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Transactivation of HIV by Human Spumaretrovirus

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Abstract

To study the activation of HIV by human spumaretrovirus (HSRV) the long terminal repeats (LTRs) of HSRV, HIV1 and HIV2 were examined with respect to their ability to function as transcriptional promoters in virus infected and uninfected cells. Transient transfections using plasmids in which the LTRs of the three viruses were coupled to the bacterial chloramphenicol acetyltransferase (CAT) gene revealed (i) the level of cat gene expression directed by the HSRV LTR was markedly increased in HSRV infected cells compared to uninfected cells, (ii) cat gene expression driven by the HIV1 LTR, but not by the HIV2 LTR could be enhanced upon HSRV infection, whereas (iii) neither in HIV1 nor in HIV2 infected cells an effect on HSRV LTR driven cat gene expression was detected.

Introduction

It has been shown previously that the HIV LTR may be activated in trans by other viruses, and it has been suggested that this transactivation may be one factor leading to the progression from an asymptomatic status of infection to clinical AIDS disease (1). The HIV LTR may be transactivated by DNA viruses (2), hepatitis B virus (3), and HTLVI tax-gene product (4), and in this case the genomic region of the HIV LTR responsible for transactivation has been mapped, (NFKB-MOTIF) (5). Thus, the study of transactivation of HIV by other viruses may (i) be of some value in the understanding of progression to clinical disease and may (ii) lead to a better understanding of viral replication.

HSRV belongs to the foamy subgroup of retroviruses (6). Foamy viruses are ubiquitous in monkeys, bovines, and felines, all of which are known to harbor lentiviruses closely related to HIV (7). There is only little knowledge on human foamy virus infections, but epidemiological data suggest natural virus presence in East African populations (8), a region with relatively high incidence of HIV infection (9). The genome of HSRV has been molecular cloned and sequenced (10). Additional to gag, pol and env the HSRV genome bears sequences in the central and 3′ region of the genome that are believed to have regulatory functions similar to the other human retroviruses (10). Especially a homology between the deduced amino acid sequence of one of the 3′ open reading frames of HSRV and the HIV-2 tat protein suggests that HSRV encodes for a viral transactivator (11).

Materials and Methods

Cells and viruses

HSRV was grown on baby hamster kidney cells (BHK-21) as described (12). HIV1 and HIV2 were grown on H9 T cells as described (13). Cells were maintained in medium supplemented with 5% fetal calf serum, glutamine and antibiotics.

Plasmids

Parent plasmid for the LTR cat constructs was pSV2cat (14). Deletion of a 500 bp AccI/HindIII fragment containing the SV40 enhancer/promoter and insertion of BglII or HindIII-linkers led to plasmids p0cat-Bg and p0cat-H, respectively. A 1.5 kb BglII fragment from pHSRV-B52 (9) containing the 3′ LTR of HSRV was inserted in sense orientation into p0cat-Bg leading to pHSRVcat. pHIV1cat was derived by inserting a 750 bp HpaI/HindIII fragment from pHXB-2D (15) containing the 5′ LTR of HIV1

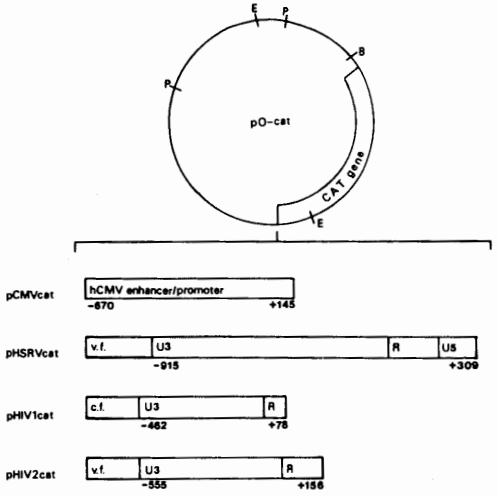

Fig. 1: LTR cat plasmids used for transfection experiments

into p0cat-H. pHIV2cat was a generous gift of Dr. L. Montagnier. Control plasmid pCMVcat was constructed by inserting a 815 bp HindIII fragment from pRR23 containing the human cytomegalovirus immediate early gene promoter/enhancer into p0cat-H. Plasmids are shown in Fig. 1.

Transfections and CAT assays

Uninfected and HSRV infected BHK-21 cells were transfected with 10 μg of plasmid DNA as described (14). Cells were harvested 48 hrs after transfection and processed as described (14). Cat assays were performed with equal amounts of protein, as determined using a commercial protein assay (Biorad), 0.1 μCi ^{14}C-chloramphenicol (Amersham) and 0.8 mM acetyl-CoA. Unacetylated chloramphenicol was separated from acetylated chloramphenicol by thin layer chromatography, and quantification was done by cutting out the spots from the chromatography plate and liquid scintillation counting.

RNA extraction and Northern blotting

RNA was extracted from pHSRVcat transfected cells by the guanidinum isothiocyanate method (16). Poly A^+ RNA was selected on oligo (dT) cellulose, RNA was run on formaldehyde containing agarose gels, and blotted onto nylon membranes (DuPont). Filters were hybridized with a cat specific probe as described (17) and exposed to X-ray film (DuPont).

Results

Demonstration of transactivation in HSRV infected cells

Uninfected and HSRV infected BHK-21 cells were transfected with pHSRVcat. RNA was extracted from transfected cultures and poly A^+ RNA was hybridized with a cat specific probe. As shown in Fig. 2 cat mRNA could only be observed in HSRV infected cells (lane b), but not in uninfected cells (lane a). The filter was rehybridized with a β-actin probe to demonstrate equal amounts of RNA in both lanes. The percentage of acetylated chloramphenicol from total input chloramphenicol was determined from CAT assays performed with lysates from cultures tansfected in parallel. The conversion rate was 64.6 % in HSRV infected cells vs. 0.4 % in uninfected cells.

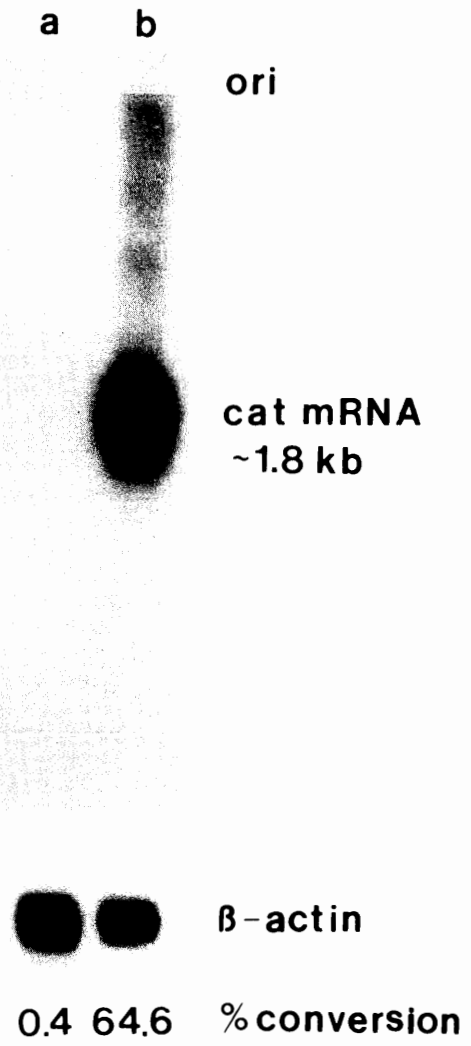

Fig. 2: Northern blot of cat mRNA in BHK-21 cells (a), and HSRV infected BHK-21 cells (b) transfected with pHSRVcat

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Plasmids pHSRVcat, pHIV1cat and pHIV2cat were transfected in HSRV infected and uninfected cells. Transfection with pSV2cat and pCMVcat seved as control. Table 1 shows a summary of three independent transfections and CAT assays. While transfection of control plasmids into uninfected cells results in expected levels of CAT activity, the LTRs are silent (HSRV) or almost silent (HIV1 and HIV2) in these

Table 1: Compilation of CAT assays

	BHK-21 % acetylation	rel. %	BHK-21/HSRV % acetylation	rel. %	H9/HIV1 % acetylation	H9/HIV2 % acetylation
pSV2cat	39.7	1.00	79.3	1.00	n.d.	n.d.
pCMVcat	40.9	1.03	95.6	1.20	n.d	n.d.
pHSRVcat	0.7	0.02	57.7	0.73	0.4	0.3
pHIV1cat	2.3	0.06	33.0	0.42	77.5	n.d.
pHIV2cat	2.2	0.06	6.8	0.09	n.d.	87.5

n.d. = not done

cells. In HSRV infected cells expression from the control plasmids is enhanced by a factor of about 2, while enhancement with pHSRVcat was found to be about 80 fold. Respective values for pHIV1cat were 14 fold enhancement, and for pHIV2cat were 3 fold enhancement. To normalize the CAT activity obtained from transfection with different plasmid all values were expressed in relation to the CAT activity obtained with pSV2cat. Following this calculation transactivation in HSRV infected cells was found to be 36.5 fold (with pHSRVcat), 7 fold (with pHIV1cat) and 1.5 fold (with pHIV2cat).
To test whether either HIV1 or HIV2 infection may lead to transactivation of the HSRV LTR, plasmids were transfected into HIV1 or HIV2 infected H9 cells. As shown in Table 1 transfection of HIV1cat and HIV2cat into HIV1 or HIV2 infected cells leads to the expected expression of CAT activity, while neither in HIV1 nor in HIV2 infected cells an effect on HSRV LTR driven cat gene expression could be detected.

Conclusion

Here we have presented evidence for a transactivator in HSRV infected cells able to enhance activity of the HSRV and HIV1 LTR, but not of the HIV2 LTR. While Northern blotting of cat mRNA from transfected cultures suggests that transactivation of the HSRV LTR mainly occurs at the transcriptional level, the mechanism of transactivation of the HIV1 LTR is still unclear. We found that transfection of control plasmids resulted in a higher level of cat activity in infectd cells than in uninfected cells. This effect may either be due to an easier transfectability of HSRV infected cells than in uninfected cells or may be the result of transactivation by an indirect mechanism as described for pSV2cat and HTLV I tax (18). Further experiments are necessary to answer this question. To study the mechanism by which HSRV transactivates its own and the HIV1 LTR we have constructed an infection molecular clone for HSRV (A. Rethwilm et al., manuscript in preparation) and are currently constructing deletion mutants of this clone to map the HSRV specific transactivator.

Acknowledgement

This study was supported by the Bundesministerium für Forschung und Technologie (BGA II-017-86).

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