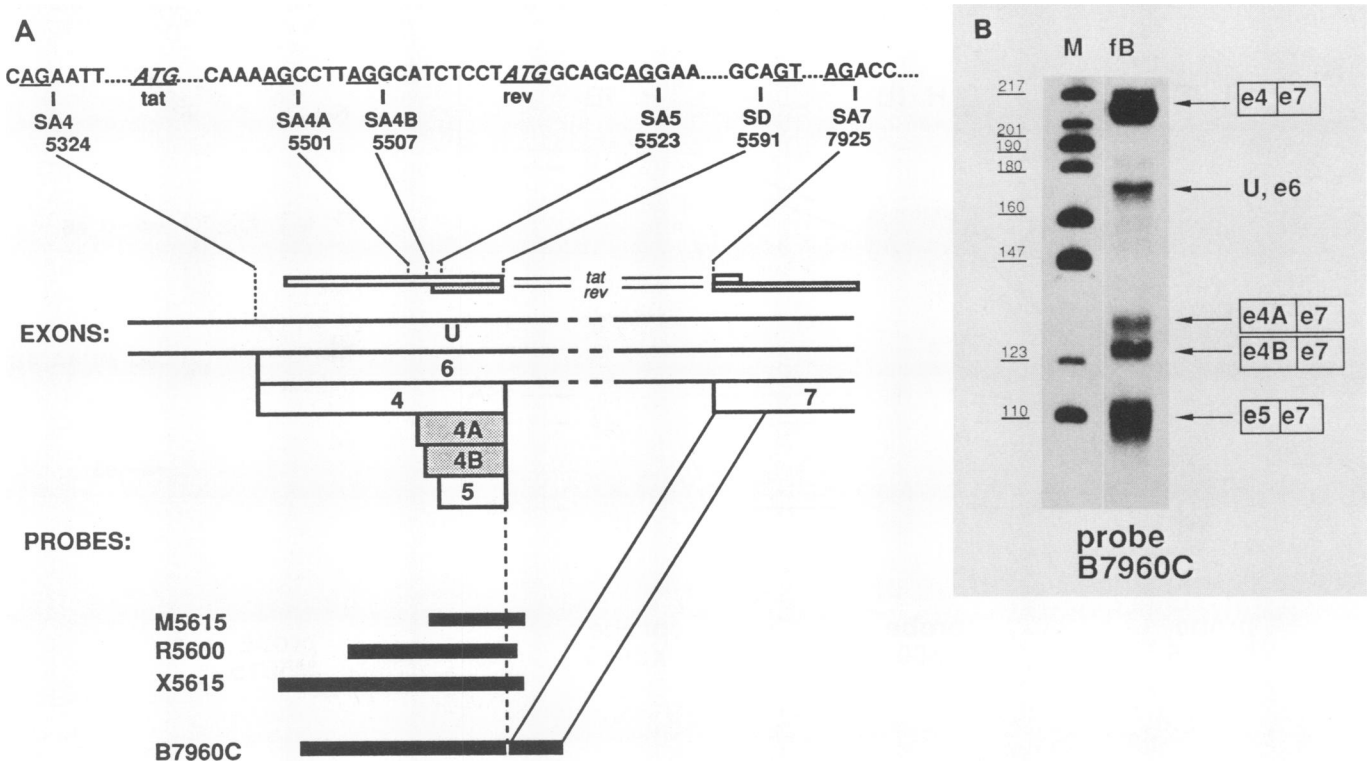

FIG. 2. Identification of the *tat*, *rev*, and *nef* mRNAs by S1 nuclease protection analysis. (A) The sequence of the area of splice sites 4, 4A, 4B, 5, and 7 found in the middle portion of HIV-1 is shown at the top. Also shown are the *tat* and *rev* open reading frames, the location of AUG initiation codons, and the arrangement of the HIV-1 exons in the middle portion of the genome. The probes used in Fig. 2 and 3 (▬) are named according to the region they represent: M5615 indicates a probe comprising nt 5501 (*Mst*II site, M) to 5615; R5600, nt 5441 (*Rsa*I site, R) to 5600; X5615, nt 5388 (*Xho*I site, X) to 5615. Probe B7960C was derived from a cDNA template and comprises nt 5405 (*Bst*NI site, B) to 7960, lacking the region of the intron nt 5591 to 7925. The numbering system follows the corrected HXB2 sequence as given by Myers (38, 42–44; G. N. Pavlakis, unpublished data), where +1 is the mRNA start site. (B) Total mRNA isolated from HeLa cells transfected with the *rev* proviral mutant fB was hybridized to probe B7960C and digested with S1 nuclease. The protected fragments were separated on a 6% acrylamide-urea gel (lane fB). The different bands corresponding to differentially spliced mRNAs are indicated by the names of the exons. U indicates unspliced mRNA. Note that the cDNA probe generated longer protected fragments for the spliced mRNAs than for the unspliced mRNA. Lane M contains radiolabeled pBR322/*Msp*I-digested marker.

tively). These results showed that Rev either expressed by the HXB2 provirus or supplied by *rev* expression plasmids suppressed the levels of all the multiply spliced species and increased the levels of the unspliced and intermediate mRNA species. The negative effect of Rev on the levels of Tat and Nef were seen only when these proteins were expressed by the provirus. Rev does not affect the expression of these proteins when they are expressed from cDNA constructs (data not shown).

Regulatory protein measurements. To compare the levels of the various mRNAs with the levels of produced proteins, we measured Tat, Rev, and Nef by immunoprecipitations with monospecific antisera produced as described previously (14, 19, 55). Figure 4 (middle panel) demonstrates that the intact provirus HXB2 produced low levels of Tat, whereas the *rev* mutant provirus fB produced high levels. The levels of immunoprecipitated Rev protein in the same experiment are shown in the top panel. The intact provirus HXB2 produced low levels of Rev. fB produced a nonfunctional mutant Rev, which lacked the domain used to produce the anti-Rev antibody and could not be detected. Addition of Rev in *trans* by cotransfection of fB and pL3crev, a Rev-producing plasmid (14), drastically reduced the levels of Tat. Cotransfection of fB and pMcrev resulted in intermediate levels of Tat (middle panel). pL3crev and pMcrev produced high and intermediate levels of Rev, respectively (top panel). There was an inverse relationship between the levels of Tat and Rev: when high levels of Rev were present, very little Tat could be detected; lower levels of Rev resulted in higher levels of Tat; and in the absence of Rev, very high levels of Tat were detected (compare middle and top panels).

HXB2 contains a stop codon within the *nef* reading frame, resulting in a truncated Nef of 124 amino acids (Nef124). Therefore, the results presented here have been derived in the absence of an intact Nef. The absence of Nef does not inhibit the production of infectious virus and the propagation of virus in tissue culture (2, 30, 33). We also measured Nef124 in cells transfected with HXB2 or fB by immunoprecipitation (Fig. 4, bottom panel). Barely detectable levels of Nef124 were present in HXB2-transfected cells. In contrast, Nef124 was easily detected in fB-transfected cells. The presence of Rev produced by pL3crev suppressed Nef124 expression to levels similar to those of HXB2 (bottom panel, right lane). A smaller Nef-related band was also present, possibly the result of translation initiation at an internal methionine codon at position 20 (2).

The S1 mapping experiments presented above demonstrate that the levels of all the mRNAs that express Tat, Rev,

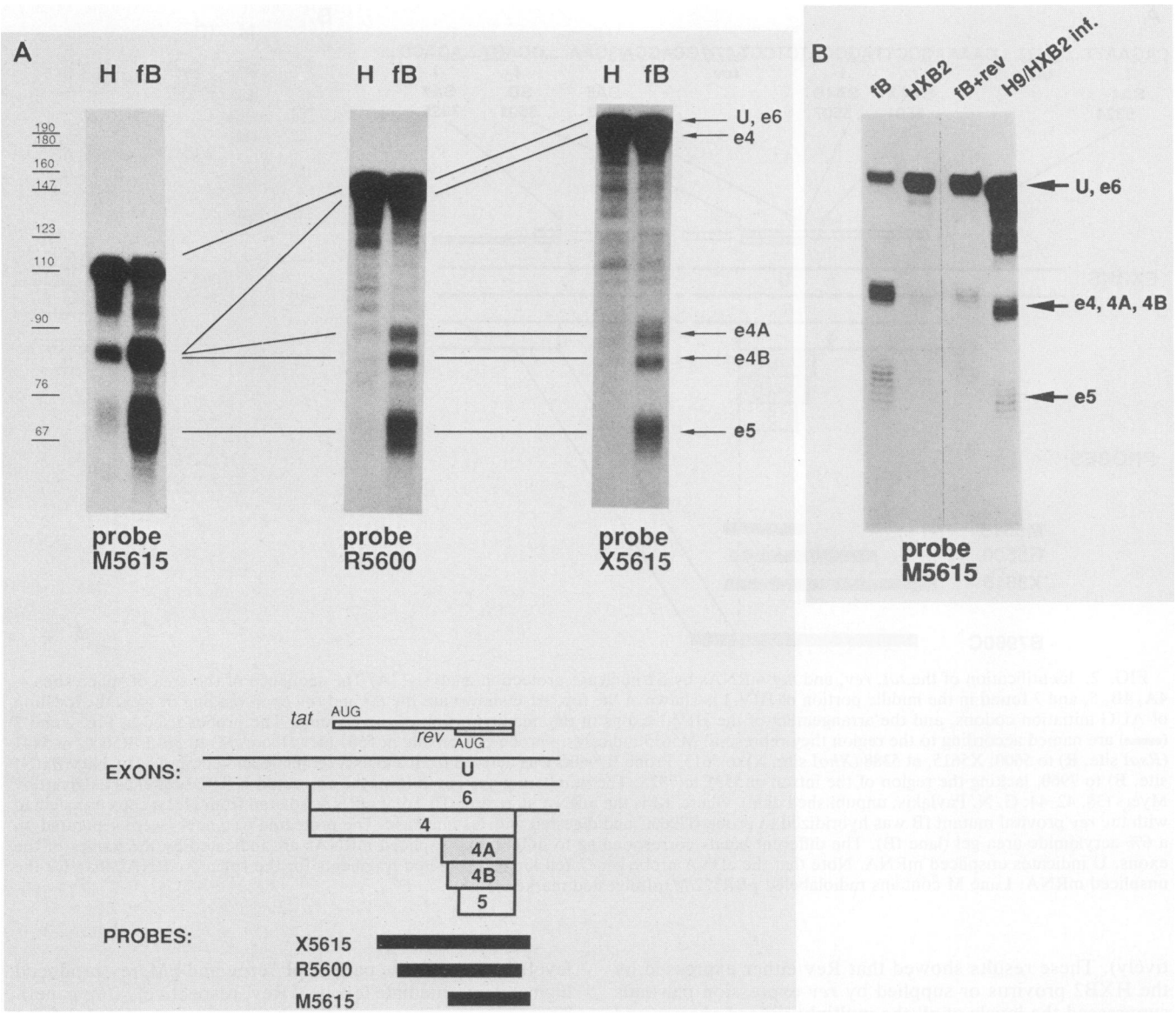

FIG. 3. Regulation of multiply spliced viral RNAs by Rev. (A) Total RNA from cells transfected with HXB2 (lanes H) or fB (lanes fB) proviral clones was hybridized with the probes indicated at the bottom of each panel (Fig. 2A) and were digested with S1 nuclease as described in Materials and Methods. The S1 probes distinguish between exons 4, 4A, 4B, 5 and the unspliced or exon 6-containing RNAs. The assignment of the bands to specific exons (e), which was done by using combinations of probes, is shown at the right. The lines between the panels connect bands produced by the same exons. The intensities of the short S1 nuclease-protected bands varied with the probe size. The size of the protected fragments was estimated by comparison with radiolabeled DNA markers, run on the same gels. The positions of the fragments of the labeled pBR322/*Msp*I-digested marker are indicated at the left. (B) The presence of Rev in *trans* lowers the levels of *tat*, *rev*, and *nef* mRNAs to HXB2 levels. Total mRNA was analyzed by S1 mapping with probe M5615 from cells transfected with fB, HXB2, fB and the *rev*-producing plasmid L3crev (fB+rev), or HXB2-infected H9 cells (H9/HXB2 inf.). In general, the same bands were observed in transfected and infected cells. Some additional bands were observed from infected cells, but their variation from experiment to experiment suggested that they were probably caused by degradation of long mRNA molecules.

and Nef are decreased in the presence of Rev. Comparison of the Tat, Rev, and Nef levels with the levels of all the mRNAs that express these proteins allows the conclusion that Rev down regulates the expression of all three regulatory proteins by acting at the mRNA level.

In addition to the two-exon Tat of 86 amino acids generated from the multiply spliced mRNAs (Fig. 1, row d), HIV-1 can produce a one-exon Tat of 72 amino acids from an intermediate-sized, RRE-containing mRNA such as the one shown in Fig. 1, row b. The Tat open reading frame terminates in the intron immediately after the splice site (nt 5591), and the produced protein is functional. It has been proposed that the one-exon Tat might be responsible for maintenance of transactivation at the steady state (34). Therefore, it might be argued that Rev would not affect the overall levels of Tat but only the ratios of two-exon to one-exon Tat. To measure the levels of these proteins, cell lines chronically infected with HIV_{IIIB} or HIV_{HXB2} or

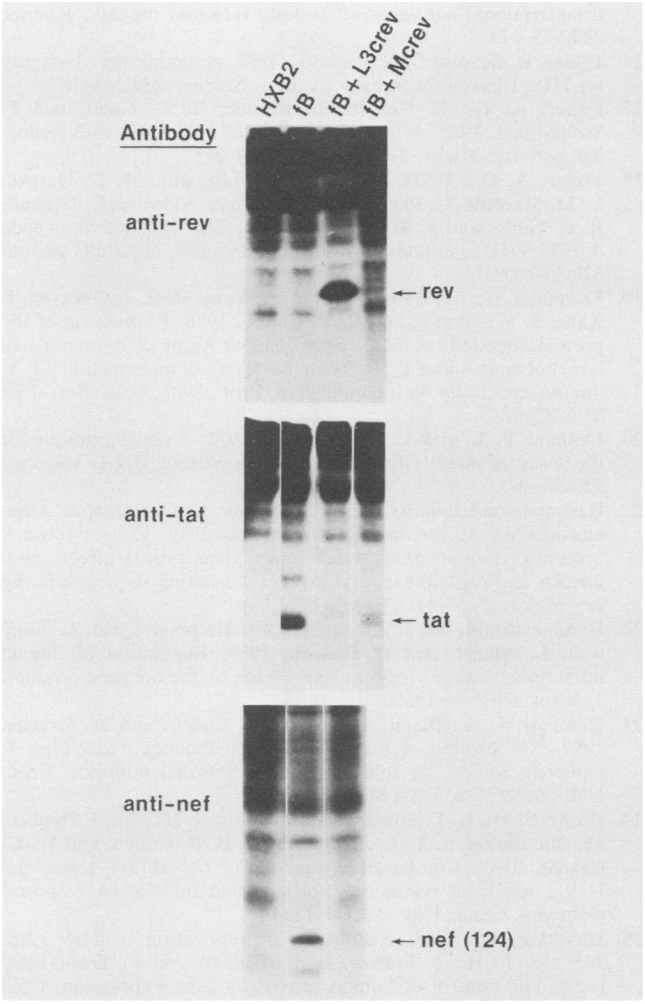

FIG. 4. Detection of Tat, Rev, and Nef by immunoprecipitation. HeLa cells were transfected with various plasmids as indicated at the top. At 36 h posttransfection, the cells were labeled, the cell lysates were divided into four parts, and proteins were immunoprecipitated with either nonimmune rabbit serum (data not shown) or anti-Rev (top panel), anti-Tat (middle panel) or anti-Nef (bottom panel) antisera. For the transfection fB+Mcrev, the immunoprecipitation with anti-Nef is not shown. The locations of Tat, Rev, and Nef are indicated by arrows. The one-exon Tat protein could not be detected in these experiments. In addition to the 124-amino-acid truncated Nef, fB produced a second Nef of approximately 10 kilodaltons, probably the result of initiation from an internal methionine. The sizes of the proteins were determined by comparison with radiolabeled protein size markers run on the same gels (data not shown).

transfected by the infectious proviral clone HXB2 were biosynthetically labeled and the proteins were immunoprecipitated with an anti-Tat antiserum (data not shown). The two-exon Tat was the predominant form both in infected lymphocytes and in HeLa cells transfected with the infectious proviral clone HXB2. These results indicate that Rev cannot completely suppress the generation of multiply spliced mRNAs.

DISCUSSION

The three groups of mRNAs producing primarily Tat, Rev, and Nef can be distinguished by the presence of exons 4, 4A or 4B, and 5, respectively. The S1 mapping experiments presented here demonstrate that the levels of all the mRNAs that express Tat, Rev, and Nef are decreased in the presence of Rev. The comparison of mRNA and protein levels for Tat, Rev, and Nef allows the conclusion that Rev down regulates the expression of all three regulatory proteins by lowering the levels of the mRNAs. Down regulation of Nef by Rev has recently been reported (1).

The two-exon Tat is the predominant form detected in infected cells and is therefore primarily responsible for transactivation. The accumulation of the one-exon Tat seen by others (34) could be the result of the truncated HIV mRNAs produced in those experiments. Despite the existence of mRNAs that can produce one-exon Tat, Rev decreased the total levels of Tat (Fig. 4).

By directly measuring the various mRNAs and proteins produced by the provirus, we have verified the model of feedback regulation proposed by us and other investigators (12, 14, 25, 54). HIV-1 uses an elaborate regulatory feedback mechanism involving three proteins, Tat, Rev, and Nef. Rev acts in the nucleus as a traffic signal to direct viral mRNA containing RRE away from splicing and toward transport and translation in the cytoplasm (14). By promoting the transport of RRE-containing mRNA, Rev decreases the pool of RNAs available for splicing. Therefore, Rev down regulates the levels of its own expression indirectly, as well as the levels of Tat and Nef. These data suggest a model in which the multiply spliced mRNAs accumulate early after infection, whereas later the presence of Rev results in the accumulation of RRE-containing mRNAs. Such a temporal switch in RNA accumulation was reported recently (30). These observations support the model that HIV has an early phase dedicated to the production of large amounts of regulatory proteins and a late phase, induced by Rev, leading to the production of structural proteins and virus particles.

The interactions between Tat, Rev, and Nef are important for the final outcome of viral infection. The use of intact proviral DNA, rather than truncated subgenomic fragments, allows the conclusion that similar regulatory events occur in HIV-infected cells. Two advantages may result from this regulation: first, HIV-1 can produce appropriate levels of many multiply spliced mRNAs, resulting in the production of additional proteins. Second, HIV-1 can regulate the levels of its own expression via viral factors, acquiring more control over the outcome of viral infection.

The control of splicing is necessary for all retroviruses, since they must produce at least two mRNAs, spliced and unspliced, from one promoter. The spliced mRNA produces Env proteins, while the unspliced mRNA produces Gag and Gag-Pol polyproteins and is also encapsidated in the virion as genomic RNA. The mechanism by which retroviruses produce balanced levels of these mRNAs is not known in detail. In Rous sarcoma virus, intron sequences appear to affect the levels of viral splicing (29). It is interesting that both families of human retroviruses (HIV and HTLV) use viral regulatory proteins (Rev and Rex, respectively) for the control of mRNA production. The recent finding that Rev of HIV-1 can be replaced by Rex of HTLV-1 (13, 45) indicates that a common cellular pathway is affected by both proteins. Examination of the function of these proteins suggests that this common pathway involves the mRNA transport from the nucleus to the cytoplasm (10, 13, 14, 21, 22, 24, 27, 28, 31, 35, 45, 51).

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ADDENDUM IN PROOF

Using PCR analysis, Arrigo et al. (S. J. Arrigo, S. Weitsman, J. D. Rosenblatt, and I. S. Y. Chen, J. Virol. **63:** 4875–4881, 1989) have proposed similar conclusions; however, they did not identify all the multiply spliced species of HIV-1 encoding the different proteins.

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