Activation of gene expression by human herpesvirus 6 is reporter gene-dependent

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Infection with human herpesvirus 6 (HHV-6) was found to up-regulate expression of human immunodeficiency virus and human T cell leukaemia virus type I (HTLV-I) long terminal repeat sequence (LTR), and herpes simplex virus type I (HSV-1) gD chloramphenicol acetyltransferase (CAT) constructs transfected into the T cell line, J.Jhan. Activation by HHV-6 was due to one or more viral proteins produced early in infection and, in the case of the HTLV-I LTR, was synergistic to induction mediated by the HTLV-I tax gene product. Neither the HTLV-I enhancer nor basal promoter elements of the HSV-1 gD gene were essential for activation and no increase in accumulated HTLV-I mRNA was observed due to HHV-6 infection. Induction by HHV-6 was found to be dependent on the reporter construct used, because the CAT gene and, to a lesser extent, the HSV-1 thymidine kinase gene were responsive to HHV-6 infection although no significant activation of growth hormone constructs was observed. Our results bear a strong resemblance to those obtained for the Epstein–Barr virus BMLF1 gene, indicating that the major HHV-6 trans-activator may be a homologue of this gene.

Introduction

Human herpesvirus 6 (HHV-6) was first detected by Salahuddin et al. (1986) and has been isolated from patients with immunoproliferative disorders and retrovirus infections (Downing et al., 1987; Tedder et al., 1987; Agut et al., 1988; Lopez et al., 1988). HHV-6 nucleotide sequences have been detected also in tissue samples from patients with lymphoid malignancies (Jarrett et al., 1988; Josephs et al., 1988). Serological investigations have shown that the majority of the adult population is seropositive for HHV-6 and that the virus is acquired in infancy (Briggs et al., 1988; Saxinger et al., 1988; Clark et al., 1990). HHV-6 is the causative agent of exanthem subitum, a common childhood infection (Yamanashi et al., 1988). The virus is able to infect populations of lymphoid cells in vitro, and virus and DNA sequences have been detected in saliva (Pietroboni et al., 1988). In vitro, the virus can be propagated in a number of lymphoblastoid cell lines (Tedder et al., 1987; Lusso et al., 1988). Sequence analysis of a 21 kb fragment shows that HHV-6 is more closely related to human cytomegalovirus than to the gammaherpesviruses Epstein–Barr virus (EBV) and herpesvirus saimiri (Lawrence et al., 1990).

To date, studies relating to gene activation by HHV-6 have concentrated on its interaction with human immunodeficiency virus (HIV), because HHV-6 is considered to be a possible cofactor in the development of acquired immunodeficiency syndrome. Ensoli et al. (1989) and Lusso et al. (1989) have found that HHV-6 can productively co-infect CD4+ T cells causing an accelerated c.p.e. and increased HIV expression. However, studies by Pietroboni et al. (1988) and Lopez et al. (1988) found that HHV-6 inhibits HIV replication, as shown by decreased reverse transcriptase activity and virus titre. Infection of cells transfected with HIV long terminal repeat sequence (LTR)–chloramphenicol acetyltransferase (CAT) constructs caused increased CAT activity, suggesting that HHV-6-responsive elements are located within the LTR (Horvat et al., 1989; Lusso et al., 1989). Further studies (Ensoli et al., 1989) showed parallel increases of mRNA and mapped potential HHV-6-responsive elements to a region of the HIV LTR containing the NFkB binding sites.

Our aim was to study the effect of HHV-6 infection on transcription directed by the human T cell leukaemia virus type I (HTLV-I) LTR, as there is the potential for the two viruses to interact in vivo because both have similar cell tropisms and HHV-6 has been isolated from patients with retroviral infections. In addition, we hoped to gain a greater understanding of the replicative cycle of HHV-6. The results obtained show that although expression of HTLV-I LTR–CAT constructs is up-
regulated by HHV-6 infection, this effect is not due to specific HTLV-I sequences and is likely to be mediated by a post-transcriptional mechanism. These conclusions are supported by additional studies using constructs containing the HIV LTR and a family of plasmids containing defined lesions in the herpes simplex virus type 1 (HSV-1) glycoprotein D (gD) gene promoter. In no case were specific promoter sequences found to be essential for the activation of gene expression during infection with HHV-6.

Methods

Cell lines and virus stocks. A Jurkat cell line (J. Jhan) was used for transfection assays and virus growth. C8166 (Salahuddin et al., 1983) cell lines were used for S1 analysis. Both T lymphocyte culture cell lines were maintained in RPMI 1640 containing 10% fetal bovine serum, 400 international units/ml penicillin and 400 μg/ml streptomycin. Infections were carried out using the A72 isolate of HHV-6 (Tedder et al., 1987). HHV-6-infected cells were identified using an indirect immunofluorescence assay (Clark et al., 1990).

DNA transfections. DNA was transfected into cells using the DEAE-dextran method. The DNA (4 μg of recorder construct plus 6 μg pUC8 or trans-activator gene) was mixed with 1 ml 25 mM-Tris-HCl pH 7.4, 137 mM-NaCl, 5 mM-KCl, 0.6 mM-Na2HPO4, 0.7 mM-CaCl2, 0.5 mM-MgCl2 and 400 μg/ml DEAE-dextran and added to 2×106 cells. The cells were incubated at 37°C for 90 min and then 15%, DMSO was added for 3 min. Following washing, 10 ml of medium or 5 ml of medium plus 5 ml of supernatant from infected cells was added and the cells were incubated for 48 h at 37°C. The TCD50 of the infected cell medium was approximately 1:12 unless otherwise stated.

Reporter gene assays. CAT assays were carried out by incubating cytoplasmic cell extracts with 14C-labelled chloramphenicol and acetyl coenzyme A as described previously (Garman et al., 1982). Media and cytoplasmic extracts were assayed for growth hormone using the Allegro immunoassay system (Biogenesis). Thymidine kinase (TK) assays were carried out as described (Cordingley et al., 1983). All results were calculated from readings within the linear range of the assay.

S1 nuclease mapping. Cytoplasmic RNA (20 μg) was precipitated with 5 × 104 Cerenkov counts of 5'-radiolabelled probe complementary to nucleotides 333 to 409 of the HTLV-1 genome. The pellet was resuspended in 30 μl 1 M-NaCl, 0.17 M-HEPES pH 7.5 and 0.33 M-EDTA, heated to 75°C for 30 min and then incubated overnight at 55°C. S1 nuclease (300 units) in a final concentration of 0.28 M-NaCl, 50 mM sodium acetate pH 4.5, 4.5 mM-ZnSO4, was added and the mixture was incubated at 37°C for 1 h. The reaction was stopped by adding 10 μg tRNA and EDTA to 5 mM, and then the RNA was ethanol-precipitated. The samples were dissolved in formamide dye, boiled and electrophoresed on an 8% denaturing polyacrylamide gel.

Plasmid constructions. HIV and HTLV-I LTR-CAT constructs have been described previously (Arya et al., 1985; Felber et al., 1985). pH2 CAT was constructed by inserting the 1-8 kb NdeI-BamHI LTR fragment from HTLV-I LTR-CAT into the vector pC19H (Marsh et al., 1984). pTax is a cDNA clone of the HTLV-I tax gene, derived from the C91PL cell line, in a vector flanked by the Moloney murine leukaemia virus LTRs (Cross et al., 1984). pgD CAT and deletion mutants were derived from the pERD series (Everett, 1986) by removing the gD promoter region as an SstI-HindIII fragment and inserting it into a suitable site in a vector derived from pBLW2 (Everett, 1984). Other plasmid constructs which have been described previously are pTKCAT (Luckow & Schutz, 1987), pTKGH (Selden et al., 1986), pTK1 (Cordingley et al., 1983) and pPRV IE (Campbell & Preston, 1987). pgDGH was constructed by inserting the HindIII fragment of pgDCAT into the same site of pGKH (Selden et al., 1986). NFTA-CAT was constructed by inserting a double-stranded oligonucleotide, residues −110 to −78 of the HIV LTR, between the SphI/Xhol sites of pSVgD. The SphI and Xhol sites of pSVgD were filled in and the plasmid was religated to form TA-CAT, which has the CAT gene under the control of the gD TATA box.

Results

Activation of HTLV-I LTR-CAT by HHV-6

In order to determine whether infection with HHV-6 could increase expression of an HTLV-I LTR-CAT construct, as had been previously found for HIV LTR constructs, transfection experiments were carried out. HIV and HTLV-I LTR-CAT constructs were introduced into J. Jhan cells (a T lymphocyte cell line) by using the DEAE-dextran transfection method. Following transfection, medium was added to the cells from uninfected or HHV-6-infected cultures. The cells were incubated for 2 days at 37°C, harvested and the CAT activity was assayed. An exceptionally marked increase in CAT activity was observed from both the HTLV-I and HIV LTR-CAT constructs (Fig. 1). In order to characterize this effect further, we studied the nature of the HHV-6 protein involved, the requirement for specific promoter sequences and the stage during the expression of the gene at which activation occurs.

To determine the time course of HHV-6 activation, cells were transfected with HTLV-I LTR-CAT, as described previously, and mock-infected or infected with HHV-6. The cells were harvested after various time intervals between 1 and 4 days and the CAT activity was measured. It was found that maximum activation occurred between 1 and 2 days after the addition of HHV-6 (Fig. 2). The progression of infection, as monitored by the percentage of cells reacting with sera from infected patients, reached a maximum after 3 days of infection. These results indicate that one or more viral proteins which are present at early times of infection and subsequently decline in activity are responsible for the activation of gene expression.

Transcription mediated by the HTLV-I LTR is up-regulated by tax, a 40K protein encoded by HTLV-I (Sodroski et al., 1984; Felber et al., 1985). The HTLV-I LTR (Fig. 3) contains three 21 bp repeat sequences which, when present in two or more copies, can act as a target for tax (Shimotohno et al., 1986; Brady et al., 1987). tax does not interact directly with the repeat sequences but mediates LTR activation through cellular DNA-binding factors (Jeang et al., 1988; Montagne et
Activation of gene expression by HHV-6

Fig. 1. Activation of promoter-CAT constructs in transfected J. Jhan cells. Plasmids used for transfections were HIV LTR-CAT (lanes 1 and 2), HTLV-I LTR-CAT (lanes 3 and 4) and pH2 CAT (lanes 5 and 6); cells were infected with HHV-6 (lanes 2, 4 and 6). The percentage acetylation of chloramphenicol/μg protein/h of assay is shown. Results represent the average of three experiments. Ac. Cm., acetylated chloramphenicol; Cm., chloramphenicol.

Fig. 2. Time course of induction of HTLV-I LTR-CAT in transfected J. Jhan cells by HHV-6 infection. Closed circles represent fold increase in CAT activity due to HHV-6 infection as compared to mock-infected transfected cells, following correction for cell death due to infection. Open circles denote percentage of infected cells which react with antiserum from an infected donor.

al., 1990). To examine the effect of co-activation with tax and HHV-6, cells were transfected with HTLV-I LTR-CAT and either mock-infected, infected with HHV-6 or cotransfected with a plasmid carrying the HTLV-I tax gene. In this experiment, infection was carried out with diluted HHV-6 medium in order to obtain similar levels of induction by HTLV-I and tax. The results (Fig. 4) show that when both methods of activation are used together a very strong response is observed (69.5-fold induction), which is greater than the sum of the individual activations of 7.7- and 7.2-fold. As an additional control, HTLV-I LTR-CAT was cotransfected with the vector which contains the regulatory sequences of pTax but lacks the tax coding region. No stimulation was obtained with this plasmid in the absence or presence of HHV-6 medium (data not shown). The results obtained show that tax and HHV-6 act synergistically and imply that they have different mechanisms of activation.

To study the sequences required for HHV-6 activation we constructed a deletion mutant of HTLV-I LTR-CAT by removing sequences upstream of a position 55 bp from the initiation site. The resultant plasmid, pH2 CAT (Fig. 3), contains the TATA box but lacks upstream regulatory elements such as the 21 bp repeat sequences. When pH2 CAT was introduced into cells its expression was increased 41.8-fold by HHV-6 infection, whereas in the same set of experiments HTLV-I LTR-CAT was stimulated 52.0-fold (Fig. 1), although basal levels of pH2

Fig. 3. The 5' LTR of the HTLV-I genome. The mRNA start site and relative positions of the 21 bp repeats (R) and TATA box (T) are indicated. Diagrams of plasmids show HTLV-I sequences (open bars) and CAT sequences (solid bars).

Fig. 4. CAT assays showing induction of HTLV-I LTR-CAT with HHV-6 and tax. J. Jhan cells were transfected with HTLV-I LTR-CAT (lanes 1 to 4), cotransfected with pTax (lanes 3 and 4) and infected with HHV-6 (lanes 2 and 4). Average fold increase in CAT activity (for three experiments) compared to cells transfected with HTLV-I LTR-CAT alone is shown. Ac. Cm., acetylated chloramphenicol; Cm., Chloramphenicol.
CAT expression were lower. This result showed that sequences upstream of residue −55 were not required for HHV-6 activation.

**HHV-6 infection does not increase levels of HTLV-I mRNA**

The C8166 cell line (Salahuddin et al., 1983) contains integrated defective HTLV-I proviruses, one of which is able to synthesize the doubly spliced form of mRNA which encodes tax; later species of RNA and infectious virus are not produced. To study the effect of HHV-6 on HTLV-I RNA levels, cytoplasmic RNA was isolated from cells which had been infected for 2 days with HHV-6 from uninfected cells and was analysed by S1 nuclease mapping. A probe complementary to residues 333 to 409 of the HTLV-I genome (Seiki et al., 1983) which spanned the mRNA start site was hybridized to the RNA and electrophoresed on a denaturing gel following S1 digestion. Both samples of C8166 RNA (Fig. 5, lanes 2 and 3) gave rise to a hybrid band of 58 bp which corresponds to correctly initiated HTLV-I mRNA. The intensity of this band was not greater in the HHV-6-infected sample, showing that infection did not affect levels of accumulated HTLV-I mRNA. It is possible that activation of an integrated promoter may differ from that of a transfected plasmid; however, HHV-6 infection of cells transfected with HTLV-I LTR–CAT, pgD CAT, or a plasmid expressing the rabbit β-globin gene under the control of the gD promoter, did not lead to detectable levels of mRNA transcripts from the transfected plasmids (data not shown). This contrasts with the situation during HSV-1 infection (Everett, 1984).

**Activation of gene expression during HHV-6 infection does not require specific promoter regulatory sequences**

A number of herpesvirus trans-activators mediate transcriptional regulation via interaction with transcription factors which bind to basal promoter elements (Abmayer et al., 1985; McKnight & Tjian, 1986). To investigate the promoter sequence required for activation by HHV-6, a series of fine deletion mutants of the HSV-1 gD gene (Everett, 1983, 1984) were utilized (Fig. 6). The constructs were transfected into J. Jhan cells and infected with HHV-6 or mock-infected. Increases in CAT activity due to HHV-6 expression are shown in Table 1. The level of activation of pgD CAT constructs was very high and, although some variation was observed (e.g. pgD CAT is activated 4400-fold and plasmid 25/4, which has a deletion in the TATA box, is activated 1300-fold), this difference is small compared to the total level of induction. These results show that no essential promoter sequences are required for activation.

To determine further whether regulatory sequences containing NFkB binding sites play a role in HHV-6 activation, we constructed vectors which consisted of the CAT gene under the regulation of the TATA box, with
HHV-6 activation is dependent on gene construct

We examined the effect of HHV-6 infection on the expression of different constructs under the regulation of the HSV-1 TK promoter to determine whether the nature of the reporter gene played any role in HHV-6 activation. The TK–CAT constructs, like all other CAT constructs tested, were strongly induced by HHV-6 infection (160-fold) (Table 2). However, the construct in which the growth hormone (GH) gene was under the control of the TK promoter, pTKGH, was only induced 1-3-fold, showing that this construct had a very low, if any, response to infection. The TK gene regulated by its own

Table 1. Activation of gD deletion mutants by HHV-6

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Basal activity (× 10⁻²)</th>
<th>+ HHV-6 infection</th>
<th>Average induction (fold) × 10⁻³</th>
</tr>
</thead>
<tbody>
<tr>
<td>pgD CAT</td>
<td>6.8† (3.6–10.0)‡</td>
<td>299† (245–325)‡</td>
<td>4.4</td>
</tr>
<tr>
<td>129/84</td>
<td>10.4 (5.3–15.5)</td>
<td>520 (417–635)</td>
<td>5.0</td>
</tr>
<tr>
<td>129/68</td>
<td>1.3 (0.8–1.8)</td>
<td>67 (40–79)</td>
<td>5.2</td>
</tr>
<tr>
<td>67/56</td>
<td>1.0 (0.8–1.2)</td>
<td>29 (21–44)</td>
<td>2.9</td>
</tr>
<tr>
<td>67/27</td>
<td>1.1 (0.6–1.6)</td>
<td>18 (11–32)</td>
<td>1.6</td>
</tr>
<tr>
<td>46/34</td>
<td>4.9 (2.6–7.8)</td>
<td>279 (235–315)</td>
<td>5.7</td>
</tr>
<tr>
<td>46/27</td>
<td>2.2 (1.2–3.2)</td>
<td>132 (112–320)</td>
<td>6.0</td>
</tr>
<tr>
<td>23/22</td>
<td>1.5 (0.6–2.4)</td>
<td>37 (30–56)</td>
<td>2.5</td>
</tr>
<tr>
<td>25/4</td>
<td>1.7 (1.4–2.0)</td>
<td>32 (26–58)</td>
<td>1.3</td>
</tr>
<tr>
<td>pBLW2§</td>
<td>1.0 (0.6–1.4)</td>
<td>2 (2–2)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* Expressed as acetylation (%)/μg protein/h assay.
† Average values.
‡ Range of values.
§ pBLW2 is the vector into which gD promoter sequences were cloned.

Table 2. Activation of different reporter gene constructs by HHV-6

<table>
<thead>
<tr>
<th>Plasmid*</th>
<th>Activation†</th>
<th>CAT activity‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>pTKCAT</td>
<td>0.12§ (0.10–0.15)</td>
<td>140-8</td>
</tr>
<tr>
<td>pTKCAT + HHV-6</td>
<td>19.30 (14.13–32.51)</td>
<td>112</td>
</tr>
<tr>
<td>pTKCAT + pPRV IE</td>
<td>2.47 (1.21–3.76)</td>
<td>20-6</td>
</tr>
<tr>
<td>pTKGH</td>
<td>7.3 (5.7–9.4)</td>
<td>2-6</td>
</tr>
<tr>
<td>pTKGH + HHV-6</td>
<td>8.7 (5.2–10.1)</td>
<td>1.2</td>
</tr>
<tr>
<td>pTKGH + pPRV IE</td>
<td>106-6 (65.8–147-4)</td>
<td>14-6</td>
</tr>
<tr>
<td>pTK1</td>
<td>984 (731–1237)</td>
<td>731</td>
</tr>
<tr>
<td>pTK1 + HHV-6</td>
<td>12397 (9823–14971)</td>
<td>12-6</td>
</tr>
<tr>
<td>pTK1 + pPRV IE</td>
<td>20961 (15621–26301)</td>
<td>21-3</td>
</tr>
<tr>
<td>pGD CAT</td>
<td>0.036 (0.03–0.122)</td>
<td>0.03</td>
</tr>
<tr>
<td>pGD CAT + HHV-6</td>
<td>176.5 (140-2–202-6)</td>
<td>4902-7</td>
</tr>
<tr>
<td>pGD CAT + pPRV IE</td>
<td>8.7 (4.6–15.2)</td>
<td>241-7</td>
</tr>
<tr>
<td>pGDGH ND††</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>pGDGH + HHV-6</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>pGDGH + pPRV IE</td>
<td>5.6 (2.3–7.4)</td>
<td>2.3</td>
</tr>
</tbody>
</table>

* Plasmid transfected into J. Jahn cells with either HHV-6 or pPRV IE.
† Average fold increase in activity due to HHV-6 infection or pPRV IE transfection.
‡ CAT activity expressed as acetylation (%)/μg protein/h of assay.
§ Values are the average of three experiments.
‖ Range of values used to calculate average value.
¶ GH concentration, ng/ml.
** TK activity expressed as c.p.m./μg protein/h of assay.
†† ND, Not detectable above background levels of GH.

and without sequences between residues -110 and -78 of the HIV LTR, which contains two copies of the NFkB binding site. Both constructs (Fig. 7) responded strongly to HHV-6 and the effect of the NFkB site was found to be negligible.

Fig. 7. Effect of NFkB binding sites on induction by HHV-6. (a) Diagram of CAT constructs. NFTA-CAT contains sequences from -110 to -78 of the HIV LTR. (b) CAT assays from transfected J. Jahn cells. Cells were transfected with TA-CAT (lanes 1 and 2) and NFTA-CAT (lanes 3 and 4) and infected with HHV-6 (lanes 2 and 4). The infected medium had approximately one-eighth of the usual titre. Average fold increase of infected compared to uninfected samples is shown. Ac. Cm., acetylated chloramphenicol; Cm., chloramphenicol.

and without sequences between residues -110 and -78 of the HIV LTR, which contains two copies of the NFkB binding site. Both constructs (Fig. 7) responded strongly to HHV-6 and the effect of the NFkB site was found to be negligible.
rabies virus trans-activator, and levels of expression were increased 20.6-, 14.5- and 21.3-fold respectively. This control shows that there is no defect in pTKGH and that higher levels of GH can be expressed. An additional study showed that no significant increase in GH expression occurred following infection when the cells or media were harvested at times between 1 and 4 days (results not shown).

Using the gD deletion constructs, we showed that no specific promoter elements were essential for activation by HHV-6 (Table 1). However, the CAT vector (pBLW2) was 27-fold less responsive than the constructs containing gD promoter sequences (Table 1). It was therefore possible that the gD promoter was activated by a second mechanism which depended on the presence of some promoter elements and therefore occurred at the level of transcription; expression of pgD CAT was increased 4400-fold due to HHV-6 infection (Table 1). To determine whether a proportion of this increase was mirrored by GH activation, pgDGH was created. Expression of this construct was not detectable with or without HHV-6 infection thus showing HHV-6 to have, at most, a minor effect on this construct; cotransfection with pPRV IE boosted expression of pgDGH to a detectable level (Table 2).

To show that the effect on the CAT gene was not due to an effect on the activity or stability of the CAT enzyme in the assay, extracts from pgD CAT-transfected cells were mixed in equal proportions with extracts from HHV-6-infected cells (Fig. 8). No increase in activity was observed compared to that seen with transfected cells mixed with non-infected cell extracts, showing that increased CAT activity is due to elevated gene expression.

### Discussion

Our results show that activation by HHV-6 depends on the reporter gene used and that the role of the promoter or enhancer sequences are, at most, of minor importance. Activation of gene expression by HHV-6 is most likely to occur post-transcriptionally because any transcriptional effect would be expected to be independent of the reporter gene used. Ensoli et al. (1989), whose study of activation centred on the HIV LTR, reach strikingly different conclusions. They found no induction of HTLV-I LTR-CAT constructs by HHV-6 and they observed also that HHV-6 infection increased the level of HIV mRNA, whereas we found no increase in accumulated HTLV-I mRNA. In addition, Ensoli et al. mapped the HHV-6-responsive elements to between 105 and 80 bp upstream of the HIV mRNA start site; these elements serve as the binding sites for the NFkB transcription factor. We found that no specific promoter sequences were necessary for activation, including those containing NFkB binding elements (Fig. 8). The variation between our results and those of Ensoli and co-workers is unlikely to be due to differences in experimental procedure, as we have repeated experiments using the methods described by Ensoli et al. (1989), who used cells preinfected with HHV-6 for transfection. This change in procedure made no difference to the ubiquitous nature of CAT activation. We have also carried out experiments in which plasmids were transfected into a second Jurkat cell line, or into HUT 78 cells. Again, non-specific activation of CAT constructs by HHV-6 was observed (data not shown), indicating that our results were unlikely to be cell type-dependent. It therefore seems most plausible that the differences between our data and those of Ensoli et al. (1989) are due to differences in the activity or expression of trans-activating polypeptides between the two virus strains. It should be noted, however, that the effect observed in both studies is the average stimulation of a non-co-ordinately infected cell population and the net result may be due to a number of trans-activating polypeptides.

Our results, however, bear a strong similarity to those obtained by Kenney et al. (1988, 1989) and Markovitz et al. (1989) who studied the EBV BMLF1 gene, which encodes an immediate early trans-activator. These workers found activation of all CAT constructs by BMLF1, including those with no recognizable eukaryotic promoter, and they observed no increase in CAT mRNA levels. Most significantly, they found that only CAT constructs and not GH constructs are induced by BMLF1. The similarity between these results and those obtained for HHV-6 suggests that the HHV-6 trans-activator may be a homologue of BMLF1. The BMLF1 gene is a member of a family of herpesvirus genes which
are related by weak homology and positional relationships (Davison & Scott, 1986; McGeoch et al., 1988; Nicholas et al., 1988; B. Barrell et al., personal communication). The cloning and sequencing of the HHV-6 genome which is now in progress (Lawrence et al., 1990; Littler et al., 1990) will determine whether HHV-6 encodes a BMLF1 homologue and should enable the identification and study of all HHV-6 trans-activating genes.

The mechanism whereby HHV-6 or EBV BMLF-1 activate CAT gene expression is not known. Differences in the level of expression from different recorder gene constructs may not be due solely to the nature of the coding sequences because CAT, TK and GH plasmids also differ in leader sequences, polyadenylation signals and number of introns. Although the highly spliced nature of the GH gene may play some role in the lack of responsiveness to HHV-6, this is unlikely to be the only important factor as we found that HSV-1 TK, an unspliced gene, is only weakly responsive and Markovitz et al. (1989) note that the unspliced β-galactosidase gene is not induced by EBV BMLF1. Differences in leader sequences and polyadenylation signals may account for the variation in induction levels between different CAT constructs. For example, HTLV-1 LTR–CAT is up-regulated 52-fold whereas pgD CAT is induced 4400-fold. HHV-6 induction may therefore occur by stabilizing mRNA, allowing preferential expression of unspliced mRNA or preferential use of specific polyadenylation signals, or even during the translation process.

It is probable that the CAT gene mirrors some property of a target viral or cellular gene the expression of which is up-regulated by the HHV-6 trans-activator during the course of infection.

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