Comparison of the Rev Transactivation of Feline Immunodeficiency Virus in Feline and Non-Feline Cell Lines

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ABSTRACT. The Rev protein of feline immunodeficiency virus (FIV) differentially transactivates the expression of viral structural proteins by allowing the accumulation of unspliced and singly spliced viral mRNA in cytoplasm via the Rev response element (RRE) at the end of env. To investigate the role of rev gene of FIV for the virus life cycle and cell tropism, we constructed the Rev expression plasmids, and functional activity of the Rev was assayed by using chloramphenicol acetyltransferase (CAT) assay system in feline and non-feline cell lines. Although the FIV Rev protein showed high transactivity to result in enhanced CAT production in a feline cell line, the productions of the CAT in non-feline cell lines were significantly lower than that in the feline cell line. These results indicate that specific cellular factor(s) present in feline cell line is required for the FIV Rev full-action and also suggest that the Rev action plays one of the important roles in determining the FIV cell tropism.—KEY WORDS: CAT assay, FIV, Rev transactivity.


The lentiviruses have been isolated from various species of primates, felids and ungulates [10]. The viruses have tropisms specific for cell type and species, which are caused by several limiting steps precluding the normal viral replication cycle. The first block of the virus infection exists in the step of the viral entry that is dependent on the presence of a number of specific factors in the target cells, such as human CD4 molecule which is bound by human immunodeficiency virus type 1 (HIV-1) envelope [7]. Another block of the viral replication exists in the step of viral gene expression. This step also crucially determines the virus tropism, because the virus promoter is activated by several transactivation factors including cellular factors [5].

Feline immunodeficiency virus (FIV) has a highly species-specific tropism and can not infect to other species in vivo. Recently, we have demonstrated that virion production from FIV provirus-transfected primate cell lines was much less efficient when compared with that from the provirus transfected feline cell line [9]. This phenomenon is at least in part due to a decreased activity of the FIV long terminal repeat (LTR), which is essential for the virus gene expression. Additionally, the FIV gene expression is regulated by a regulatory gene, rev, which is encoded within the FIV genome [6, 11]. The Rev acts via a cis-acting RNA sequence designated as Rev response element (RRE) located at the 3' end of the env gene, and is required for the expression of unspliced and singly spliced mRNAs that encode the viral structural proteins [6, 11].

Recently, it was reported that cellular factor(s) is required for HIV-1 Rev action [16-18], and HIV-1 RRE contributes to cell type specific viral tropism [3]. These findings suggested that interaction between the Rev protein and RRE is important not only for the viral replication but also determination of the virus tropism. However, to date, the role of the FIV Rev including in the virus tropism is not identified. In this study, as one approach for determining the role of rev gene for the cell tropism of FIV, we analyzed the rev gene activity in feline and non-feline cell lines.

To evaluate the rev gene activity of the FIV, we used chloramphenicol acetyltransferase (CAT) assay system [12]. Since the Rev protein promotes cytoplasmic expressions of genes which contain the RRE, plasmids carrying splice sites, CAT gene and RRE can produce CAT at high levels only when the Rev protein is present [6]. Thus, the plasmid, pRSpCAT-RREef [6] which contained RRE fragment of FIV TM2 strain [8] in downstream of the CAT gene was used for reporter plasmid in this study (Fig. 1B).

Because the FIV LTR showed a very weak promoter activity in non-feline cell lines [9], we used pRSVs vector [11] which contains the Rous sarcoma virus (RSV) LTR and the simian virus 40 (SV40) small t intron and polyadenylation signal for efficient expression of the FIV Rev in non-feline cell lines. Two Rev expression effector clones were constructed from two different cDNA clones of FIV TM2 strain which encode intact ORF-A and/or rev genes (Fig. 1A) by insertion of the necessary DNA fragment into the pRSvSV. Cloning and sequence of the cDNA clones were described previously [15]. Thus, plasmid pF-A/rev contains entire ORF-A [13] and rev coding sequences, and expresses both ORF-A and Rev proteins. This expression plasmid was constructed to evaluate whether the transcript which has bicistronic coding capacity can translate the Rev equally to the monocistronic transcript or not. A plasmid pF-rev contains only rev coding sequences (Fig. 1B). Another plasmid pF-revR contains the antisense orientation of the rev gene and used as negative control effector (Fig. 1B). Parallel transfection with the constitutive CAT expression vector, pRSvSV-CAT (previously referred to as pRSpCATARADA) [12], served as positive control for transfection efficiency.

First, to determine the ability of the two Rev expression plasmids to enhance CAT production, 2 μg of the pRSpCAT-RREef was cotransfected with 5 μg of either the pF-A/rev or the pF-rev in CRFK cells (feline kidney cells) [2] by calcium phosphate coprecipitation method [14]. The cells were grown in Dulbecco's modified Eagle's medium

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supplemented with 10% foetal calf serum. CAT activity in the cells was assayed two days after transfection by thin-layer chromatography method as described previously [12]. For positive control, 2 \( \mu \)g of pRVSV-CAT was also cotransfected with 5 \( \mu \)g of pRVSV. As shown in Fig. 2, both Rev expression plasmids showed high activity to result in enhanced CAT production in CRFK cells. The levels of the activation by these expression plasmids were almost the same as that by pRVSV-CAT. On the other hand, this enhancement was not observed by the negative effector, pF-revR. These results demonstrated that the FIV Rev expression plasmids derived from the two different cDNAs clones efficiently expressed the Rev protein and the expression of the ORF-A product did not

Fig. 1. Map of the FIV cDNAs and the expression plasmids used in this study. (A) Schematic representation of FIV genome is shown at the top. The splicing patterns of the two different cDNAs encoding rev gene are also shown. The location of the RRE at the end of env gene is shown by box. (B) Schematic representation of the expression plasmid (pRVSV), three effector plasmids and a reporter plasmid (pRSpCAT-RRE). t and A indicate the SV40 small t intron and polyadenylation signal, respectively. SD and SA indicate splice donor and splice acceptor sites, respectively.

Fig. 2. CAT activities directed by the Rev expression plasmids in CRFK cells. pRSpCAT-RRE (2 \( \mu \)g) was cotransfected with pF-A/rev, pF-rev, or pF-revR (5 \( \mu \)g), and CAT activity in the cell lysates (60-min reaction) 48 hr later was determined. CAT activity in the cells cotransfected with pRVSV-CAT (2 \( \mu \)g) and pRVSV (5 \( \mu \)g) was also shown for the positive control. A representative result from thin-layer chromatography, separating \([^{14}C]\)chloramphenicol (CM) from its acetylated forms (Ac-CM), is shown. The percent conversion of chloramphenicol to its acetylated forms is indicated on the below. A/rev, pF-A/rev; rev, pF-rev; revR, pF-revR; RVSV, pRVSV-CAT.

89.4  73.3  12.0  98.0
% conversion

Ac-CM
CM
Fig. 3. Relative CAT activities in feline and non-feline cell lines. The abilities of the pF-Rev, pF-Rev, and pF-RevR to activate pRSpCAT-RREf in the cell lines were shown as the CAT activity relative to that of pRVSv-CAT. Three independent experiments were performed, and the average are presented. Symbols of the bars are indicated on the right.

 affect the function of the Rev protein in CRFK cells. Next, to investigate whether the Rev protein is equally functional in non-feline cell lines including Vero (monkey kidney cells), COS (monkey kidney cells transformed by SV40) [4], HeLa (human epithelioid carcinoma cells), and SW480 (human colon carcinoma cells) [1] cells, the CAT assay was also carried out. The CAT productions in these cell lines were compared with that in CRFK cells, and the ability of the Rev to activate pRSpCAT-RREf in these cell lines was shown as the CAT activity relative to that of pRVSv-CAT. As shown in Fig. 3, although the pRVSv-CAT efficiently expressed the CAT production in non-feline cell lines, the levels of CAT activities by the Rev expression plasmids in these cell lines were significantly low when compared with that in CRFK cells. Especially, the relative CAT activities in HeLa and SW480 cells were 6- to 30-fold less than that in CRFK cells. This experiment was repeated at least three times, and we always obtained the low level of CAT activities in the non-feline cell lines.

The most important purpose of this work is to investigate whether the FIV Rev can act in non-feline cell line or not. The results of the experiment in Fig. 3 clearly demonstrated that functional abilities of the FIV Rev protein in the non-feline cell lines were much less than that in feline cell line. From this cell type specific ability of the Rev protein, it was suggested that the specific cellular factor(s) is required for the Rev full-action, as observed in a HIV-1 Rev [16-18]. From this point of view, it is likely that rev gene also plays one of the key roles for determination of the tropism of the virus. Therefore, identification of such cellular factor(s) which interacts with the FIV Rev protein is important to understand the mechanisms of the viral replication and tropism.

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