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The Blocks to Human Immunodeficiency Virus Type 1 Tat and Rev Functions in Mouse Cell Lines Are Independent

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Rodent cells present two blocks precluding the expression of the human immunodeficiency virus type 1 (HIV-1) genome. First, the viral protein Tat is only poorly active in these cells. Second, when the HIV-1 provirus is integrated in the genome of mouse cells, it electively fails to express the viral structural proteins, indicating a block to Rev action. Both defects can be complemented by fusion of the infected mouse cells with uninfected human cells. Because the production of high levels of Rev is dependent on Tat-mediated transactivation and because both Tat and Rev bind the viral transcript, it has been hypothesized that the two blocks found in rodent cells might be linked. In the present work, we demonstrate that overexpression of Rev in mouse cell lines does not relieve their block in HIV-1 structural-gene expression. In addition, we show that this defect is also present in human-mouse cell hybrids which contain human chromosome 12 and support Tat function. On that basis, we conclude that the blocks to HIV-1 Tat and Rev action in mouse cell lines are independent and result from the absence of distinct cellular elements that are critical for HIV-1 gene expression.

The growth of human immunodeficiency virus (HIV), like that of all viruses, is crucially dependent on the presence of a number of specific factors in the infected cell. Identifying such cellular factors is important to unravel the mechanisms of viral replication. In that respect, it is instructive to analyze the cell- and species-specific tropism of viruses such as HIV type 1 (HIV-1). Mice are not a natural target for HIV-1 infection, and murine cells have been shown to present at least four limiting steps that preclude a normal viral replicative cycle. The first two of these blocks are located at the level of viral entry: mouse CD4 does not bind the HIV-1 envelope (14), and rodent cells transfected with human CD4 do bind but fail to internalize the virus (16). Two additional blocks have a dramatic influence on the expression of the HIV-1 proviral DNA in murine cells, both in a quantitative and in a qualitative manner. First, the viral protein Tat, which is the major transactivator of the HIV-1 long terminal repeat (LTR) and which greatly amplifies viral expression and production, has been shown to be poorly active in mouse cells (8, 21). It was further demonstrated that a cellular mechanism that supports Tat function is associated with the presence of human chromosome 12 in human-rodent hybrid cells (8, 21). Second, using HIV-1-derived viruses pseudotyped with the envelope of the murine leukemia virus (HIV[MLV] pseudotypes), we have demonstrated that several rodent cell lines, including NIH 3T3 cells, could not support the function of *rev*, the viral regulatory gene which, in human cells, induces the cytoplasmic export of the incompletely spliced HIV-1 mRNAs that encode the viral structural proteins (33). Most importantly, we could complement the *rev* defect by fusing the infected murine cells with uninfected human cells. We concluded that HIV-1 tropism was partly a consequence of a *trans*-acting human cellular factor critical for Rev function.

In apparent contradiction of these data, the Rev-dependent production of the HIV-1 envelope gp160 was successfully obtained in *Drosophila melanogaster* cells by using both an insect expression vector which contained a human tissue plasminogen activator (tPA) gene fused in frame with the gp160-coding sequence and a *rev* expression vector (9). Also, in the mouse cell lines ltk$^-$ and NIH 3T3, Rev expressed in *trans* was found to induce the production of chloramphenicol acetyltransferase (CAT) from a construct in which the reporter gene was placed within an intron that was bordered by functional HIV-1 splice sites and that contained the HIV-1 Rev-responsive element (RRE) (18). However, it was noted in this and another study (35) that Rev function in NIH 3T3 cells was diminished, relative to its function in primate cells similarly tested. Finally, Jolicoeur et al. demonstrated that HIV-1 particles were efficiently produced in the milk and epididymal fluid of transgenic mice containing the complete HIV-1-coding sequences, fused to the mouse mammary tumor virus LTR (10).

These conflicting results have generated a large amount of controversy regarding the existence of species-specific limitations to Rev function. On numerous instances, it has been argued that the Rev-negative phenotype that is observed in mouse cells containing the HIV-1 provirus was secondary to the defect in Tat transactivation present in this species, resulting in insufficient levels of the Rev protein. To address these points, we reexamined the block to HIV-1 structural-gene expression in mouse cell lines infected with HIV(MLV) pseudotypes. Specifically, we asked (i) whether this block could be overcome by expressing high levels of Rev and (ii) whether it was associated with (and perhaps a consequence of) the Tat defect previously described for these cells. The results showed that the block to Rev function previously identified in murine cell lines is independent of Rev concentration and of Tat function.

We have previously demonstrated that Rev function is dependent on the accumulation of this viral protein above a critical threshold (23). The failure to reach such a threshold is responsible for a form of HIV-1 proviral latency, in which infected cells exhibit an aberrant pattern of RNA expression,

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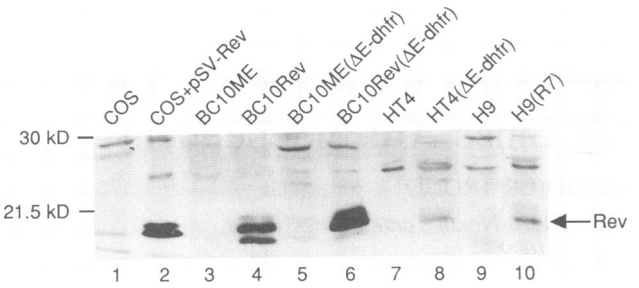

FIG. 1. Analysis of *rev* expression. Levels of Rev protein were compared by Western blotting in equal numbers of various primate and murine cells. Lanes: 1, mock-transfected COS cells; 2, COS cells transfected with the *rev*-expressing plasmid pSV-Rev; 3, BC10ME cells; 4, BC10Rev cells; 5, BC10ME cells transduced with the HIV-1-derived ΔE-dhfr provirus; 6, ΔE-dhfr-transduced BC10 Rev cells; 7, HeLa-derived HT4 cells; 8, ΔE-dhfr-transduced HT4 cells; 9, human T-lymphoid H9 cells; 10, HIV-1-infected H9 cells.

with mostly multiply spliced viral RNAs expressed in the cytoplasm (24). We called this phenotype a blocked-early-stage pattern of viral expression, because it recapitulated the pattern observed in the early phase of a lytic infection (12). At least in some of the cells with such a phenotype, the primary cause of latency appears to be transcriptional, because provision of large amounts of Rev in *trans* results in shifting viral RNA expression from a blocked early stage to a fully productive mode (36). In mouse cells infected with HIV(MLV) pseudotypes, high levels of the multiply spliced mRNAs, which encode the viral regulatory proteins Tat, Rev, and Nef, were observed (33). This suggested that a low concentration of Rev was not responsible for the failure to express the viral structural genes in this setting. Nevertheless, we asked whether the overproduction of Rev would allow the expression of the HIV-1 structural genes in such cells. The BC10Rev cells (a gift from S. Chang, Viagene, Inc., San Diego, Calif.) were derived from a BALB/c mouse fibroblast cell line, BC10ME, transduced with a replication-defective HIV-1 *rev*-expressing murine retroviral vector. HIV(MLV) pseudotypes were generated by cotransfecting COS cells with pΔE-dhfr, an envelope-defective HIV-1-derived construct which expresses a mutant dihydrofolate reductase gene from the viral LTR and which confers methotrexate resistance to transduced cells (34), and pSV-AMLVenv, a plasmid that induces large quantities of the amphotropic MLV envelope gene, from the simian virus 40 promoter (a gift from D. Littman, University of California at San Francisco) (22). BC10ME and BC10Rev cells were exposed to the filtered supernatant of the transfected COS cells in the presence of 8 μM Polybrene (Sigma) and were selected in 0.2 μM methotrexate (amethopterin; Sigma). Resistant colonies were obtained, named BC10ME(ΔE-dhfr) and BC10Rev(ΔE-dhfr), respectively, and maintained in 2 μM methotrexate. Cells were grown and analyzed both as clones and as populations, with similar results (only results obtained with populations are given in this paper). Rev levels in these cells, as well as in primate cells containing the HIV-1 provirus or a *rev* expression plasmid, were first analyzed by Western blotting (Fig. 1). In all cases, equivalent numbers of cells were lysed on ice in 10 mM Tris-Cl (pH 7.4), 1% Nonidet P-40, 0.5% sodium dodecyl sulfate (SDS), and 150 mM NaCl. DNase I (100 U) (Boehringer Mannheim) was added to the samples, which were incubated for 30 min at 37°C. After centrifugation, protein concentrations were

TABLE 1. HIV-1 p24 antigen production by murine, human, and hybrid cells transduced with the ΔE-dhfr provirus

Cell line	p24 (pg/ml)[a]
BC10ME(ΔE-dhfr)	35
BC10Rev(ΔE-dhfr)	140
A9(ΔE-dhfr)	7
915(ΔE-dhfr)	21
HT4(ΔE-dhfr)	50,000

[a] Cells (2×10^6) were plated in 10 ml of growth medium, and HIV-1 p24 antigen produced in the supernatant after 48 h was measured by ELISA; the measurements were repeated on numerous occasions without significant variability.

measured with the bicinchoninic acid protein assay reagent (Pierce) according to the manufacturer's instructions. Aliquots of the cell lysates were mixed with 4× sample buffer (4% SDS, 50 mM Tris-Cl [pH 7.0], 24% glycerol, 0.01% bromophenol blue) and heated to 100°C for 3 min. Proteins were electrophoresed onto an SDS-polyacrylamide gel (15% polyacrylamide) and transferred to an Immobilon P membrane (Micron Separations Inc.) in 0.01 M CAPS (3-cyclohexylamino-1-propanesulfonic acid) (Sigma) buffer (pH 11.0) containing 5% methanol. Rev was immunodetected with a rabbit polyclonal antiserum (1:250) raised against a C-terminal Rev peptide (a gift from M. L. Hammarskjöld, State University of New York at Buffalo) and a goat anti-rabbit alkaline phosphatase conjugate (1:2,000) (Fisher). The results showed that BC10Rev expressed high levels of Rev (Fig. 1, lane 4). Infection with HIV(MLV) pseudotypes had little effect on these levels (Fig. 1, lane 6), showing that Rev was largely derived from the murine retroviral vector in BC10Rev(ΔE-dhfr) cells. Accordingly, very low amounts of Rev were detected in BC10ME(ΔE-dhfr) cells (Fig. 1, lane 5). Importantly, Rev levels in BC10Rev cells exceeded those observed in HT4(ΔE-dhfr), a human HeLa cell derivative transduced with the HIV-1-derived ΔE-dhfr provirus (Fig. 1, lane 8), and in H9(R7), a human T-lymphoid cell line infected with HIV-1 (Fig. 1, lane 10), both of which exhibit a Rev-permissive phenotype (see below). HIV-1 p24 antigen concentrations in the supernatants of BC10ME(ΔE-dhfr), BC10Rev(ΔE-dhfr), and HT4(ΔE-dhfr) cells were then measured by a sensitive enzyme-linked immunosorbent assay (ELISA) (DuPont) (Table 1). BC10Rev(ΔE-dhfr) cells released an average of 140 pg of HIV-1 p24 antigen per ml, whereas BC10ME(ΔE-dhfr) cells produced 35 pg/ml. These numbers were in striking contrast to the large amounts found in the supernatant of the HT4(ΔE-dhfr) cell line, a human HeLa cell derivative transduced with the same HIV-1-derived provirus (34), which contained 50,000 pg/ml. To assess more directly the function of Rev in the mouse cells, we examined the RNAs expressed from ΔE-dhfr by Northern (RNA) blot analysis (Fig. 2). Cytoplasmic RNAs were isolated from cell populations stably transduced with the HIV-1-derived provirus. Cells were washed with phosphate-buffered saline (PBS) once, chilled on ice, and then lysed in a solution containing 10 mM Tris-Cl (pH 7.5), 10 mM NaCl, and 1% Nonidet P-40 for 4 min. Nuclei and debris were removed by centrifugation at 4°C. RNA was extracted with buffered phenol (pH 6.5) containing 1% SDS and was precipitated in ethanol. Northern blot analysis was performed as previously described (33), with slight modifications. Twenty micrograms of RNA was denatured in a solution containing 1× MOPS buffer (20 mM MOPS [morpholine propanesulfonic acid; pH 7.0], 5 mM sodium acetate

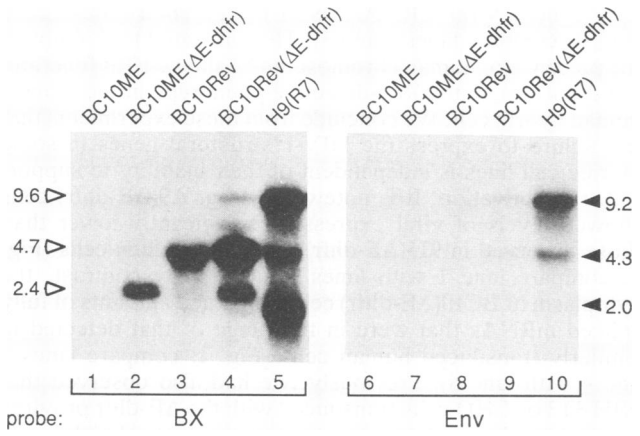

FIG. 2. Northern blot analysis of cell lines transduced with an HIV-1-derived provirus. Cytoplasmic RNAs from the ΔE-dhfr-transduced, *rev*-expressing BC10Rev mouse cells (lanes 4 and 9) and from the *rev*-negative BC10ME cells (lanes 2 and 7) were analyzed by Northern blotting. The corresponding cells without the HIV-1-derived provirus were used as negative controls (lanes 1, 3, 6, and 8). H9 cells infected with the HIV-1_{HXB2}-derived R7 virus (31) served as a positive control (lanes 5 and 10). The BX probe (lanes 1 to 5), complementary to the 3′ portions of the HIV-1 transcript, could detect all HIV-1 RNA species and also a 4.5-kb-long RNA made from the *rev*-expressing murine retroviral vector present in BC10Rev (as seen in lanes 3 and 4). The Env probe (lanes 6 to 10) was specific for the incompletely spliced HIV-1 mRNA species. The sizes of the major classes of viral mRNAs are indicated on the left (ΔE-dhfr) and on the right (R7) sides. All mRNAs from ΔE-dhfr are approximately 400 nucleotides larger because of the *dhfr* insert (34).

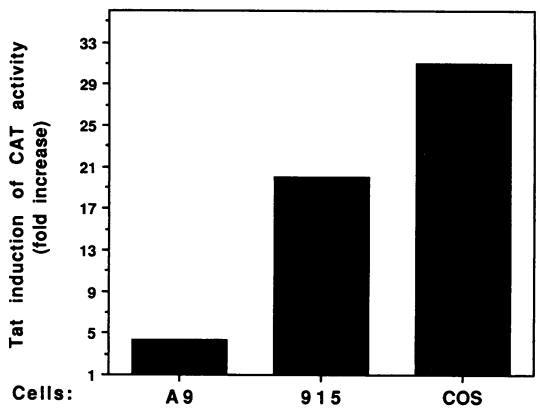

FIG. 3. Tat transactivation in mouse-human hybrids containing human chromosome 12. The 915 cells are mouse-human hybrids derived from the A9 murine fibroblast cell line. Tat induction was calculated by dividing the amounts of CAT activity obtained from cells transfected with pLTR-CAT and pC-Tat by the numbers recovered from transfections with pLTR-CAT and the control vector pC-PLK. The experiment was repeated three times, with less than 10% variability.

[NaOAc], 0.5 mM EDTA), 6.5% formaldehyde, and 50% formamide at 60°C for 15 min. To this solution, 2% glycerol was added, and RNA samples were analyzed by electrophoresis through a 1.2% agarose–1% formaldehyde–1× MOPS gel bed, in 1× MOPS buffer. RNA was transferred to NitroPure-supported nitrocellulose membranes (Micron Separations Inc.) in 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). RNA was cross-linked to the membrane with UV light (12 μJ) by using a Stratalinker UV Crosslinker (Stratagene). The membranes were prehybridized for 2 h at 60°C in a solution containing 50% formamide, 5× SSCPE (1× SSCPE is 1× SSC, 13 mM KH_2PO_4, and 1 mM EDTA), 5× Denhardt's solution, 0.1% SDS, 250 μg of salmon sperm DNA per ml, and 500 μg of yeast tRNA per ml. Hybridizations were performed overnight in the same solution at 60°C with ^{32}P-labelled RNA probes complementary to nucleotides 8896 to 8474 (BX) or 6850 to 6833 (Env) of the HIV-1_{HXB2} sequence (25). The BX probe could detect all viral mRNA species, whereas the Env probe was specific for the singly spliced and unspliced mRNAs. After hybridization, the membranes were washed as previously described (32) and exposed to X-ray film. The results demonstrated that BC10Rev(ΔE-dhfr) cells exhibited a blocked-early-stage pattern of viral RNA expression, similar to that observed in BC10ME(ΔE-dhfr) cells, with large amounts of multiply spliced but very little unspliced viral mRNA in the cytoplasm (Fig. 2). Taken together, these data indicate that the Rev-defective phenotype observed in HIV(MLV) pseudotype-infected murine cell lines is independent of the levels of Rev expressed in these cells.

Murine cell lines infected with HIV(MLV) pseudotypes exhibit a Rev-defective phenotype. By contrast, Rev has been found to be functional in similar cells, when tested within the context of heterologous expression systems, independent of the HIV-1 5′ LTR (18, 35). Transactivation of the HIV-1 LTR by the viral protein Tat has previously been shown to be defective in rodent cells. Therefore, the Rev defect detected in our system could be secondary to this block in Tat function. From the results described above, it was unlikely that it would be due to a quantitative defect in Rev production. However, alternative connections between the Tat and Rev proteins, which have both been shown to bind the viral mRNA (for a review, see reference 26), could be envisioned. It has been shown that the defect in Tat function in rodent cells is no longer observed with hybrid proteins that contain the activation domain of Tat fused to heterologous RNA-binding proteins (1). This indicates that the murine block to Tat action is due to a lower affinity of Tat for the *trans*-acting response RNA (TAR) in this species. If Tat normally directed the viral RNAs toward a specific pathway, where Rev could act upon them to promote the cytoplasmic export of the incompletely spliced species, then the defective binding of Tat in mouse cells could be responsible for a Rev-negative phenotype. To test this hypothesis and to ask whether Tat and Rev defects were linked, we took advantage of human-mouse cell hybrids with a demonstrated ability to support Tat transactivation. The 915 cell line (obtained from E. Stanbridge, University of California at Irvine) is a mouse-human hybrid derived from mouse A9 fibroblasts which contains human chromosome 12 (21). We first verified that Tat transactivation was functional in 915 cells by cotransfecting plasmid pLTR-CAT, which produces the CAT gene from the viral LTR (20), with the vector pC-Tat, in which Tat is expressed from the cytomegalovirus immediate-early promoter (33) (Fig. 3). A9, 915, and COS cells were transfected with 8 μg of pLTR-CAT plus 2 μg of either pC-Tat or the control vector pC-PLK (33) in 10-cm^2 plates with 200 μg of DEAE-dextran per ml and with 100 μM chloroquine added in the COS cells. An MLV promoter-driven β-galactosidase-expressing plasmid (pBAG) was cotransfected to normalize the results for transfection efficiency. All cells were shocked with 10% dimethyl sulfoxide in PBS for 1 min and incubated in growth medium for 48 h. CAT assays were performed by a modified diffusion method,

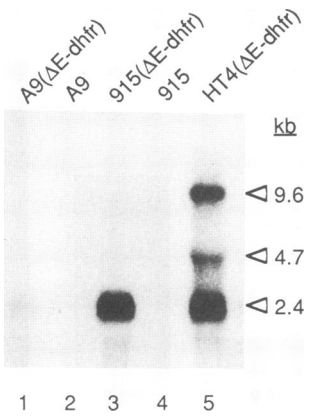

FIG. 4. Expression of the HIV-1-derived provirus in Tat-permissive mouse-human hybrids. Cytoplasmic RNAs from A9(ΔE-dhfr) (lane 1), 915(ΔE-dhfr) (lane 3), and the human control HT4(ΔE-dhfr) (lane 5) cells were analyzed by Northern blotting with the BX probe as a detector. Uninfected A9 (lane 2) and 915 (lane 4) cells were used as controls. The molecular sizes of the major classes of viral mRNAs are indicated on the right.

as described previously (28). Briefly, cells were washed with ice-cold PBS and lysed in 0.25 M Tris-Cl by being frozen and thawed three times. Cell debris was pelleted by centrifugation. Cell extracts (3 to 30 μl) were mixed with 100 mM Tris-Cl (pH 7.8) to 50 μl in a plastic 5-ml scintillation vial, and the mixture was heated for 15 min at 65°C to inactivate deacetylases. A 200-μl volume of freshly prepared CAT reaction mixture containing 0.125 M Tris-Cl (pH 7.8), 1.25 mM chloramphenicol (Sigma), 0.2 μCi of ^{3}H-labelled acetyl coenzyme A (4.2 Ci/mmol; 50 μCi/ml) (Amersham), and distilled H_2O was added, and the mixture was overlaid with 4 ml of Econofluor (Du Pont) and incubated at 37°C for 90 min. Radioactivity was measured with a Beckman LS6000LE scintillation counter. The β-galactosidase assays were performed by mixing 30 μl of the cell extracts with 470 μl of buffer containing 0.1 M Na_2PO_4, 10 mM KCl, 1 mM $MgSO_4$, and 100 μl of 2 mg of *o*-nitrophenyl-β-D-galactopyranoside (Sigma) per ml, and incubating the mixtures at 37°C. The reaction was stopped with 500 μl of 1 M Na_2CO_3, and the optical density at 420 nm was read. As illustrated in Fig. 3, the results confirmed that the presence of human chromosome 12 conferred Tat permissiveness to the hybrids. Tat-mediated transactivation was only 4-fold in the parental A9 cells, whereas it was more than 20-fold in 915 cells and 30-fold in COS cells. We therefore infected the A9 and 915 cells with HIV(MLV) pseudotypes, using the HIV-1-derived replication-defective (ΔE-dhfr) construct as described before. Methotrexate-resistant cells were then selected and analyzed for the production of HIV-1 p24 antigen by ELISA. The result showed that both cell lines produced exceedingly low levels of viral antigen (Table 1). Although the 915(ΔE-dhfr) cells secreted three times more p24 than the A9(ΔE-dhfr) cells (21 versus 7 pg/ml), this was still more than 2,000-fold less than the amounts found in the supernatant of the similarly transduced human cells HT4(ΔE-dhfr). A Northern blot analysis of viral cytoplasmic RNAs from these cells was most revealing (Fig. 4). A9(ΔE-dhfr) cells exhibited low levels of mostly fully spliced viral mRNA (Fig. 4, lane 1). Although the ΔE-dhfr provirus was expressed at significantly higher levels in 915(ΔE-dhfr) cells, the cytoplasm of the hybrid cells contained almost exclusively multiply spliced viral mRNAs, as in the parental mouse cells (Fig. 4; compare lanes 1 and 3). This indicated that although the presence of human chromosome 12 allowed Tat function, it did not rescue the Rev-defective phenotype in the mouse-human hybrid cell. We conclude from these experiments that the failure to express the HIV-1 structural genes in some murine cell lines is independent of their inability to support Tat transactivation. It is noteworthy that A9(ΔE-dhfr) cells showed levels of viral expression significantly lower than those observed in 915(ΔE-dhfr) or HT4(ΔE-dhfr) cells (Fig. 4; compare lane 1 with lanes 3 and 5). By contrast, the cytoplasm of BC10(ΔE-dhfr) cells contained amounts of fully spliced mRNAs that were in the range of that detected in similarly transduced human cells (Fig. 2; compare lanes 2 and 4 with lane 5). Previously, we had also observed that NIH 3T3 or CHO cells transduced with the ΔE-dhfr provirus and isolated by methotrexate selection expressed high levels of fully spliced viral mRNAs, in spite of their known inability to support Tat function (33). Because methotrexate resistance is conferred to cells transduced with ΔE-dhfr by translation of a 2-kb mRNA promoted in the HIV LTR, it is likely that the difference observed between the A9 cells on the one hand and the BC10, 3T3, and CHO cells on the other hand is due to a lower sensitivity of the A9 cells to the toxic effects of methotrexate. This allows A9 cells expressing only weakly from the viral LTR to survive the selection. In contrast, higher levels of dhfr production appear to be necessary to confer methotrexate resistance to BC10, 3T3, and CHO cells.

This work indicates that the block to HIV-1 structural-gene expression, previously observed in some rodent cell lines infected with HIV(MLV) pseudotypes, is independent of the amounts of Rev produced in these cells and is not secondary to their defect in Tat function. Since we previously demonstrated that the block could be rescued by fusion of infected mouse cells with uninfected human cells, this confirms that some mouse cell lines are defective in a factor which is present in human cells and essential for full HIV-1 expression. This cellular activity apparently differs from the recently described RRE-binding factor NF-RRE, whose expression was shown to be ubiquitous, at least in mammalian cells (35).

How can these data be reconciled with other results, which suggest that Rev does function in a variety of cells, including those of mouse origin? First, some rodent cells that are different from the ones described in this paper appear to support the expression of the HIV-1 structural genes from proviral constructs. For instance, high levels of the HIV-1 Gag and Env proteins were found in several tissues of transgenic mice containing the complete HIV-1-coding sequence fused to the mouse mammary tumor virus LTR (10). Also, one rat embryonal fibroblastoid cell line was shown to support the production of viral particles when it was transfected with an HIV-1 proviral construct (19). Therefore, the block to Rev function may be tissue rather than species specific. It is well-established that alternative splicing of a number of cellular genes is regulated by factors which are expressed in a tissue-specific manner (for a review, see reference 15). To our knowledge, no human cell line that cannot support Rev function has yet been identified. In addition, tissue-specific differences cannot account for all of the discrepancies between our results and those of other investigators, because in some cases the cell lines tested were identical (18, 33, 35). The answer to these discrepancies must reside in the experimental systems used in either analysis. The evidence presented here relies on the study of

HIV-1 structural-gene expression, in the context of a full-length provirus. By contrast, in similar cell types, Rev was found to function in the more artificial setting of heterologous expression systems, which contained only parts of the HIV-1 transcript (18, 35). The latter approach may have only partly reconstituted the requirements for Rev action. For instance, it has been shown that Rev function is dependent on an inefficient processing of RRE-containing RNAs by the cellular splicing machinery (3). The full-length HIV-1 transcript might be spliced at a higher efficiency in some mouse cells than in human cells, preventing the action of Rev. Systems based on HIV-1 subgenomic fragments may not reconstitute such differences, since multiple *cis*-acting sequences have been shown to play a role in splice site selection of cellular as well as retroviral pre-mRNAs (5, 7, 11, 13, 30). Interestingly, it was reported that splicing of the encoded Tat pre-mRNA from a subgenomic Tat expression vector appeared to be significantly more efficient in the murine ltk$^-$ cells than in COS cells (18). It is instructive to note that Rous sarcoma virus also demonstrates species-specific differences in the expression of its various mRNAs (2). There is a marked increase in the efficiency of splicing of the Rous sarcoma virus RNA in NIH 3T3 cells compared with that in chicken embryo fibroblasts, resulting in the accumulation of *src* mRNA in the murine cells. This phenomenon is thought to account for the lack of permissiveness of mouse cells for Rous sarcoma virus replication.

Alternatively, and not exclusively, the viral *cis*-acting repressor sequences, which have been shown to govern the Rev requirement of the HIV-1 incompletely spliced mRNAs (4, 6, 17, 27, 29), could act as more-potent nuclear retention signals in some murine cells than in primate cells. Such sequences, called CRS or INS, have been shown to cover large regions of the *gag* and *pol* and, to a lesser extent, *env* mRNAs (6, 27, 29). Importantly, all of these sequences were not included in those expression systems which suggested that Rev could function in nonprimate cells (9, 18, 35). Whether the Rev-defective phenotype observed in mouse cells is due to an accelerated splicing of the HIV-1 transcript or alternatively to a more-efficient nuclear retention of the CRS-containing mRNAs, it is interesting to note that this phenomenon is recessive in human-mouse somatic-cell hybrids (33). This indicates that human cells contain a *trans*-acting factor which either slows down the splicing or enhances the transport of the viral transcript.

In conclusion, these studies underscore the complexity of the viral and cellular elements involved in mediating the regulation of HIV-1 gene expression by Rev. Some rodent cells present a defect in one or several factors that are essential for the function of this viral protein in the context of the provirus, and these cellular components are distinct from the Tat cofactors also missing in such cells. In addition, these results emphasize that a number of virus and cell factors condition Rev function. Although in vitro approaches which aim at simplifying the study of these various factors are of tremendous value in understanding the mechanisms of Rev action, experiments done in the context of the full-length provirus might still at times better reflect the subtleties involved in the differential regulation of HIV-1 gene expression during viral replication.

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