Trans-activation of Long Terminal Repeat Sequence-mediated Gene Expression Is Not a Property of Type D Retrovirus Replication

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SUMMARY

Several retroviruses encode trans-acting factors which activate gene expression directed by long terminal repeat (LTR) sequences and play a role in the positive feedback regulation of virus replication. We have examined two Mason-Pfizer monkey virus (MPMV) strains for their ability to produce and respond to such factors. Plasmids with the LTR of either MPMV or type D retrovirus/New England (D/NE) were fused to the bacterial chloramphenicol acetyltransferase (CAT) gene. Introduction of these plasmids into several different human cell lines gave rise to significant CAT activity, demonstrating the strong transcriptional promoter activity of these LTRs. However, little or no increase in CAT activity was found upon transfection of these plasmids into MPMV- or D/NE-infected cell lines as compared with uninfected cell lines. Furthermore, CAT activity was not enhanced in uninfected cells by cotransfecting either a functional MPMV DNA clone, a plasmid expressing the human T-lymphotropic retrovirus trans-activator genes, tat-1 or tat-3. These data show that the property of trans-activation of LTR-mediated gene expression is a function in the replication of only certain retroviruses.

Mechanisms which regulate transcription of eukaryotic genes include both cis- and trans-acting elements (Dynan & Tjian, 1985; McKnight & Tjian, 1986). Trans-acting transcriptional factors have been described that are active for genes derived from cells, DNA viruses and RNA viruses including retroviruses. A potent trans-acting transcriptional factor (tat) has been described for both type I and II human T-lymphotropic viruses (HTLV) (Sodroski et al., 1984; Cann et al., 1985). In these cases, tat increases long terminal repeat (LTR)-directed transcription of heterologous genes about 100-fold. The tat-1 and tat-2 factors are encoded by double-spliced mRNAs which include an open reading frame in the 3' portion of the viral genome (Cann et al., 1985; Sodroski et al., 1985c; Seiki et al., 1986). Unlike tat-1 and tat-2, the major effect of tat-3 appears to be at a post-transcriptional level (Cullen, 1986; Okamoto & Wong-Staal, 1986; Wright et al., 1986). However, positive feedback regulation of virus replication appears to result in all cases (Chen et al., 1985; Dayton et al., 1986; Fisher et al., 1986).
Fig. 1. MPMV and D/NE LTR CAT plasmids. SP65-CAT3 was constructed by digesting pSVO-CAT (Gorman et al., 1982b) with BamHI, treating with Klenow fragment of *Escherichia coli* DNA polymerase I, digesting with HindIII, and cloning the 1-7 kb fragment between the *PvuII* and HindIII sites of SP65 (Promega). Clone MPMV-ΔHin includes a full proviral copy of the MPMV genome with flanking cellular sequences constructed by blunt-ending the *EcoRI* insert with the Klenow fragment of *E. coli* DNA polymerase and cloning into the similar blunt-ended *EcoRI* and *PvuII* sites of pBR322 (Barker et al., 1986). MPMV-ΔHin was grown in *dam*- *dem*-- bacteria, and digested with *EcoRI* and *NarI* and the 1-8 kb fragment was cloned into *EcoRI* and *AccI*-digested SP65 (Promega), yielding clone MPMV-LTR. MPMV-LTR was digested with *PstI* and the 1-5 kb fragment with LTR sequences cloned into the *PstI* site of SP65-CAT3 in the correct or incorrect transcriptional orientation relative to the CAT gene in plasmids MPMV-CAT3 and MPMV-CAT4, respectively. Clone D398 includes a permuted copy of a DNA intermediate form of D/NE cloned at the *SstI* site of pAT153 (Desrosiers et al., 1985). It was digested with *PvuII* and *NarI* and the 3-0 kb fragment including LTR sequences cloned into *SmaI* and *AccI*-digested SP65 generating clone NE-LTR. NE-LTR was digested with HindIII and the 1-1 kb fragment cloned into the HindIII site of pSVO-CAT (Gorman et al., 1982b), in the same or opposite transcriptional orientation relative to the CAT gene in clones NE-CAT1 and NE-CAT2, respectively. Arrows within boxes indicate LTRs and their transcriptional orientation. Abbreviations: A, *AccI*; B, *BamHI*; H, HindIII; E, *EcoRI*; N, *NarI*; P, *PstI*; S, *SacI*; Sm, *SmaI*; V, *PvuII*.

It is not yet clear to what extent other retroviruses may use trans-activation to regulate their expression. Several characteristics of type D retroviruses justify investigation of possible trans-activation in cells infected by this sub-family of retroviruses. Recent sequencing data have revealed potential extra open reading frames in macaque type D retrovirus genomes (Power et al., 1986; Sonigo et al., 1986). Also, a magnesium preference of the reverse transcriptase is shown by all retroviruses that have been found thus far to trans-activate, and the type D retroviruses also have a magnesium-preferring reverse transcriptase (Rho et al., 1981; Rey et al., 1984; Stromberg et al., 1984). Furthermore, type D retroviruses are similar to some of the known trans-activating retroviruses in their ability to induce a chronic immunodeficiency syndrome (Marx et al., 1986). We therefore examined whether trans-activating factors could be detected in cells infected by type D retroviruses.

Plasmids were constructed utilizing the entire Mason-Pfizer monkey virus (MPMV) or type D retrovirus/New England (D/NE) LTR fused to the chloramphenicol acetyltransferase (CAT) gene in the correct (MPMV-CAT3 and NE-CAT1) or incorrect (MPMV-CAT4 and NE-CAT2) transcriptional orientation (Fig. 1). These plasmids include the entire LTR sequences as well as 112 of 251 nucleotides and 113 of 259 nucleotides transcribed into the 5' untranslated region of the *gag* mRNAs of MPMV and D/NE, respectively (Power et al., 1986; Sonigo et al., 1986). These plasmids were then used to measure possible trans-activating factors in MPMV- or D/NE-infected or transfected cells.

In uninfected Raji cells, no CAT activity was seen in cultures transfected with SP65-CAT3 which lacks a transcriptional promoter (Table 1). Significant levels of activity were seen,
Table 1. Effect of plasmids on the relative CAT activity of various cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Ratio CAT activity in infected compared to uninfected cell lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmid</td>
<td>Raji</td>
</tr>
<tr>
<td>pSV2-CAT</td>
<td>1.00</td>
</tr>
<tr>
<td>RSV-CAT</td>
<td>1.66</td>
</tr>
<tr>
<td>NE-CAT1</td>
<td>0.87</td>
</tr>
<tr>
<td>NE-CAT2</td>
<td>0.14</td>
</tr>
<tr>
<td>MPMV-CAT3</td>
<td>2.20</td>
</tr>
<tr>
<td>MPMV-CAT4</td>
<td>0.07</td>
</tr>
<tr>
<td>SP65-CAT3</td>
<td>0.08</td>
</tr>
</tbody>
</table>

*In each experiment, 5 μg of the indicated plasmid was transfected by the DEAE-dextran method (Queen & Baltimore, 1983) for the Raji cell lines, or by the calcium phosphate precipitation method (Van der Eb & Graham, 1980) for the HeLa and SV80 cell lines. Cells transfected with calcium phosphate were shocked with glycerol 24 h after addition of DNA. Transfected cells were harvested 48 h after transfection. Lysates were prepared and CAT assays performed as described previously (Gorman et al., 1982b) but with 2.4 mM-acetyl coenzyme A, 0.05 μCi [14C]chloramphenicol, and 20 μl of cell extract per assay. CAT assays were performed for 16 h with the lysates from Raji cells, and for 2 to 60 min with lysates from HeLa and SV80 cells. All experiments were performed three times and representative data are shown. The standard error for simultaneous transfections and CAT assays performed in triplicate was 6%.† ND, Not determined.

However, with plasmids pSV2-CAT or RSV-CAT, which have the CAT gene fused to the simian virus (SV)40 early enhancer and promoter or the Rous sarcoma virus LTR, respectively (Gorman et al., 1982a,b). A similar level of activity was observed in cultures transfected with MPMV-CAT3 and NE-CAT1, but no CAT activity was detected after transfection of MPMV-CAT4 or NE-CAT2. D/NE-infected Raji cells transfected with NE-CAT1 showed 2.23-fold more CAT activity than did the uninfected Raji cells. In contrast, the ratio of CAT activity directed by MPMV-CAT3 in infected compared to uninfected Raji cells was 0.57. In multiple repetitions of this experiment, less than three-fold differences in CAT activity were observed with each of the plasmids transfected into D/NE-infected compared to uninfected Raji cells.

Similar results were obtained with these plasmids after transfection into uninfected or MPMV-infected HeLa or SV80 cells (Table 1). In HeLa cells, CAT activity was two-to-threefold higher in the infected cells, whereas in SV80 cells a five- to sixfold decrease in CAT activity was noted. Similar alterations in CAT activity as a result of MPMV infection were noted in cells transfected with RSV-CAT.

Enhancement of LTR-mediated gene expression was also examined in uninfected cells cotransfected with a tat-1-expressing plasmid, a functional HIV proviral clone expressing tat-3, or a functional MPMV proviral clone (Table 2). Cotransfection of the tat-1 plasmid with NE-CAT1 or MPMV-CAT3 produced little or no increase in CAT activity. Significantly greater increases (three- to 11-fold) of HTLV-I-CAT-directed activity were noted after cotransfection of the tat-1 plasmid. In HTLV-I-infected cells, levels of NE-CAT1- or MPMV-CAT3-directed CAT activity were comparable to those in the SV80 cell line. In contrast, the HTLV-I-CAT-mediated activity was 30- to 300-fold greater in the HTLV-I-infected cell line compared to the SV80 cell lines (Table 2) or the uninfected lymphoid cell line HUT-78 (data not shown).

In HeLa cells, cotransfection of a functional MPMV proviral DNA clone caused a moderate suppression (2.5- to 4.5-fold) of activity directed by NE-CAT1 or MPMV-CAT3. This is similar to the suppression of activity resulting from cotransfection of the MPMV proviral clone with RSV-CAT, HTLV-I-CAT or the HIV LTR CAT plasmid (C15-CAT). Cotransfection with a HIV proviral DNA clone caused a 2.5-fold increase in activity with NE-CAT1, but a 1.3-fold decrease in activity directed by MPMV-CAT3. This is to be compared with the 40-fold increase...
Table 2. Effect of a functional HIV or MPMV DNA clone or a tat-l-expressing DNA clone on MPMV and D/NE LTR-directed gene expression as determined by relative CAT activity

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Relative CAT activity</th>
<th>Uninfected SV80 cells</th>
<th>MPMV-infected SV80 cells</th>
<th>HTLV-l-infected MT2 cells</th>
<th>Uninfected HeLa cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>- + tat-1</td>
<td>- + tat-1</td>
<td>- + tat-1</td>
<td>- + MPMV-AHin</td>
</tr>
<tr>
<td>pSV2-CAT</td>
<td>1:00</td>
<td>1:00</td>
<td>1:00</td>
<td>1:00</td>
<td>1:00</td>
</tr>
<tr>
<td>RSV-CAT</td>
<td>1:48</td>
<td>1:80</td>
<td>0:51</td>
<td>1:84</td>
<td>1:54</td>
</tr>
<tr>
<td>NE-CAT1</td>
<td>5:12</td>
<td>2:70</td>
<td>0:88</td>
<td>1:10</td>
<td>4:37</td>
</tr>
<tr>
<td>NE-CAT2</td>
<td>0:10</td>
<td>0:08</td>
<td>0:05</td>
<td>0:05</td>
<td>ND†</td>
</tr>
<tr>
<td>MPMV-CAT3</td>
<td>6:34</td>
<td>6:48</td>
<td>1:27</td>
<td>5:03</td>
<td>7:00</td>
</tr>
<tr>
<td>MPMV-CAT4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>HTLV-l-CAT</td>
<td>0:09</td>
<td>0:32</td>
<td>0:03</td>
<td>0:33</td>
<td>9:51</td>
</tr>
<tr>
<td>C15-CAT</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SP65-CAT3</td>
<td>0:03</td>
<td>0:02</td>
<td>0:01</td>
<td>0:02</td>
<td>0:09</td>
</tr>
</tbody>
</table>

* Transfections and assays were performed as described in Table 1 except for those with MT2 cells which were transfected by the DEAE-dextran method. Five µg of each CAT plasmid was cotransfected with 5 µg of MPMV-AHin, the tat-l-expressing plasmid (Felber et al., 1985), the tat-3-expressing plasmid HXB2 (Seigel et al., 1986), pSV2neo (Southern & Berg, 1982) or SP65HP. SP65HP was derived from SP65 by deleting a 0.4 kb fragment between the HinII and PvuII sites. Plasmid C15-CAT includes the U3 and R regions of the HIV LTR fused to CAT (Sodroski et al., 1985b). All experiments were performed three times and representative data are shown here.

† ND, not determined.

in activity directed by the HIV LTR CAT plasmid, when cotransfected with the tat-3-expressing plasmid.

These data demonstrated that the transcriptional activity of both the MPMV LTR and D/NE LTR are comparable to those of the SV40 early region and RSV LTR in several human cell lines. Although MPMV was isolated from a rhesus monkey with a breast carcinoma (Chopra & Mason, 1970), the activity of the MPMV LTR differs from that of the mouse mammary tumour virus LTR (Majors & Varmus, 1983), in that it is not inducible by glucocorticoids (data not shown).

MPMV and D/NE do not produce significant levels of trans-activation of LTR-mediated gene expression in each of the three cell lines examined, which include epithelial, fibroblastic and lymphoid cell types. Despite the diversity of cell types examined, we cannot exclude the possibility that trans-activation may occur in some other cells. Furthermore, the MPMV and D/NE LTRs do not respond to trans-activators encoded by the HTLV-1 or HIV genomes. It should be recognized, however, that our plasmid constructs included only viral LTR sequences and the possibility that sequences elsewhere in the viral genome mediate trans-activation cannot be excluded by these data. Except for these caveats, we would conclude that trans-activation of LTR-directed gene expression is not a property of the replication of all retroviruses. This suggests that this activity is restricted to particular retroviruses, and is possibly related to particular features of their life cycle or pathophysiology.

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REFERENCES

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