Analysis of sequences important for herpes simplex virus type 1 latency-associated transcript promoter activity during lytic infection of tissue culture cells

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We describe the analysis of the herpes simplex virus type 1 (HSV-1) latency-associated transcript (LAT) promoter using an HSV-1-based vector system. Sequences under investigation for LAT promoter activity were analysed for their ability to direct chloramphenicol acetyltransferase gene expression, either in transfection assays or following their insertion into an HSV-1 vector from which the endogenous LAT promoter sequences had been removed. The analysis mapped the main determinants of LAT promoter activity during lytic infection of tissue culture cells to a 277 bp region between −279 and −2 relative to the recognized 5’ end of the primary 8.3 kb transcript. The LAT promoter constructs behaved similarly in the context of the virus genome and in the plasmid-based transfection assays. Comparison of the relative activities following infection of fibroblast and neuroblastoma cell lines indicates that sequences upstream from −279 are important for LAT promoter activity in neurons.

Introduction

A major feature of the biology of herpes simplex virus type 1 (HSV-1) is its capacity to establish a latent infection in peripheral nervous system ganglionic neurons and to reactivate periodically causing recrudescent disease (for a review, see Hill, 1985).

The extent and the nature of any expression from latent HSV genomes was unknown until Stevens et al. (1987) showed by in situ hybridization and Northern blotting that latency-associated transcripts (LATs) could be detected in ganglia from mice that were latently infected with HSV-1. The LATs map to the long repeat region of the genome and are transcribed in the opposite direction to, and partially overlap with, IE-1 mRNA (Rock et al., 1987b; Wechsler et al., 1988a, b; Spivack & Fraser, 1987; Wagner et al., 1988a; Stevens et al., 1987; Gordon et al., 1988; Steiner et al., 1988). Subsequent work by a number of groups has refined our knowledge of the HSV-1 LATs and shown that LATs are also encoded by a number of other herpesviruses including pseudorabies virus (Cheung, 1989) and bovine herpes virus type 1 (Rock et al., 1986, 1987a).

It is now known that the HSV-1 LATs include at least three overlapping non-polyadenylated RNAs which share common 5’ and 3’ ends (Rock et al., 1987b; Wagner et al., 1988a, b; Wechsler et al., 1988; Spivack & Fraser, 1988), but differ in size through differential splicing (Wagner et al., 1988b; Wechsler et al., 1988a, b; Spivack & Fraser, 1988). LAT RNAs have also been detected, although they are much less abundant, during acute infection of mice and in tissue culture (Spivack & Fraser, 1987, 1988; Wagner et al., 1988b). In lytically infected tissue culture cells, the only detectable species is 2.0 kb in size (Spivack & Fraser, 1988; Wagner et al., 1988b), whereas in latently infected tissue there are additional species of 1.5 kb and 1.45 kb (Spivack & Fraser, 1987; Rock et al., 1987b; Wagner et al., 1988a, b; Wechsler et al., 1988b). The 2 kb LAT is not now thought to be a primary transcription product but is believed to be a stable intron excised from an 8.3 kb transcript (Dobson et al., 1989; Mitchell et al., 1990a; Zwaagstra et al., 1990) (designated m-LAT), which extends from 660 bp upstream of the 2 kb LAT to the first consensus polyadenylation signal in the short terminal repeat just downstream from the immediate (IE)-3 gene. Devi-Rao et al. (1991) demonstrated that LAT is uncapped, and that its 3’ end maps to a canonical splice acceptor site, and Farrell et al. (1991) showed that when a 2.4 kb HSV-1 fragment encompassing the 2 kb IAT was cloned into the lacZ gene, splicing occurred giving rise to a processed lacZ transcript and a 2 kb ‘LAT’ intron.

Various studies have demonstrated that lytic and latent phase LAT expression requires promoter elements...
situated immediately upstream from the mapped 5' end of m-LAT. This promoter region contains a series of consensus elements such as a TATA box and potential Sp1 binding motifs, and the more recently identified cAMP response element (CRE) (Leib et al., 1991) and LAT promoter binding factor (LPBF) motif (Zwaagstra et al., 1991), both of which appear to play a major role in LAT promoter regulation. The majority of these studies have involved plasmid expression systems in transient transfection assays in various cell types. However, a definitive identification of all cis-acting elements responsible for LAT transcription can only come from examination of the LAT promoter in the context of the virus genome. To date, such studies have been limited to examination of mutant viruses with various portions of the LAT promoter deleted (Javier et al., 1988; Dobson et al., 1989; Steiner et al., 1989; Mitchell et al., 1990b). In particular, Dobson et al. (1989) showed that a virus with a 203 bp deletion that removed the region surrounding the TATA box (-137 to +66 relative to the m-LAT cap site) abolished detectable LAT transcription during latency.

In this study, we have cloned various LAT promoter sequences and examined their ability to drive expression of an adjoining reporter gene, for chloramphenicol acetyltransferase (CAT), in transient expression assays in a plasmid expression vector and, when inserted into the virus genome, in lytic infection of tissue culture cells.

Methods

Viruses and cells. BHK-21 clone 13 cells (Macpherson & Stoker, 1962), cultured in the Glasgow modification of Eagle's medium containing 10% calf serum (ETC10), were used routinely for growth and titration of virus stocks, viral gene expression experiments and short-term transfection assays. C1300 Neuro-2A cells (Augustus-Tocco & Sato, 1969), a murine neuroblastoma cell line, cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 4 mM-l-glutamine, 100 units/ml penicillin and 100 mg/ml streptomycin were used for viral gene expression experiments. The wild-type virus used in superinfection experiments was HSV-1 strain 17.

Plasmid construction. Plasmid pLAT1CAT contains a 1094 bp fragment which extends from -279 to +812 of the LAT sequence. To construct pLAT1CAT, the 1091 bp HindII-HindII fragment was isolated from pGX48 (Perry et al., 1991). The construct pLAT1CAT, the 1091 bp fragment was purified, blunt-ended with T4 DNA polymerase, ligated into HindII-digested pUC8 DNA and a plasmid containing the insert in the correct orientation was selected (pLAT6). The LAT6 sequence was isolated as a SalI/BglII fragment and ligated into SalI/BglII-digested pF35 DNA (pLAT6CAT). To construct pLAT6CAT, which contains 391 bp of LAT sequence from the HindII site at -279 to the Nael site at +111, pLAT1CAT was digested with Nael and BglII, blunt-ended with T4 DNA polymerase and religated. This treatment removed all of the LAT sequences downstream from the Nael site at +111 (pLAT6CAT). To construct plAT6CAT, which contains 798 bp of LAT sequence from the DraI site at -800 to the PvuI site at -2, the 661 bp SalI/EcoRV fragment was excised from plAT6-HindIII + SalI [consisting of the 1620 bp DraI (-800)–HindII (+812) fragment cloned into pUC19 with the pUC19 HindIII site converted to a SalI site by linker insertion] and ligated into SalI/EcoRV-digested pLAT6 (pLAT60). The LAT60 sequence was isolated from pLAT60 as a SalI/BglII fragment and ligated into SalI/BglII-digested pF35 DNA (pLAT60CAT).

Other constructs used as controls in the following expression assays were as follows: pgDCAT (Everett, 1986) has the CAT gene under the control of the HSV-1 glycoprotein D (gD) promoter and pF310 (Rixon & McLauchlan, 1990) has the CAT gene under the control of the HSV-2 IE-4/5 gene promoter. These are examples of HSV early and IE promoters respectively and are normally active during the lytic stages of HSV infection. Plasmid pF35 was used as a negative control and has no promoter driving CAT activity.

Construction and screening of recombinant virus. This procedure was carried out essentially as described by Rixon & McLauchlan (1990).

Transfection of plasmid DNA into cells. Plasmid DNA was transfected into BHK-21 cells using a modified version of the calcium phosphate technique described by Shen et al. (1982). Plasmid DNA (3 pl) was made up to 57 pl in deionized H2O, and 66 pl of 2 x HeBS (260 mM-NaCl, 9.8 mM-KCl, 1.6 mM-Na2HPO4, 11 mM-D-glucose, 42 mM-HEPES pH 7.15) was added. To this 9 pl of 2 M-CaCl2 was added, the samples were immediately vortexed and left at room temperature for 10 min. The medium was then removed from 80% confluent cell monolayers grown in 35 mm Petri dishes and retained (conditioned medium). Cells were overlaid with the calcium phosphate precipitate and incubated at 37 °C for 45 min with intermittent rocking. Plating medium (18.75 ml conditioned medium, 20 ml ETC10, 2 ml 2 x HeBS, 0.25 mM CaCl2; the latter added just before use) was added (2 ml) to each monolayer and the incubation continued for a further 3 to 4 h at 37 °C. The plating medium was then removed from the cells and 25% (v/v) DMSO in 1 x HeBS applied for 4 min at room temperature (Stow & Wilkie, 1976). The DMSO was removed and the cells were washed twice with ETC10. 2 ml of fresh ETC10 was then added and the monolayers were incubated at 38.5 °C. Infection with virus, when required, was performed 1 h after DMSO boosting. Incubation was continued overnight at 38.5 °C.

CAT assays. Cell extracts were prepared by washing in PBS, then scraping into 2 ml of TEN (150 mM-NaCl, 40 mM-Tris–HCl pH 7.5, 1 mM-EDTA) and transferring to 15 ml Falcon tubes. The cells were pelleted at 2000 r.p.m. at 4 °C for 1 min in a Sorvall RT6000B centrifuge, resuspended in 75 ml 0.25 mM-Tris–HCl pH 7.8 and sonicated. The sonicates were transferred to 1.5 ml reaction vials and centrifuged at 10000 r.p.m. for 2 min to remove cell debris. The supernatants were stored at -20 °C. CAT assays were performed essentially as described by Seed & Sheen (1988). Assay mixtures containing 1 pl 25 mM-n-butyryl-CoA, 0.5 μl [14C]chloramphenicol (45 μCi/mmole), 5 μl 250 mM-
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Tris–HCl pH 7.8 and 18.5 µl H2O together with 20 µl undiluted or diluted [to prevent the reaction going beyond the linear range (30%) of conversion] sonicate. After incubation for 30 or 60 min at 37°C, the products were extracted with 150 µl tetramethylpentadecane (TMPD)/xylene (2:1 mixture respectively). The percentage conversion to product was determined by comparing the counts present in 140 µl of the TMPD/xylene extract to the total counts in the assay (i.e. the total number of counts in a parallel control reaction). The protein concentration of the extract was determined by the method of Bradford (1976). CAT activities were expressed as the percentage [14C]chloramphenicol converted to the butyrylated form per µg protein per hour.

Results

The sequence numbering convention used here is such that the mapped 5' end of the 8.3 kb m-LAT (Zwaagstra et al., 1990; Devi-Rao et al., 1991) i.e. the T residue in the sequence 5' . .GATCG.. 3', at position 118802 of the published HSV-1 sequence (Perry & McGeoch, 1988) is referred to as position +1.

Promoter activity of the LATCAT plasmids

Fig. 1 shows a schematic representation of the region containing the 5' end of the 8.3 kb LAT and upstream promoter elements. In order to assay for promoter activity exhibited by these sequences, a series of DNA fragments were cloned into the CAT expression vector pFJ5 (Rixon & McLauchlan, 1990; see Methods). The locations of these fragments and relevant sequence elements are indicated in Fig. 1. The promoter activities of the constructs were determined by quantification of CAT activity in short-term transfection assays in BHK cells as described in Methods. The results illustrated in columns 2 and 3 of Table 1 give the mean of at least four independent determinations along with the standard error of the mean. The CAT activities from each transfection experiment were calculated as percentage conversion per hour per µg protein, and then normalized relative to the CAT activity exhibited by pLAT1CAT whose activity was assigned the nominal value of 1.

As observed from the results in Table 1, the control plasmids pgDCAT and pFJ10 exhibit relatively high levels of constitutive activity, with pFJ10 being 11-fold more active than pgDCAT. Plasmid pLAT1CAT exhibited a level of activity (about 23% of pgDCAT) which indicates that the LAT1 fragment contains sequences that can perform as a promoter in this assay. This activity is abolished when the 5' end of the LAT1 sequence is deleted to +65 (data not shown). The results for pLAT4CAT, pLAT8CAT and pLAT6CAT demonstrate that as the 3' end of the LAT sequence is deleted towards the putative promoter elements, the level of CAT activity increases, with the level of pLAT6CAT activity being greater than that exhibited by pgDCAT. This observation is consistent with the notion that these elements form a bona fide promoter which exhibits a distance effect, i.e. promoter activity increases as the distance between the promoter and reporter gene decreases. The small increase in activity exhibited by pLAT60CAT over LAT6CAT indicates that sequences upstream from the HindIII site at −279 contribute only slightly to promoter activity in this assay.

Effect of virus infection on promoter activity

In addition to the transient expression assays detailing

![Fig. 1. Schematic representation of LAT promoter fragments. The top line indicates the relative positions of promoter elements and the 5' ends of both m-LAT and the 2 kb LAT (LAT intron). Below that are indicated regions of loci conserved between the HSV-1 and HSV-2 nucleotide sequences as identified by McGeoch et al. (1991). The LAT1 fragment extends from −279 to +812, LAT4 from −279 to +265, LAT8 from −279 to +111, LAT6 from −279 to +2 and LAT 60 from −800 to −2.](image)

**Table 1. Relative CAT activities of promoter/reporter gene constructs**

<table>
<thead>
<tr>
<th>Plasmid construct</th>
<th>Uninfected cells</th>
<th>s.e.m. (±)</th>
<th>Infected cells*</th>
<th>s.e.m. (±)</th>
<th>Induction (fold increase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pFJ5</td>
<td>0.11</td>
<td>0.04</td>
<td>0.92</td>
<td>0.15</td>
<td>8.30</td>
</tr>
<tr>
<td>pFJ10</td>
<td>48.32</td>
<td>8.50</td>
<td>612.30</td>
<td>89.59</td>
<td>12.70</td>
</tr>
<tr>
<td>pgDCAT</td>
<td>4.40</td>
<td>0.79</td>
<td>534.90</td>
<td>94.80</td>
<td>121.56</td>
</tr>
<tr>
<td>pLAT1CAT</td>
<td>1.00</td>
<td>0.28</td>
<td>3.69</td>
<td>0.54</td>
<td>3.69</td>
</tr>
<tr>
<td>pLAT4CAT</td>
<td>1.28</td>
<td>0.29</td>
<td>4.03</td>
<td>1.00</td>
<td>3.14</td>
</tr>
<tr>
<td>pLAT8CAT</td>
<td>2.79</td>
<td>0.27</td>
<td>13.39</td>
<td>1.95</td>
<td>4.70</td>
</tr>
<tr>
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<td>1.73</td>
<td>47.11</td>
<td>19.30</td>
<td>9.20</td>
</tr>
<tr>
<td>pLAT60CAT</td>
<td>8.80</td>
<td>1.17</td>
<td>52.54</td>
<td>20.70</td>
<td>5.90</td>
</tr>
</tbody>
</table>

* Cells were infected at 6 h post-transfection at an m.o.i. of 5 with wild-type HSV-1 strain 17.
constitutive promoter activity of the LAT promoter clones, the effect on promoter activity of infecting the transfected BHK-21 cells with HSV-1 was investigated. The results are shown in columns 4 and 5 of Table 1 and the level of induction above constitutive activity is indicated in column 6.

Infection resulted in very high levels of CAT activity for pgDCAT and pFJ10. However, although their final levels were similar, pgDCAT showed a much greater response to infection (120-fold greater induction) than pFJ10 (13-fold) as would be expected for early and IE promoters in response to HSV-1 trans-acting factors. The activity of the control plasmid pFJ5 also increased following infection, an effect probably due to the presence of cryptic promoters in the vector sequence. The LATCAT plasmids all exhibited relatively low increases in CAT induction in response to infection when compared with the gD promoter. pLAT1CAT was induced only 3.7-fold. With pLAT4CAT, pLAT8CAT and pLAT6CAT the infected cell promoter activity again increased as the distance between the 3' end and the promoter elements was reduced, consistent with the distance effect described above. pLAT6CAT demonstrated the greatest effect showing a 9.2-fold greater induction, which was similar to that of pFJ10 although the final level was still less than a tenth of the activity exhibited by either of the lytic cycle promoter constructs following infection. The level of activity exhibited by pLAT6CAT and pLAT60CAT-transfected cells when infected were similar, although the extent of induction above constitutive activity was 66% less for pLAT60CAT. This again suggests that sequences upstream from the HindIII site at -279 contribute little to LAT promoter activity in BHK cells, and demonstrates that all of the sequences involved in the response to virus infection are confined to the LAT6 fragment.

Activity of promoter/reporter gene constructs in context of the virus genome

To determine whether the activities of these constructs in transient expression assays accurately reflected their behaviour when present in a native context, the LAT promoter/reporter gene fusions described above were inserted into the virus genome using the direct ligation vector system described by Rixon & McLauchlan (1990). Briefly, this system utilizes an HSV-1 variant (1802) in which a unique XbaI restriction enzyme cloning site was engineered at an intergenic position in the short unique genome region (between U5 genes 8/9 and 10/11/12). A series of plasmids was designed for use with this vector (one being pFJ5 used in the above transfection assays) which allowed protein coding sequences to be placed under the control of various promoters, and then isolated as XbaI fragments for insertion into the vector. It was shown that in these circumstances, IE and early HSV promoters behaved as expected in both wild-type and temperature-sensitive versions of the vector (Rixon & McLauchlan, 1990).

To prevent the possibility of genome rearrangements, a version of the 1802 vector was created which had both copies of LAT (from TRL and IRL respectively) deleted between the DraI site (HSV-1 sequence position 118002; also the 5' terminus of the LAT60 clone) to the HpaI site (position 120468) 370 bp downstream from the 3' end of the IE-1 mRNA. This vector (designated 1804) is therefore deleted for all of the endogenous HSV-1 sequences present in the pLATCAT plasmids.

The XbaI fragments from plasmids pLAT1CAT, pLAT4CAT, pLAT8CAT, pLAT6CAT and pLAT60CAT were isolated, purified and introduced into the 1804 vector as described in Rixon & McLauchlan (1990). All of these 1804-derived viruses were given the prefix ‘v’, e.g. the virus derived from pLAT1CAT was designated vLAT1CAT. The integrity of the genomic structure of all recombinant viruses was verified by Southern blots (data not shown). One-step growth curve experiments performed in BHK cells demonstrated little difference in growth characteristics between the recombinant and wild-type viruses (data also not shown). These viruses were used to examine LAT promoter activity during lytic infection of BHK-21 and C1300 Neuro-2A neuroblastoma cell lines.

CAT expression from recombinant viruses

(i) BHK-21 cells

The levels of CAT activity produced by the vLATCAT viruses along with controls vFJ5, vFJ7 and vFJ10 were determined at 6, 12, 24 and 48 h post-infection (p.i.) at an m.o.i. of 5 at 37 °C. vFJ7 has the HSV-2 ribonucleotide reductase small subunit (early gene) promoter driving the CAT gene (Rixon & McLauchlan, 1990). A typical set of data is shown in Fig. 2. For the sake of clarity, the entire data set is presented on a logarithmic plot (Fig. 2a), in addition the data from the vLATCAT viruses alone are presented on a linear plot (Fig. 2b).

Fig. 2(a) shows that the CAT activities exhibited by the lytic cycle promoters in vFJ7 and vFJ10 are at least 10-fold greater than those exhibited by the strongest promoter of the vLATCAT viruses (vLAT6CAT). This parallels the results obtained with pgDCAT and pFJ10 in the transfection experiments and demonstrates that the ribonucleotide reductase promoter in vFJ7 and the gD promoter in pgDCAT are of comparable potency. Fig. 2(b) shows that in accordance with the results obtained by transfection, vLAT4CAT exhibits slightly increased levels of activity over vLAT1CAT, and that
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Fig. 2. CAT activities produced by recombinant 1804-derived LAT promoter/CAT fusion viruses after infection of BHK-21 cells. (a) CAT activities produced by vFJ5, vFJ7, vFJ10 and vLATCAT viruses shown on a logarithmic scale. (b) CAT activities produced by vFJ5, vLATCAT and other viruses shown on a linear scale. BHK-21 cells were infected at an m.o.i. of 5 and incubated at 37°C. CAT activities were determined at 6, 12, 24 and 48 h p.i. and expressed as the percentage [%14C]chloramphenicol converted per h per µg protein. Symbols: Δ, vFJ5; ▲, vFJ7; ■, vFJ10; ●, vLAT1CAT; ○, vLAT4CAT; ●, vLAT6CAT; ▼, vLAT8CAT; ▲, vLAT60CAT.

Activity increases further with vLAT8CAT and vLAT6CAT. vLAT60CAT has slightly less activity than vLAT6CAT, further demonstrating that sequences upstream from position -279 contribute little to LAT promoter activity in BHK-21 cells.

These results therefore demonstrate that the LAT promoter constructs perform in a similar manner to that noted in transfection assays when present in the context of the HSV-1 genome and also that the sequences sufficient and necessary for LAT promoter expression during lytic infection of tissue culture cells lie within the 277 bp HinfI–PvuI LAT6 sequence.

Fig. 3. CAT activities produced by recombinant 1804-derived LAT promoter/CAT fusion viruses after infection of C1300 Neuro-2A neuroblastoma cells. (a) CAT activities produced by vFJ5, vFJ7, vFJ10 and vLATCAT viruses shown on a logarithmic scale. (b) CAT activities produced by vFJ5, vLATCAT and other viruses shown on a linear scale. C1300 Neuro-2A cells were infected at an m.o.i. of 5 and incubated at 37°C. CAT activities were determined at 6, 12, 24 and 48 h p.i. and expressed as the percentage [%14C]chloramphenicol converted per h per µg protein. Symbols: Δ, vFJ5; ▲, vFJ7; ■, vFJ10; ●, vLAT1CAT; ○, vLAT4CAT; ●, vLAT6CAT; ▼, vLAT8CAT; ▲, vLAT60CAT.

(ii) C1300 Neuro-2A cells
Since the LAT gene is the only HSV-1 gene active during latency in neurons, the behaviour of the vLATCAT viruses in a neuron-derived cell line was examined. In this study, the C1300 Neuro-2A mouse neuroblastoma cell line (Augusti-Tocco & Sato, 1969) was employed.

The levels of CAT activity produced by the vLATCAT viruses and by vFJ5, vFJ7 and vFJ10 were again determined at 6, 12, 24 and 48 h p.i. A typical set of data is shown in Fig. 3 which again, for the sake of clarity, is presented on both logarithmic and linear plots. As was
observed with BHK-21 cells, the HSV lytic cycle promoters demonstrated much greater activity than the vLATCAT viruses. The level of activity was similar to that exhibited in BHK-21 cells (although direct comparisons of promoter activity in different cell lines are difficult to make). The activity of the lytic cycle promoters, and the IE-4/5 promoter in particular, is intriguing since at up to 6 h p.i., Kemp & Latchman (1989) could detect virtually no IE mRNA in HSV-1-infected C1300 Neuro-2A cells. The IE-4/5 promoter activity observed here could be explained as a result of a higher m.o.i. overcoming the non-permissiveness of C1300 Neuro-2A cells. Indeed, Kemp & Latchman (1989) demonstrated that increasing both the amounts of virus and the incubation time led to a majority of cells staining with anti-Vmw175 antibody of 48 h p.i.

The pattern of CAT activity exhibited by the vLATCAT viruses (Fig. 3b) differed significantly between C1300 Neuro-2A and BHK-21 cells. Although the relative activities displayed by vLAT1CAT, vLAT4CAT, vLAT8CAT and vLAT6CAT were essentially similar, the activity exhibited by vLAT60CAT in C1300 Neuro-2A cells was over threefold greater than that of vLAT6CAT. This contrasts with the situation in BHK-21 cells, and suggests that sequences upstream from position -279 (the 5' end of LAT6) enhance activity in neuroblastoma cells, and may therefore contain elements which contribute to neuron-specific expression of the LAT gene.

Discussion

The LAT promoter region has been extensively studied using short-term transfection assays. These studies have shown that the core promoter involved in constitutive expression comprises the region containing the conserved blocks 3 to 6 (Fig. 1) identified by McGeoch et al. (1991) from comparison of the sequences of HSV-1 and HSV-2. Data presented here (Table 1) which demonstrate that the main determinants of in vitro promoter activity map to a 277 bp region between -279 and -2 (the LAT6 clone) agree with those of a number of other groups (Javier et al., 1988; Dobson et al., 1989; Steiner et al., 1989; Batchelor & O'Hare, 1990; Zwaagstra et al., 1989, 1990, 1991; Devi-Rao et al., 1991). Zwaagstra et al. (1989) initially identified the LAT promoter using a construct similar to pLAT6CAT in Vero cells, and Batchelor & O'Hare (1990) mapped the main determinants of LAT promoter activity in HeLa cells to the 140 bp region between -138 and +2. They further showed that this activity increased only marginally (twofold) on addition of sequences between -138 and -320. Sequences downstream from the 5' end of m-LAT are not necessary for lytic phase expression. Thus, Devi-Rao et al. (1991) noted that the promoter activity in rabbit skin cells of a 352 bp fragment from -362 to -11 was reduced fivefold upon addition of sequences from -11 to +701. Similarly, pLAT6CAT exhibited fivefold higher activity than pLAT1CAT (-279 to +812) in BHK-21 cells (Table 1). The inverse relationship between the length of downstream sequence and promoter activity that is evident from Table 1 suggests that this effect may simply be a consequence of increasing the separation of the promoter and the CAT open reading frame. However, it is interesting to note that this region contains the conserved sequences 7, 8 and 9 identified by McGeoch et al. (1991), and a direct role for these in modulating LAT promoter activity cannot be ruled out.

McGeoch et al. (1991) identified conserved sequences (1 and 2) upstream from the recognized promoter elements. These sequences also appear to have little effect on constitutive promoter activity. Thus, the results in Table 1 show that an additional 520 bp upstream from the HindIII site at -279 (LAT60; -800 to -2) conferred only a marginal effect on LAT promoter activity (increasing it by 50%). Batchelor & O'Hare (1990) also found that upstream sequences extending from -608 to -320 had only a slight effect, resulting in a minor decrease in activity. A more dramatic effect was observed by Zwaagstra et al. (1990), who found that addition of a further 331 bp (−612 to −281) or 1652 bp (−1933 to −281) upstream from their basic LAT promoter construct (−279 to −2) reduced promoter activity by threefold in BHK cells and mouse L cells and six- to 12-fold in Vero and CV-1 cells.

The results obtained following infection largely confirmed these findings. All the LATCAT plasmids showed increased activity after infection with the greatest effect being observed with pLAT6CAT (9.2-fold increase; Table 1). This can be compared with the HSV lytic promoters for gD (early) which has a similar basal level but is induced to a much greater degree (160-fold) or with the IE-4/5 (pFJ10) promoter which is induced to a similar degree (10-fold) but has a much higher basal level. Devi-Rao et al. (1991) found that their LAT promoter clones behaved in a fashion similar to those described here, with the LAT6 counterpart (the −363 to −12 EagI fragment), being stimulated 3.5-fold. However, Batchelor & O'Hare (1990) found that promoter activities exhibited by their LAT clones (the −608 to −2 PvuII fragment and −138 to +64 PstI fragment) were four- to fivefold lower after infection. These differing results are perhaps a consequence of cell-specific effects; Batchelor & O'Hare (1990) used HeLa cells for their assays, but Devi-Rao et al. (1991) used rabbit skin cells.

Addition of upstream sequences to the LAT6 clone (in LAT60) did not significantly alter the response to HSV-1 infection and these results, as a whole, indicate that
sequences outside the core promoter region (−279 to −2) exert only a minor influence (depending on laboratory and cell line) upon LAT promoter activity in lytically infected non-neuron derived cell lines.

Transfection assays are clearly useful for mapping promoters and providing an insight as to how they are regulated; however, a proper understanding of the LAT promoter can only come from its characterization in the context of the HSV-1 genome. The system we used in this study allows the introduction of promoter/reporter gene constructs directly into a LAT-deleted HSV-1 vector, thus allowing the examination of LAT promoter activity during the infection of tissue culture cells. This should therefore give a more valid comparison of these constructs than that provided by transfection studies. In general, the results with the recombinant viruses support the conclusions obtained from the transfection studies, with the individual LATCAT promoter constructs demonstrating the same activities relative to each other and to the viral lytic promoters. However, the most interesting results were the differing behaviours of vLAT6CAT and vLAT60CAT following infection of BHK-21 fibroblast and C1300 Neuro-2A neuroblastoma cells. In BHK-21 cells the CAT activity induced by vLAT60CAT was marginally less than that of vLAT6CAT (Fig. 2). However, in C1300 Neuro-2A neuroblastoma cells vLAT60CAT activity was more than three times that of vLAT6CAT. Thus the region from −800 to −279 appears to contain sequences which are important for activity of the LAT promoter in neuronal cells. A similar conclusion was reached by Batchelor & O’Hare (1990) who showed using transfection assays that the region from −608 to +1 was an eightfold more potent promoter in IMR-32 neuroblastoma cells than the region from −138 to +1, but was only twice as potent in HeLa cells. Similarly, Zwaagstra et al. (1991) showed that sequences upstream of −279 increased promoter activity up to threefold in immortalized and neuronal cells. Comparison of the HSV-1 and HSV-2 sequences identified two conserved regions in this portion of the genome (conserved regions 1 and 2 in McGeoch et al., 1991). It is interesting to speculate that sequences within these regions are responsible for the enhanced promoter activity in neurons but this has yet to be established unambiguously. Although it is apparent from these studies that sequences upstream of −279 are important for neuronal specificity of the LAT promoter, other studies have demonstrated the importance of downstream sequences as well. Notably, Batchelor & O’Hare (1992) have identified sequences between −258 and −161 that are responsible for a 15-fold enhancement of LAT promoter activity in IMR32 neuroblastoma cells. Similarly, Devi-Rao et al. (1991) demonstrated a sixfold reduction of LAT promoter activity in C1300 Neuro-2A cells following deletion of sequences between −258 and −161 and Zwaagstra et al. (1991) observed a fourfold increase in expression in neuronal cells that was conferred by a region encompassing −283 to −162.

We are currently utilizing the 1804 vector system to characterize the LAT promoter in animal latency systems. In these studies, we have replaced the CAT reporter gene with the lacZ gene, thus allowing the use of histochemical analysis to allow detection of promoter activity in individual cells in latently infected tissues. This should allow a definitive mapping of the minimal 5’ ends of the LAT promoter necessary for LAT gene expression in neurons during latency.

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Javier, R. T., Stevens, J. G., Dissette, V. B. & Wagner, E. K. (1988). A herpes simplex virus transcript abundant in latently infected neurons but this has yet to be established unambiguously. Although it is apparent from these studies that sequences upstream of −279 are important for neuronal specificity of the LAT promoter, other studies have demonstrated the importance of downstream sequences as well. Notably, Batchelor & O’Hare (1992) have identified sequences between −258 and −161 that are responsible for a 15-fold enhancement of LAT promoter activity in IMR32 neuroblastoma cells. Similarly, Devi-Rao et al. (1991) demonstrated a sixfold reduction of LAT promoter activity in C1300 Neuro-2A cells following deletion of sequences between −258 and −161 and Zwaagstra et al. (1991) observed a fourfold increase in expression in neuronal cells that was conferred by a region encompassing −283 to −162.

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