The EMBO Journal vol.9 no.12 pp.4155-4160, 1990

# A human cell factor is essential for HIV-1 Rev action

**Didier Trono[1] and David Baltimore[2]**

Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142, and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

[1]Present address: Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA
[2]Present address: The Rockfeller University, 1230 York Avenue, New York, NY 10021, USA

Communicated by D.Baltimore

**To examine the restriction of HIV growth in murine cells, we infected NIH 3T3 cells with HIV pseudotyped by Moloney murine leukemia virus. The virus, which carried a dominant selectable marker under the control of the HIV LTR, gave large numbers of resistant clones, showing that murine cells are permissive for HIV uncoating, reverse transcription, nuclear transport and integration. However, we found that several murine cell lines, as well as CHO cells, could not support the function of *rev*, the viral regulatory gene which, in human cells, induces the cytoplasmic expression of the incompletely spliced class of HIV mRNAs that encode the viral structural proteins. Transfection of the HIV-infected murine cells with a HTLV-1 *rex*-expressing vector failed to rescue the *rev*$^-$ phenotype, indicating that the block extended to *rex* function. Most importantly, we could complement the *rev* defect by fusing the infected murine with uninfected human cells. We conclude that HIV tropism is partly a consequence of a *trans*-acting cellular factor critical for Rev function.**

*Key words:* human immunodeficiency virus/human T cell leukemia virus/Rev/Rex/tropism

## Introduction

Identifying mechanisms responsible for cell and species specific tropism of viruses may reveal cellular factors that are crucial for their replication. Murine cells are not a natural substrate for infection with human immunodeficiency virus (HIV) and present at least three limiting steps precluding a normal viral life cycle. First, they do not bind HIV, apparently because of species specific properties of human CD4, the HIV receptor (Landau *et al.*, 1988). Second, there must be another entry component because expression on murine cells of human CD4 allows HIV-1 binding but not infection (Maddon *et al.*, 1986). Third, murine cells transfected with the HIV-1 provirus produce virus particles much less efficiently than transfected human cells (Levy *et al.*, 1986), at least in part due to a decreased *trans*-activation of the HIV-1 long terminal repeat (LTR) by the Tat protein (Hart *et al.*, 1989). As such, murine cells constitute an attractive substrate to help identify species specific human cell components that are essential for the various steps of the HIV life cycle.

With the long-term objective of identifying cellular factors for HIV growth, we attempted to characterize better HIV restriction in murine cells through the use of HIV pseudotypes. When two different enveloped viruses infect the same cell, the progeny can contain a mixture of the two envelope glycoproteins, resulting in pseudotyped viruses with altered tropism (for a review, see Zavada, 1982). Taking advantage of this phenomenon, we established stable human cell lines which produce HIV pseudotypes by virtue of the expression of an envelope-defective HIV-1 provirus carrying a dominant selectable marker, and of a vector encoding the envelope glycoprotein of Moloney murine leukemia virus (MoMLV). Upon infection of murine NIH 3T3 cells with the supernatant from such cells, high numbers of resistant clones were obtained, showing that murine cells are permissive for the early steps of the HIV-1 life cycle, including uncoating, reverse transcription, nuclear transport and integration. However, the pattern of HIV specific gene expression in the murine cells indicate a severe block to the function of Rev, the HIV-1 *trans*-acting protein which, in human cells, induces the cytoplasmic expression of the incompletely spliced classes of RNA encoding the viral structural proteins (Feinberg *et al.*, 1986; Sodroski *et al.*, 1986; Malim *et al.*, 1988; Felber *et al.*, 1989; Hammarskjold *et al.*, 1989). The same phenotype was also observed in a mouse mammary epithelial cell line, in a mouse T cell line and in Chinese hamster ovary cells. Murine cells also did not support the action of Rex, the functional equivalent of Rev in the unrelated Human T Cell Leukemia Virus HTLV-1 (Inoue *et al.*, 1987; Hidaka *et al.*, 1988). Most importantly, the block to Rev function could be complemented by fusion of infected murine with uninfected human cells. We therefore conclude that the function of HIV-1 Rev, and apparently also of HTLV-1 Rex, is critically dependent on the presence of human *trans*-acting cellular factors, which are unavailable in murine cells.

## Results

### *Murine cells are permissive for the early steps of the HIV-1 life cycle*

The HT4(ΔE-dhfr) cell line [previously described as HT4(WT-ΔE-dhfr) in Trono *et al.*, 1989] is a HeLa cell derivative that constitutively expresses an Env$^-$ HIV-1 provirus in which the *Nef* reading frame has been replaced by the sequence encoding a mutated dihydrofolate reductase gene (*dhfr**) (Simonsen and Levinson, 1983; Feinberg,M.B. and Baltimore,D., in preparation). As a consequence, methotrexate resistance is conferred to cells by translation of a 2 kb mRNA promoted in the HIV LTR, which is Rev independent in its formation because it is terminally spliced. To create HIV(MoMLV) pseudotypes, we transfected

(HT4(ΔE-dhfr) cells with pCRIP-gag$^-$2, a construct encoding the Moloney murine leukemia virus envelope (Danos and Mulligan, 1988), using pSV2-His cotransfection to provide a selection for transfected cells (Hartman and Mulligan, 1988). Histidinol resistant cell clones were isolated and designated HT4(MoE-ΔE-dhfr). The supernatant of HT4(MoE-ΔE-dhfr) was used to infect both NIH 3T3 cells and, as a control, EJ-A1 cells, a human bladder carcinoma cell line that expresses the murine ecotropic retroviral receptor (Albritton *et al.*, 1989); cells were then selected in 0.2 μM methotrexate. HT4(MoE-ΔE-dhfr) supernatant was found to induce $\sim 5 \times 10^2$ methotrexate-resistant 3T3(ΔE-dhfr) colonies per ml, ~40 times less than the number of methotrexate-resistant EJ-A1(ΔE-dhfr) colonies (Table I, line 1). It appears that once entry is bypassed through the use of HIV pseudotypes, NIH 3T3 cells are permissive for the early steps of the HIV-1 life cycle, i.e. disassembly, reverse transcription, nuclear transport, integration and expression of the proviral DNA. The lower number of colonies obtained in murine cells compared with human cells following infection with HIV(MoMLV) pseudotypes might reflect a lower average level of HIV-driven gene expression, precluding the isolation of cells in which retroviral integration is not optimal, and therefore dhfr production not sufficient.

Surprisingly, we found that the supernatants of HT4(ΔE-dhfr) and HT4(WT-dhfr), a cell line expressing an Env$^+$ version of the same provirus (Trono *et al.*, 1989), were also infectious in EJ-A1 cells and to a lesser extent in 3T3 cells (Table I, lines 2 and 3). However, only HT4(WT-dhfr) supernatant induced a cytopathic effect in CEM cells, whereas only HT4(ΔE-dhfr) supernatant could infect HT4-6C cells, the CD4$^+$ HeLa cell line from which all the HT4(dhfr) lines were made (Chesebro and Wehrly, 1988). Also, we found that preincubation of both supernatants with an antiserum reacting with the murine amphotropic retroviral envelope glycoprotein dramatically reduced their ability to infect EJ-A1 cells (not shown). These results demonstrate that HT4-6C, in which CD4 was introduced by means of a retroviral vector, is heavily contaminated with a murine amphotropic retrovirus, as recently revealed (Chesebro, 1989). As such, upon infection with HIV, HT4-6C produces HIV pseudotypes whose very broad tropism represents a significant biohazard, strongly limiting its use as a diagnostic tool.

### *Murine cells do not support Rev function*

To study further the HIV-1 life cycle in murine cells, methotrexate-resistant cells were grown and analyzed both as clones and as populations, with similar results. We first measured the amount of HIV specific p24 antigen in the supernatant of those cells. 3T3(ΔE-dhfr) released an average 0.1 ng/ml of p24, whereas values in the supernatant of EJ-A1(ΔE-dhfr) averaged 20 ng/ml. The same 1:200 ratio between murine and human cells was observed when p24 was measured in the cytoplasm, indicating that the defect in 3T3 was not at the level of virus assembly or release. Still, methotrexate concentration could be increased to >50 μM without affecting 3T3(ΔE-dhfr) growth, suggesting that HIV-driven gene expression was significant in these cells. To explain this apparent paradox, we examined whether murine cells might be unable to support Rev function, and present a specific defect in the cytoplasmic

**Table I.** Infection of murine and human cells with HIV pseudotypes

| Donor | Recipient | | | |
|---|---|---|---|---|
| | NIH 3T3[a] | EJ-A[a] | HT4-6C[a] | CEM[b] |
| HT4(MoE-ΔE-dhfr) | $5 \times 10^2$ | $2 \times 10^4$ | ND[c] | ND[c] |
| HT4(ΔE-dhfr) | 50 | $2 \times 10^3$ | 0 | − |
| HT4(WT-dhfr) | 3 | $4 \times 10^2$ | $5 \times 10^2$ | +++ |

[a]Adherent recipient cells were incubated for 24 h with the filtered supernatant of donor cells, trypsinized and placed in methotrexate selection (0.2 μM for 3T3 and EJ-A1, 2 μM for HT4-6C); resistant colonies were later stained with crystal violet and counted.
[b]After infection, CEM cells were observed for the appearance of cytopathic effect (CPE) and followed by indirect immunofluorescence, using serum from an HIV-infected individual as detector antibody. As early as 3−4 days following incubation with HT4(WT-dhfr) supernatant, CEM showed major CPE and a large percentage of immunofluorescence-positive cells; by contrast, both phenomena were completely absent at >4 weeks following incubation of CEM with HT4(ΔE-dhfr) supernatant.
[c]ND, not determined.

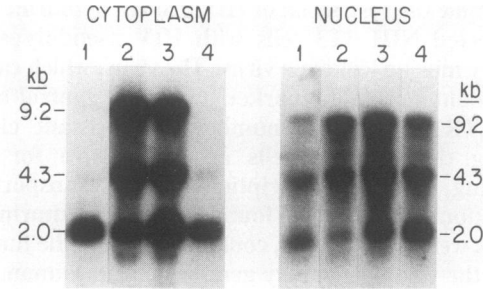


**Fig. 1.** Northern blot analysis of (HIV-dhfr) cells RNA, showing that 3T3(ΔE-dhfr) cells exhibit a Rev$^-$ phenotype. HT4(WT-dhfr), (ΔE-dhfr) and (V-dhfr) are HeLa cell derivatives that express an Env$^+$, Env$^-$ or Rev$^-$ provirus, respectively (Trono *et al.*, 1989). **Left panel**: cytoplasmic RNA; **right panel**: nuclear RNA. Lane 1, 3T3(ΔE-dhfr); 2, HT4(ΔE-dhfr); 3, HT4(WT-dhfr); 4, HT4(V-dhfr).

export of incompletely spliced HIV mRNAs, resulting in a low production of structural proteins like p24. This was confirmed by Northern blot analysis of HIV specific cytoplasmic and nuclear RNA, which showed that the pattern observed in 3T3(ΔE-dhfr) was identical to that observed in HT4(V-dhfr), a HeLa cell derivative stably transfected with a Rev$^-$ provirus (Trono *et al.*, 1989) (Figure 1 and Table II). The cytoplasm of 3T3(ΔE-dhfr) contained mostly 2 kb RNA species, at levels comparable with those seen in human cells; by contrast, in the nucleus, the larger RNA species represented a fraction of the total HIV specific signal that was much closer to that observed in human cell lines expressing a Rev$^+$ provirus, and identical to that seen in HT4(V-dhfr). Based on this result, we conclude that 3T3 cells are unable to support Rev function.

We then attempted to determine whether the block to Rev function extended to non-3T3 murine cells, and to other rodent cells. Using the same approach as before, stable cell lines expressing the HIV(ΔE-dhfr) provirus were established, and analyzed by Northern blot (Figure 2). A Rev$^-$ phenotype similar to the one in 3T3(ΔE-dhfr) was found in the mouse mammary epithelial cell line Comma-1D (lane 2), in the murine T cell line EL-4 (lane 3), as well as in a CHO cell derivative (lane 5). Some leakage in the Rev block was observed in the murine T cell hybridoma 5D5; however, even in this case, RNA of the fully spliced class was much

**Table II.** Relative representation of the three sizes of HIV RNA in (HIV-dhfr) cell lines

| RNA species | Cytoplasmic RNA (% of 2.0 kb) | | | | Nuclear RNA (% of 2.0 kb) | | | |
|---|---|---|---|---|---|---|---|---|
| | 3T3 (ΔE-dhfr) | HT4 (ΔE-dhfr) | HT4 (WT-dhfr) | HT4 (V-dhfr) | 3T3 (ΔE-dhfr) | HT4 (ΔE-dhfr) | HT4 (WT-dhfr) | HT4 (V-dhfr) |
| 9.2 kb | 4 | 100 | 40 | 5 | 53 | 254 | 152 | 52 |
| 4.3 kb | 9 | 148 | 60 | 12 | 107 | 151 | 199 | 96 |
| 2.0 kb | (100) | (100) | (100) | (10) | (100) | (100) | (100) | (100) |

Radioactivity from a Northern blot as shown on Figure 1 was measured using a Betascope 600 (Betagen). For each case, counts obtained from 4.3 and 9.2 kb RNAs are expressed as a percentage of those obtained from the 2.0 kb RNA, which is given the value of 100%.

more abundant than that of the incompletely spliced classes (lane 4). It appears therefore, that the block to Rev function extends to a variety of murine tissues, as well as to CHO cells.

### *The murine block to HIV-1 Rev function extends to HTLV-1 Rex*

The Rex protein is the functional homologue of HIV-1 Rev in HTLV-1, and induces the cytoplasmic export of HTLV-1 unspliced mRNAs encoding the viral structural proteins (Inoue *et al.*, 1987; Hidaka *et al.*, 1988). Rev and Rex are phosphoproteins localized primarily to the nucleolus of expressing cells, but share no marked nucleotide or amino acid homology, and computer-generated predictions of secondary structure reveal no significant similarity (Siomi *et al.*, 1988; Felber *et al.*, 1989; Cullen *et al.*, 1988). Nevertheless, it has been shown that the HTLV-1 Rex protein can functionally replace the Rev protein of HIV-1 (Rimsky *et al.*, 1988). This genetic complementation was found to be adequate for the rescue of a replication-defective Rev mutant of HIV-1. We therefore attempted to define whether HTLV-1 Rex could bypass the block to HIV-1 Rev function present in murine cells. For this, we compared the effect of transfecting 3T3(ΔE-dhfr) with plasmids expressing either a lethally mutated Rev gene (pSVRev*) or a wild type Rev gene (pSVRev) or the Rex gene (pcRex). As a preliminary control, each one of these constructs was first used to cotransfect COS cells with a Rev-defective HIV-1 provirus, pBamP3 (Feinberg *et al.*, 1986). In both cases, the result was assessed by measuring the production of viral p24 antigen in the cell supernatant (Figure 3). In accordance with the published data, we confirmed that Rex could complement the Rev-defective HIV-1 provirus in human cells. Even though pSVRev cotransfection was most effective, cotransfection of COS cells with pBamP3 and pcRex induced a >1500-fold increase in p24 production, compared with cells transfected with pBamP3 and the functionally silent pSVRev* (Figure 3, left). Results obtained in murine cells differed strikingly: neither pcRex nor pSVRev transfection had any stimulatory effect on p24 antigen production from 3T3(ΔE-dhfr) cells (Figure 3, right). This suggests that HTLV-1 Rex cannot induce the cytoplasmic export of HIV-1 RNAs in 3T3 cells.

To examine further whether the block to HTLV-1 Rex function in 3T3 cells was substrate specific, or extended to Rex's ability to act on HTLV-1 derived RNAs, we utilize a reporter system similar to that previously developed by Seiki *et al.* (1988). For this, two different constructs were made, expressing the chloramphenicol acetyltransferase (CAT) gene from the HTLV-1 5′ LTR (Figure 4). In the first, pSP73-HTLV1-CAT, signals required for accurate

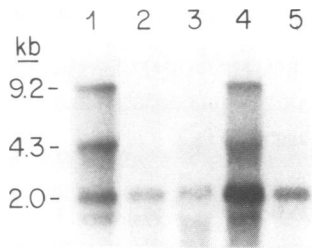


**Fig. 2.** Other murine cell types and Chinese hamster ovary cells also exhibit a Rev⁻ phenotype. Cytoplasmic RNA from stable cell lines expressing the modified HIV-1 provirus (ΔE-dhfr) was analyzed by Northern blot, as in Figure 1. Lane 1, HT4(ΔE-dhfr); 2, COMMA-1D(ΔE-dhfr); 3, EL-4(ΔE-dhfr);4, 5D5(ΔE-dhfr); 5, CHOHT4(ΔE-dhfr).

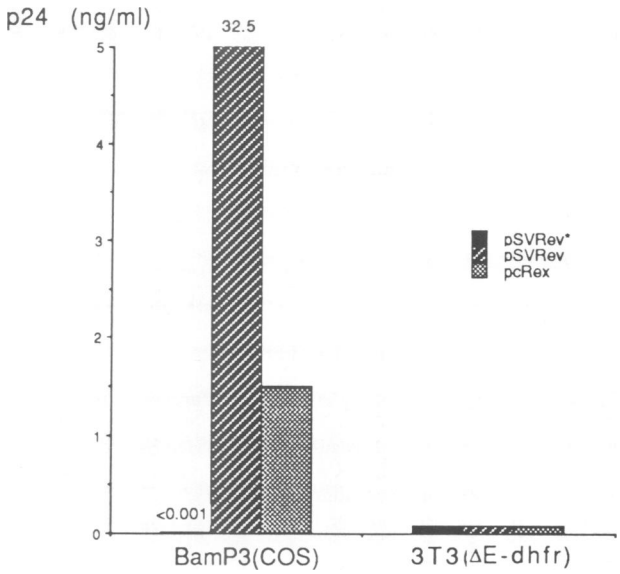


**Fig. 3.** Transfection of HIV-infected murine cells with a HTLV-1 Rex expression vector fails to complement the Rev⁻ phenotype. **Left**: cotransfection of COs cells with pBamP3, a Rev-defective HIV-1 provirus, and pSV-Rev*, pSVRev or pcRex. **Right**: transfection of 3T3(ΔE-dhfr) with the same plasmids. In all cases, pCMV-CAT was cotransfected, and results normalized for transfection efficiency.

mRNA 3′-end formation and polyadenylation were provided by a piece from the SV40 genome; in the second, pHTLV1-CAT-RXE, this sequence was replaced by a fragment from the HTLV-1 3′ LTR, containing the *cis*-acting elements which have been shown to be necessary for Rex regulation in human cells. We first cotransfected HeLa cells with pSP73-HTLV1-CAT or pHTLV1-CAT-RXE, in the presence or the absence of the Rex-expressing vector pcRex (Rimsky *et al.*, 1988); in all cases, the Tax-expressing vector pCMV-Tax was used to *trans*-activate the HTLV-1 LTR.

In accordance with published results (Seiki *et al.*, 1988), we observed a significant inhibitory effect from the HTLV-1 3′ LTR on CAT expression (Table III, line 1, compare A with C). Rex coexpression did not enhance CAT production from pSP73-HTLV1-CAT, whereas it strongly stimulated that from pHTLV1-CAT-RXE (line 1, compare A with B and C with D). By contrast, results obtained in 3T3 cells differed dramatically: pSP73-HTLV1-CAT and pHTLV1-CAT-RXE gave the same basal level of CAT activity, suggesting that the HTLV-1 3′ LTR had no negative effect in this case (line 2, compare A with C); moreover, pcRex cotransfection had no significant effect on CAT production from either vector (line 2, compare A with B and C with D). These data suggest that a factor or factors crucial to HTLV-1 as well as HIV-1 RNA processing in human cells are either absent or non-functional in murine NIH 3T3 cells.

### *Human cells contain a trans-acting Rev cofactor*

Two possibilities existed to explain the *rev*$^-$ phenotype observed in 3T3(ΔE-dhfr): it could result from the presence of a Rev inhibitor in murine cells or, alternatively, from the lack of a Rev cofactor, expressed in human cells. To address the latter possibility, we asked whether human cells could complement the Rev defect present in murine cells by providing a *trans*-acting Rev cofactor. To this end, we fused 3T3(ΔE-dhfr) with EJ-A1 cells, using polyethylene glycol (PEG). This resulted in a striking increase in p24 antigen production in the supernatant (Figure 5); the addition of PEG was crucial to obtain this increase, which could not be blocked by AZT. These properties show that the stimulation of p24 production is truly due to complementation of the defect present in 3T3 cells following fusion with human cells, and not to transfer of the HIV provirus from 3T3(ΔE-dhfr) to EJ-A1 by HIV pseudotypes.

We could also demonstrate complementation at the level of RNA, where fusion with human cells induced a dramatic increase in the cytoplasmic representation of incompletely spliced HIV RNA species in 3T3(ΔE-dhfr) (Figure 6 and Table IV). Finally, we took advantage of the constitutive expression of the neomycin resistance gene in EJ-A1 cells to select stable mouse−human hybrids, by placing the fused cells under dual methotrexate and G418 selection. These hybrids also exhibited a Rev$^+$ phenotype, at both cytoplasmic and nuclear levels (Figure 6 and Table IV).

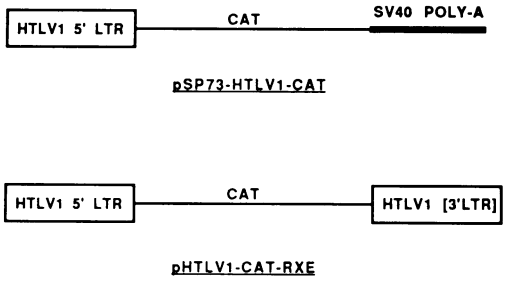


**Fig. 4.** Constructs used to assay HTLV-1 Rex function in murine cells. In pSP73-HTLV1-CAT, signals required for accurate mRNA 3′-end formation and polyadenylation were provided by a piece from the SV40 genome. In pHTLV1-CAT-RXE, this sequence was replaced by a *Hin*cII−*Eco*RI fragment from the HTLV-1 3′ LTR, containing the Rex response element and the RNA termination signals.

**Table III.** Rex-responsiveness of a HTLV-1 derived RNA in human and murine cells

| | Plasmids used | Relative amount of CAT activity in transfected cells | |
|---|---|---|---|
| | | HeLa | NIH 3T3 |
| A | pSP73-HTLV1-CAT + pCPLK | 1 | 1 |
| B | PSP73-HTLV1-CAT + pcRex | 0.6 | 1 |
| C | pHTLV1-CAT-RXE + pCPLK | 0.03 | 1.1 |
| D | pHTLV1-CAT-RXE + pcRex | 0.18 | 1.2 |

Cells were cotransfected with the plasmids indicated, plus pCMV-Tax in all cases. Cytoplasmic extracts were assayed for CAT activity 40 h later. Two separate experiments gave similar results. The activity obtained with pSP73-HTLV1-CAT + pCPLK (A) was given the value of 1 in each cell type.

## Discussion

We used HIV(MoMLV) pseudotypes carrying a dominant selectable marker under the control of the HIV LTR to create stable murine cell lines which constitutively expressed a modified HIV-1 provirus. Thus, murine cells are permissive for HIV-1 uncoating, reverse transcription, nuclear transport, integration and expression. This confirms the results described recently by Spector *et al.*, who showed that HIV (murine amphotropic retrovirus) pseudotypes could infect several murine cell lines, resulting in the production of low amounts of p24 antigen (Spector *et al.*, 1990), and by Lusso *et al.*, who demonstrated the production of HIV (murine endogenous retrovirus) pseudotypes from HIV-infected human cells that had been formerly passaged in mice (Lusso *et al.*, 1990). However, we were able to characterize better HIV-1 expression in murine cells. We thus found that the pattern of HIV specific gene expression in murine cells was indicative of a major block to Rev function, because fully spliced viral mRNA was the predominant species observed in the cytoplasm of infected cells, whereas all three major classes of RNA were represented in their nucleus. HTLV-1 Rex, which has been shown in human cells to induce the cytoplasmic export of HIV-1 as well as HTLV-1 mRNAs, could not complement the Rev-negative phenotype in 3T3 cells. Moreover, we found, using a heterologous reporter gene system, that the Rex requirement of a HTLV-1 derived mRNA was abolished in 3T3 cells. This suggests that one or several elements also essential for HTLV-1 RNA processing are either absent or non-functional in these cells. Finally, and most importantly, the Rev defect could be complemented by fusion of the infected murine with uninfected human cells. These results imply that Rev function, and therefore HIV replication, is critically dependent on the presence of a human *trans*-acting Rev cofactor (we call it RCF), which is absent or non-functional in murine cells.

We have recently suggested that a threshold amount of the Rev protein is necessary for its function (Pomerantz *et al.*, 1990). Our data shows, however, that a quantitative defect in transcription from the HIV LTR cannot account for the Rev$^-$ phenotype observed in rodent cells. Levels of HIV expression in the various cell lines were assessed on multiple occasions, by Northern blot analysis of RNA purified from numerous independently isolated clones or cell populations, expressing several different HIV derived proviruses, and obtained either by transfection or by infection

with HIV pseudotypes (not shown). In all cases, the total amount of RNA analyzed was carefully measured. We thus found that the level of HIV expression in the murine cells, as assessed by the amount of 2 kb RNA present, was not significantly different from that in human cells. This is perhaps best illustrated by our finding that 3T3(ΔE-dhfr) cells exhibited levels of 2 kb mRNA which were actually slightly higher than those present in the Rev$^-$ human cell line HT4(V-dhfr) (Figure 1, left, compare lanes 1 and 4). In addition, the most permissive of the rodent cell lines tested, the mouse hybridoma 5D5, had amounts of fully spliced mRNA that were significantly higher than those present in human cells, but still showed comparatively much lower quantities of the incompletely spliced species (Figure 2, compare lanes 1 and 4). Therefore, we conclude that the Rev defect observed in murine cells is not secondary to lower levels of HIV-driven transcription in this species.

If the lack of a single component is responsible for both Rev and Rex defect in murine cells, then this cellular factor must be key to a pathway common to both proteins. The precise function of RCF cannot be defined at this point, because of our ignorance of the mechanisms involved in Rev action. Because of their defect, murine cells may be of tremendous value in helping to understand the role of RCF and permitting its isolation.

The Rev defect revealed here indicates that a low level of HIV LTR-driven expression is not the sole explanation for the poor virus production noted after transfection of murine cells with the HIV-1 provirus, or after infection of such cells with HIV pseudotypes. This result is of primary importance for the interpretation of experiments studying HIV-1 replication or HIV-associated disease in mice models, like transgenics.

It appears finally that murine cells are defective in at least four functions essential to the HIV life cycle, two critical for viral entry and two involved in viral gene expression. Both the level of RNA synthesis, as determined by Tat, and the pattern of RNA produced, as determined by Rev, involve host cell components absent in murine cells. The narrow tropism of HIV relies therefore on a number of specific capabilities of primate cells. This is in sharp contrast with other species specific viruses, as for instance poliovirus or MLV, whose tropism is limited only by one factor, their cell surface receptor. It could be that HIV, in contrast to other viruses, emerged from an evolutionary process which for a very long time has been restricted to its current host species or close relatives.

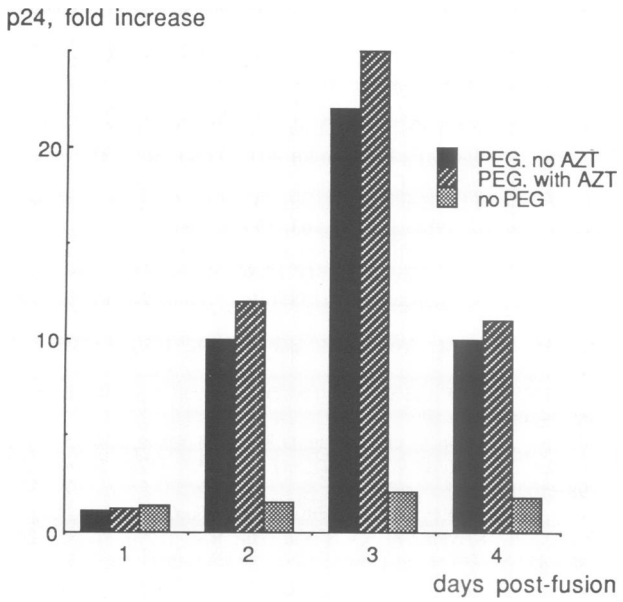


**Fig. 5.** Fusion with human cells dramatically increases the production of p24 antigen from 3T3(ΔE-dhfr) cells. HIV-1-infected murine and uninfected human cells were incubated in the presence or the absence of 1 μM AZT, and fused with polyethylene glycol, or mock-treated. p24 activity was monitored in the cell supernatant. The effect of AZT on cells not treated with PEG was negligible (not shown).

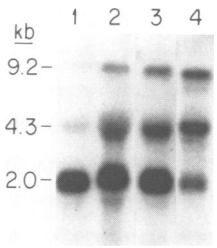


**Fig. 6.** A *trans*-acting factor present in human cells rescues the Rev$^-$ phenotype characteristic of 3T3(ΔE-dhfr) cells. Northern blot analysis of cytoplasmic RNA was performed as described in Figure 1, on AZT/PEG-treated 3T3(ΔE-dhfr) (lane 1) and 3T3(ΔE-dhfr) + EJ-A1 (lane 2) cells, 48 h after fusion (see Figure 3). Cytoplasmic (lane 3) and nuclear (lane 4) RNA was also analyzed from stable mouse–human hybrids doubly selected in methotrexate (0.2 μM) and G418 (400 μg/ml).

## Materials and methods

### *Cell cultures and infections*

HT4 cell derivatives were grown in DMEM supplemented with 10% fetal calf serum (FCS), penicillin and streptomycin. Calf serum was substituted for FCS for the maintenance of EJ-A1 and NIH-3T3 cells. 5D5 (a gift from M.Sy) is a T cell hybridoma obtained by fusing BW5147 cells with lymph node cells from auto-immune *lpr/lpr* mice; 5D5 and EL-4 were grown in RPMI with 10% FCS, penicillin and streptomycin. Comma-1D cells are mouse mammary epithelial cells (Danielson *et al.*, 1984). They were maintained in DME containing 5% FCS, 5 μg/ml insulin, penicillin and streptomycin. The CHO cell line used in this study is a derivative which

**Table IV.** Fusion of murine with human cells: effect on HIV RNA pattern

| RNA species | Cytoplasm | | | Nucleus |
|---|---|---|---|---|
| | 3T3(ΔE-dhfr) | 3T3(ΔE-dhfr) + EJ-A1[a] | 3T3(ΔE-dhfr) + EJ-A1[b] | 3T3(ΔE-dhfr) + EJ-A1[b] |
| 9.2 kb | 7 | 33 | 43 | 93 |
| 4.3 kb | 12 | 48 | 50 | 162 |
| 2.0 kb | 100 | 100 | 100 | 100 |

Ratios were calculated from Northern blot in Figure 4, as indicated in Table II.
[a]RNA harvested 48 h after fusion of 3T3 cells with EJ-A1 cells using PEG.
[b]RNA from stable mouse–human hybrids doubly selected in methotrexate and G418.

can be infected with MLV (a gift from D.Patterson); it was maintained in DME:F12 containing 10% FCS, penicillin and streptomycin. For infection with HIV(Moloney) pseudotypes, recipient cells were either exposed to the filtered supernatant from producer cells in the presence of 8 μM polybrene (Sigma), or cocultivated with the producer cells for 24 h, incubated in fresh medium for another 24 h, and split in methotrexate selection (amethopterin, Sigma, 0.2 μM for 3T3, EJ-A1, Comma-1D, EL-4, CHO and 5D5, 2 μM for HT4). HT4(MoE-ΔE-dhfr) cells were selected in $His^-$ DMEM containing 1.25 mM histidinol (Sigma).

### *Plasmids and DNA transfections*

Plasmid pSVRev expresses the HIV-HXB2 Rev cDNA in a pSV2 background (Mulligan and Berg, 1980); pSVRev* contains a translational frameshift, similar to the one present in pBamP3, which abolishes Rev function (Feinberg *et al.*, 1986). HTLV-1 derived fragments used in this study are described in Feinberg, 1987. pCPLK carries a polylinker between the CMV immediate early promoter and the SV40 RNA termination/polyadenylation signal, in a pGEM7Zf(+) background. pCMV-CAT and pCMV-Tax were constructed by inserting the CAT gene and the HTLV-1 Tax cDNA, respectively, in this polylinker. pSP73-HTLV1-CAT was obtained by replacing, in pSV2-CAT, the SV40 promoter by the HTLV-1 5′ LTR. pHTLv1-CAT-RXE was made by further substituting the SV40 polyadenylation elements by a *Hinc*2−*Eco*RI fragment from the HTLV-1 3′ LTR. Transfection derived stable cell lines were established by a modified calcium phosphate technique (Chen and Okayama, 1988). DNA transfections for transient experiments were done using a DEAE−dextran procedure, with DMSO shock and chloroquine treatment (Ausubel *et al.*, 1987). In all cases, the total amount of DNA transfected was equalized. The amount of p24 antigen in the cell supernatant was measured using a sensitive ELISA assay system (DuPont-NEN). CAT assays were performed as described (Gorman *et al.*, 1982).

### *Northern blot analysis*

Following extraction of cytoplasmic RNA by the NP40 method (Trono *et al.*, 1988), RNA was obtained from the nuclear pellet using the hot phenol technique (Queen and Baltimore, 1983). Using conditions previously described (Trono *et al.*, 1988), equal amounts of RNA were denatured, electrophoresed through a 1.1% agarose/formaldehyde gel, transferred to nitrocellulose and hybridized at 60°C with a T7 RNA polymerase generated $^{32}P$ probe complementary to nucleotides 8475−8900 of the HIV-1 genome. Filters were washed three times in 0.2 × SSC at 68°C and exposed to X-ray film. The amount of radioactivity present in each lane was also counted using a Betascope 600 (Betagen), according to the manufacturer's instructions.

### *Cell fusion*

$3.5 \times 10^5$ 3T3(ΔE-dhfr) cells were plated on 35 mm dishes, either alone or with an equal number of EJ-A1 cells, in the presence or the absence of 1 μM AZT; when dually seeded dishes reached confluence, one set of each combination was treated with PEG 1500 (Boehringer Mannheim) as previously described (Cepko *et al.*, 1984), or mock-treated. Production of p24 antigen in the supernatant was measured daily using an ELISA assay system (DuPont-NEN). Values obtained from dually seeded dishes were divided by those obtained from the corresponding singly seeded dish, to calculate the fold increase in p24 antigen production resulting from fusion of murine with human cells. Two separate experiments gave results varying by <5%. Stable mouse−human hybrids were dually selected in methotrexate (0.2 μM) and G418 (400 μg/ml).

## Acknowledgements

We thank W.J.Nir for excellent technical assistance, M.Feinberg, M.Muesing and R.Pomerantz for helpful discussions, O.Danos and R.C.Mulligan for pCRIP-$gag^-$2, W.Greene for pcRex, M.Feinberg and M.Muesing for pSV-Rev, pSVRev* and pSP73-HTLV1-CAT, J.Cunningham for EJ-A1, M.Sy for 5D5, and D.Patterson for the CHO cell derivative. This work was supported by a grant from NIH.

## References

Albritton,L.M., Tseng,L., Scadden,D. and Cunningham,J. (1989) *Cell*, **57**, 659−666.

Ausubel,F., Brent,R., Kingston,R.E., Moore,D.D., Smith,J.A., Seidman, J.G. and Struhl,K. (1987) *Current Protocols in Molecular Biology*. John Wiley & Sons, New York.

Cepko,C.L., Roberts,B.E. and Mulligan,R.C. (1984) *Cell*, **37**, 1053−1062.

Chen,C. and Okayama,H. (1988) *Mol. Cell. Biol.*, **7**, 2745−2752.

Chesebro,B. (1989) *AIDS Research and Reference Reagent Program Courier*, **89-101**, 2−3.

Chesebro,B. and Wehrly,K. (1988) *J. Virol.*, **62**, 3779−3788.

Cullen,B.R., Hauber,J., Campbell,K., Sodroski,J.G., Haseltine,W. and Rosen,C.A. (1988) *J. Virol.*, **62**, 2498−2501.

Danielson,K.G., Oborn,C.J., Durban,E.M., Butel,J.S. and Medina,D. (1984) *Proc. Natl Acad. Sci. USA*, **81**, 3756−3760.

Danos,O. and Mulligan,R.C. (1988) *Proc. Natl Acad. Sci. USA*, **85**, 6460−6464.

Feinberg,M.B. (1987) *Molecular Biology of Human Pathogenic Retroviruses*. PhD Thesis, Stanford University.

Feinberg,M.B., Jarrett,R.F., Aldovini,A., Gallo,R.C. and Wong-Staal,F. (1986) *Cell*, **46**, 807−817.

Felber,B.K., Hadzopoulo-Cladaras,M., Caldaras,C., Copeland,T. and Pavlakis,G. (1989) *Proc. Natl Acad. Sci. USA*, **86**, 1495−1499.

Gorman,C., Moffat,L.F. and Howard,B.H. (1982) *Mol. Cell. Biol.*, **2**, 1044−1051.

Hammarskjold,M.L., Heimer,J., Hammarskjold,B., Sangwas,I., Albert,L. and Rekosh,D. (1989) *J. Virol.*, **63**, 1959−1966.

Hart,C.E., Ou,C., Galphin,J.C., Moore,J., Bacheler,L.T., Wasmuth,J.J., Petteway,S.R. and Schochetman,G. (1989) *Science*, **246**, 488−491.

Hartman,S.C. and Mulligan,R.C. (1988) *Proc. Natl Acad. Sci. USA*, **85**, 8047−8051.

Hidaka,M., Inoue,M., Yoshida,M. and Seiki,M. (1980) *EMBO J.*, **7**, 519−523.

Inoue,J., Yoshida,M. and Seiki,M. (1987) *Proc. Natl Acad. Sci. USA*, **84**, 3652−3657.

Landau,N.R., Warton,M. and Littman,D.R. (1988) *Nature*, **334**, 159−162.

Levy,J.A., Cheng-Mayer,C., Dina,D. and Luciw,P.A. (1986) *Science*, **232**, 998−1001.

Lusso,P., Veronese,F.D.M., Ensoli,B., Franchini,G., Jemma,C., De Rocco,S.E., Kalyanaraman,V.S. and Gallo,R.C. (1990) *Science*, **247**, 848−852.

Maddon,P.J., Dalgleish,A.G., McDougal,J.S., Clapham,P.R., Weiss,R.A. and Axel,R. (1986) *Cell*, **47**, 333−348.

Malim,M.H., Hauber,J., Fenrick,R. and Cullen,B.R. (1988) *Nature*, **335**, 181−183.

Mulligan,R.C. and Berg,P. (1980) *Science*, **209**, 1422−1427.

Pomerantz,R.J., Trono,D., Feinberg,M.B. and Baltimore,D. (1990) *Cell*, **61**, 1271−1276.

Queen,C. and Baltimore,D. (1983) *Cell*, **33**, 741−748.

Rimsky,L., Hauber,J., Dukovich,M., Malim,M.H., Langlois,A., Cullen,B.R. and Greene,W.C. (1988) *Nature*, **335**, 738−740.

Seiki,M., Inoue,J., Hidaka,M. and Yoshida,M. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 7124−7128.

Simonsen,C.C. and Levinson,A.D. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 2495−2499.

Siomi,H., Shida,H., Nam,S.H., Nosaka,T., Maki,M. and Hatanaka,M. (1988) *Cell*, **55**, 197−209.

Sodroski,J., Goh,W.C., Rosen,C., Dayton,A., Terwilliger,E. and Haseltine,W. (1986) *Nature*, **321**, 412−417.

Spector,D.H., Wade,E., Wright,D.A., Koval,V., Clark,C., Jaquish,D. and Spector,S.A. (1990) *J. Virol.*, **64**, 2298−2308.

Trono,D., Andino,R. and Baltimore,D. (1988) *J. Virol.*, **62**, 2291−2299.

Trono,D., Feinberg,M.B. and Baltimore,D. (1989) *Cell*, **59**, 113−120.

Zavada,J. (1982) *J. Gen. Virol.*, **63**, 15−24.

*Received on April 18, 1990; revised on August 20, 1990*