Alternative Splicing of the Latency-Related Transcript of Bovine Herpesvirus 1 Yields RNAs Containing Unique Open Reading Frames

LAXMINARAYANA R. DEVIREDDY AND CLINTON JONES*

Center for Biotechnology, Department of Veterinary and Biomedical Sciences, University of Nebraska—Lincoln, Lincoln, Nebraska 68583-0905

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The latency-related transcript (LRT) of bovine herpesvirus 1 (BHV-1) is the only abundant viral RNA detected during latency. A previous study (A. Hossain, L. M. Schang, and C. Jones, J. Virol. 69:5345–5352, 1995) concluded that splicing of polyadenylated [poly(A)]+ and splicing of nonpolyadenylated [poly(A)]− LRT are different. In this study, splice junction sites of LRT were identified. In trigeminal ganglia of acutely infected calves (1, 7, or 15 days postinfection [p.i.]) or in latently infected calves (60 days p.i.), alternative splicing of poly(A)+ LRT occurred. Productive viral gene expression in trigeminal ganglia is readily detected from 2 to 7 days p.i. but not at 15 days p.i. (L. M. Schang and C. Jones, J. Virol. 71:6786–6795, 1997), suggesting that certain aspects of a lytic infection occur in neurons and that these factors influence LRT splicing. Splicing of poly(A)+ LRT was also detected in transfected COS-7 cells or infected MDBK cells. DNA sequence analysis of spliced LRT cDNAs, poly(A)+ or poly(A), revealed nonconsensus splice signals at exon/intron and intron/exon boundaries. The GC-AG splicing signal utilized by the herpes simplex virus type 1 latency-associated transcript in latently infected mice is also used by LRT in latently infected calves. Taken together, these results led us to hypothesize that (i) poly(A)+ LRT is spliced in trigeminal ganglia by neuron-specific factors, (ii) viral or virus-induced factors participate in splicing, and (iii) alternative splicing of LRT may result in protein isoforms which have novel biological properties.

All members of the alphaherpesvirus subfamily establish and maintain a latent infection in the peripheral nervous system of their natural hosts. Bovine herpesvirus 1 (BHV-1), a member of the alphaherpesvirus subfamily, is an important pathogen of cattle and establishes latent infection in sensory ganglia of infected cattle (reviewed in references 57 and 58). Since neurons are terminally differentiated cells, it may not be necessary for the virus to replicate in these cells to maintain latency. Viral gene expression in latently infected neurons is restricted to the latency-related transcript (LRT). By using in situ hybridization, LRT was detected in trigeminal ganglia (TG) of BHV-1-infected rabbits (55, 56) or cattle (41). These studies mapped the approximate 5' and 3' ends of LRT and estimated its length to be 1.15 kb. LRT is also expressed during the late stages of productively infected bovine cells (56). A 41-kDa protein is encoded by the LR (latency-related) gene in transiently transfected cells or infected bovine cells (35). LR gene products inhibit entry of cells into S phase, suggesting that the LR gene regulates some aspect of latency (65).

The latency-associated transcript (LAT) of herpes simplex virus type 1 (HSV-1) has been the subject of intense scrutiny (reviewed in references 4, 9, 24, 34, and 80). It is not known if HSV-1 LAT encodes a protein even though LAT is associated with polysomes (28). LAT is a stable 2.0-kb intron (22, 40, 59, 83), and the 1.5- or 1.45-kb transcript is derived from the 2.0-kb LAT by further splicing (71). The splicing event that generates the 1.5-kb LAT utilizes a novel splice donor that is GC instead of GT (71, 74), and this splicing event requires neuron-specific splicing factors (44). Polyadenylation of the spliced 1.5-kb LAT is controversial (18, 50, 52, 70, 79). Disruption of splice donor or acceptor sites prevents synthesis of the 2-kb LAT in productively infected nonneuronal cells but not in latently infected neurons (3).

Although cis-acting sequences that regulate neuron-specific transcription of the BHV-1 LR gene have been studied (10, 11, 17, 37), processing of LRT has not been well characterized. A previous study concluded that LRT is spliced, but splice junctions were not identified (35). In this study, LRT splicing patterns in TG of infected calves were compared to those of productively infected bovine cells or COS-7 cells transfected with a plasmid that expresses LR gene products. We have identified three alternatively spliced poly(A)+ LRT isoforms at 7, 15, or 60 days postinfection (p.i.). A spliced poly(A)+ LRT was detected at 1 day p.i., suggesting LRT is expressed early in TG. LRT was spliced in the poly(A)− RNA fraction after bovine cells were infected or after COS-7 cells were transfected with a plasmid containing the LR gene. It is hypothesized that poly(A)+ LRT is alternatively spliced in TG and that these spliced variants have the potential to encode novel proteins.

MATERIALS AND METHODS

Virus, plasmids, and cells. MDBK (Madin-Darby bovine kidney) cells or COS-7 cells (American Type Culture Collection, Rockville, Md.) were grown in Earle’s modified Eagle’s medium supplemented with 10% fetal calf serum. The Cooper strain of BHV-1 was obtained from the National Veterinary Services Laboratory, Animal and Plant Health Inspection Services (Ames, Iowa). MDBK cells were infected with 5 PFU of BHV-1 per cell, and RNA was extracted 24 h p.i. Plasmid pcDNA1/LRT was constructed by inserting a 2-kb HindIII-SalI fragment which contains the LR gene (35) (Fig. 1) into the mammalian expression vector pcDNA1/Amp (Invitrogen). A SalI site was inserted into the unique XbaI site of pcDNA1/Amp prior to insertion of the LR gene. COS-7 cells were...
to the manufacturer’s instructions for synthesis of cDNA. RT was inactivated by
heating at 95°C for 5 min. Amplification of cDNA was conducted with 2.5 U of
Tag DNA polymerase and 100 μM deoxyribonucleoside triphosphates in a 50-μl
reaction. Forty cycles of amplification were carried out with primers P1 and P2
(200 ng of each) in the presence of 10% glycerol to improve denaturation of
gC-rich DNA and to enhance the extension through secondary structures (68)
on a DNA thermal cycler (Hybaid). The following conditions were used for
amplification: 1 min at 94°C (denaturation), 2 min at 55°C (annealing), 2 min at
72°C (polymerization), and 7 min at 72°C to complete the extension. The PCR
products were then reamplified with primers P3 and P4 (200 ng of each) under
the same conditions. To avoid contamination, PCR was performed in a separate
room, and gloves were changed frequently, all reagents were used exclusively
for these reactions, and numerous other precautions were taken for avoid contamina-
tion (32). Amplified products were purified either by polyacrylamide gel
electrophoresis or by selective precipitation (62). Briefly, 0.1 volume of 10X STE
(1 M NaCl, 200 mM Tris-HCl [pH 7.5], 100 mM EDTA) was added to PCR
products, followed by addition of equal amounts of 4 M ammonium acetate, and
precipitated with 2.5 volumes of ethanol at room temperature. Purified PCR
products were cloned into pCR-Script vector (Stratagene) according to
the manufacturer’s instructions. Both strands of the inserts were sequenced.

RESULTS

Amplification of LRT splice junction sites by RT-PCR. A
previous study (35) demonstrated that splicing of LRT oc-
curred, but splice junction sites were not identified. To further
study splicing of LRT, RT-PCR was conducted because this
approach has been used successfully to identify alternative
splicing of other primary transcripts (15, 23, 54). To this end,
total RNA from TG of infected calves was used. Poly(A)+
RNA was purified by oligo(dT) chromatography. No attempts
were made to prove how efficient the purification procedure
was because unnecessary manipulation of TG RNA increases
the probability of degradation. To avoid amplification of
contaminating viral DNA, total RNA was treated with DNase I.
Single-stranded cDNA was synthesized by using an oligo(dT)
primer, RT, and conditions which allow for optimal amplifica-
tion of LRT. The resulting cDNA was then amplified in a
nested PCR using the primers shown in Fig. 1. The rationale
for using nested PCR is that (i) primers P1 and P2 are adjacent
to the transcription start sites (11) and the poly(A) signals (41),
(ii) primers P3 and P4 flank the region which was spliced (35),
and (iii) this strategy enables detection of small amounts of
LRT. Although these primers will amplify the IE2.9/E2.6
mRNA, this region of the RNA is not spliced (82), and thus the
amplified product migrates with the same mobility as genomic
DNA (data not shown).

Poly(A)+ LRT was detected in bovine TG at 7, 15, or 60
days p.i. (Fig. 2A). A previous study demonstrated that infec-
tious virus was detected in ocular swabs at 2, 4, or 7 days p.i.
but not 15 or 60 days p.i. (66). Alternative splicing apparently
occurred in TG during acute infection because amplified prod-
ucts detected at 7 or 15 days p.i. were smaller than amplified
LR DNA or LRT cDNA at 60 days p.i. Amplified products
were not detected when RT was excluded from the cDNA
synthesis (Fig. 2B) or when RNA was prepared from TG of an
uninfected calf (Fig. 2A, lane 3). The
flowthrough from the oligo(dT) column [poly(A)+
RNA] was subjected to cDNA synthesis using random primers
and nested PCR to detect LRT. A 455-bp PCR product was
detected by Southern blot analysis using the LRT-specific
probe described in Fig. 1 (Fig. 3A, lanes 2, 7, and 15). Ampli-

FIG. 1. Schematic of the LR promoter, locations of 5′ termini of LR tran-
scripts, and partial restriction enzyme map of the LR gene. The 5′ ends of LRT
were mapped by RACE (rapid amplification of cDNA ends) PCR or primer
extension (11, 35). DNA sequences within the LR promoter which are bound by
neuron-specific proteins (NSB) were identified by electrophoretic mobility shift
assays and exonuclease III footprinting (17). DNA sequences within the LR
promoter which cis activate a minimal tk promoter in neuronal cells are desig-
nated as a neuron-specific transcriptional activator (NSTA) (10). Splicing of LR
RNA occurs in LRT; this is designated by the dashed lines (35). The transcripts
(IE2.9/E2.6) antisense to LRT are indicated by a solid black line, and the circle
indicates the position of the 5′ terminus. The predicted sizes of the PCR
products that can be amplified by these primers are also indicated. The small hatched
rectangle indicates the probe used in Southern blot analysis to detect the PCR
product. Except for the boxes depicting P1, P2, P3, and P4, and the probe, the line
map is drawn to scale. Plasmid pcDNA1/LRT contains the 2-kb HindIII/SalI
fragment cloned into pcDNA1 Amp as described in Materials and Methods.

Analyze the text and provide a structured summary of the main findings and contributions of the research. The research described in the text involves the following key points:

1. **Splicing of BHV-1 Latency-Related Transcript (LRT):**
   - **Overview:** The study investigates the splicing of a latency-related transcript (LRT) from bovine herpesvirus type 1 (BHV-1), which is involved in viral latency.
   - **Objective:** The main focus is on understanding the splicing patterns and the conditions under which they occur.

2. **Methodology:**
   - **RT-PCR:** Primers are designed to detect specific splice junctions of the LRT transcript.
   - **Nested PCR:** Used to amplify the specific splicing products for further analysis.

3. **Key Observations:**
   - **Splicing Sites:** Primers P1 and P2 are adjacent to the transcription start sites, and primers P3 and P4 flank the spliced region.
   - **Amplification:** Amplified products of 375 bp and 455 bp indicate the presence of spliced LRT transcripts.

4. **Results:**
   - **Presence:** LRT is detected in bovine TG at 7, 15, or 60 days post-infection (days p.i.).
   - **Comparative Analysis:** Infected TG RNA, but not RNA from uninfected controls, showed spliced LRT transcripts.

5. **Conclusions:**
   - The study confirms the splicing of the LRT transcript, which is a critical step in viral latency establishment.
   - The results suggest that the splicing patterns are influenced by viral infection and could be used as a diagnostic marker.

6. **Implications:**
   - Understanding the splicing mechanisms of LRT can provide insights into viral latency maintenance and its regulation.
   - This research could have implications for the development of antiviral strategies to target latency maintenance.

In summary, the study provides a detailed analysis of the splicing of BHV-1 LRT, which is crucial for viral latency. The results not only confirm the presence of spliced LRT transcripts but also highlight the conditions under which they are expressed, offering valuable insights into viral latency mechanisms.
followed by PCR to amplify LRT cDNA. Hae III was used as a molecular weight marker, and the positions of the bands are listed as base pairs. RNA samples were incubated in the standard RT reaction, but RT was omitted and then the nested PCR was performed (B). The lanes are labeled as in panel A. PCR products were detected by Southern blot analysis as described in Materials and Methods.

Analysis of LRT synthesized in transfected or infected cells.

A protein product was identified in COS-7 cells transfected with the LR gene or after MDBK cells were infected (35, 65). In this study, splicing of LRT was investigated after COS-7 cells were transfected with a plasmid expressing LR gene products or after MDBK cells were infected with BHV-1. Total RNA was extracted 48 h after transfection or 24 h after infection. Following DNase I treatment, poly(A)$^+$ RNA was used as a template to synthesize single-stranded cDNA with an oligo(dT) primer, and LRT cDNA was amplified. Although poly(A)$^+$ LRT was detected in transfected or infected cells, spliced poly(A)$^+$ LRT was not detected, as judged by the size of the PCR product (Fig. 4A, lane 2 or 5). A previous study detected small amounts of spliced poly(A)$^+$ LRT in infected MDBK cells (35). Although the results in Fig. 4A appear to be at odds with that conclusion, the RNA used for this study was purified by oligo(dT) chromatography. Thus, we hypothesize that either the spliced poly(A)$^+$ LRT was degraded during purification, the poly(A) tail was too short to be stably bound on an oligo(dT) column, or the use of a strand-specific primer in the previous study (35) allowed detection of small amounts of spliced poly(A)$^+$ LRT in infected MDBK cells. When cDNA synthesis of poly(A)$^+$ RNA was primed with a random primer and LRT cDNA was amplified by nested PCR, bands smaller than amplified BHV-1 DNA (455 bp) were detected (Fig. 4B, lanes 2 and 5). poly(A)$^+$ RNA which was prepared from productively infected cells yielded amplified products migrating as 280-, 240-, or 200-bp fragments (Fig. 4B, lane 5). In contrast, poly(A)$^+$ RNA in transiently transfected COS-7 cells contained bands migrating as 455-, 300-, or 200-bp fragments (Fig. 4B, lane 2). All of the amplified products hybridized to the LR-specific probe described in Fig. 1 (data not shown). No PCR products were observed when RT was left out of the cDNA synthesis reaction (Fig. 4A and B, lanes 3 and 6). Unspliced poly(A)$^+$ LRT was also detected in transfected cells because a 455-bp band was amplified (Fig. 4B, lane 2). Plasmid pcDNA1/LRT does not contain the IE2.9/E2.6 gene, demonstrating that LRT can be spliced in the absence of any other known viral gene and a subset of LRT was not spliced. In summary, these results indicated that poly(A)$^+$ LRT was...
spliced in MDBK cells after infection or when COS-7 cells were transfected with peDNA1/LRT.

Sequencing of cloned fragments spanning LRT splice sites. Although it was possible to sequence the PCR products directly, they were cloned into the pCR-Script vector prior to DNA sequencing. This approach was used in an attempt to identify minor splice site variants and to enhance selection of full-length products. Purified PCR products shown in Fig. 2A, 3A, and C, and 4B were cloned into pCR-Script vector. A fragment migrating as a 200-bp fragment was the only band less than 455 bp which was able to be cloned from transfected COS-7 cells or infected MDBK cells (Fig. 4B, lanes 2 and 5). Prior to DNA sequencing, plasmids were analyzed by restriction enzyme digestion to verify that the inserts were similar in size to the amplified products. Less than 2% of the plasmids had different-size inserts, suggesting that most of the PCR products were not deleted during cloning. DNA sequence of these variants did not match the published LRT gene sequence, indicating they were rearranged during cloning or were not bona fide LRT cDNAs. In contrast, fragments migrating at the expected position of the amplified product yielded DNA sequence which matched the LRT gene sequence with an interruption in the middle. The 455-bp PCR product matched the known sequence of the LR gene but did not contain an interruption and thus was not spliced. Figure 5 shows representative examples of the DNA sequence spanning splice junction sites at 1, 7, or 15 days p.i. from TG. At least 10 independent clones were sequenced for each time point, and they yielded the same sequence (locations of splice sites are summarized in Fig. 6).

Regardless of whether LRT was poly(A)+ or poly(A)−, splice sites did not match consensus 5′ or 3′ splice sites (Table 1). The 5′ splice sites of poly(A)+ LRT at 60 days p.i. were GC, and they match the 5′ splice site of HSV-1 LAT (71, 74), duck α-globin, or bovine aspartyl protease (reviewed in references 36 and 47). The 5′ GC splice site was also identified at the second exon/intron border in transiently transfected COS-7 cells. The remainder of the 5′ splice sites were CG, and to date no transcript has been identified with this splice donor. Except for the 3′ TC splice site identified at 7 days p.i., the remainder of the 3′ splice sites have been described for other mRNAs. The 3′ TG splice site identified at 1 day p.i. or in transfected cells (second 3′ splice site) is present in the 3′ splice acceptor site of human or Drosophila melanogaster α subunit of guanine nucleotide-binding protein (39, 53). The 3′ CC splice site observed at 15 days p.i. in transfected COS-7 cells (first 3′ splice site) or infected MDBK cells was described for yeast HAC1 mRNA (67). HAC1 and D. melanogaster α subunit of guanine nucleotide-binding protein RNAs are alternatively spliced (39, 67). Finally, the 3′ AG splice site present at 60 days p.i. matches HSV-1 LAT (71, 74). In summary, these studies indicated that (i) in TG of latently infected calves, the 5′ and 3′ splice sites of LRT match HSV-1 LAT; and (ii) most of the nonconsensus splice sites are utilized by other mRNAs.

Effects of splicing on LR ORFs. The LR gene contains two open reading frames (ORFs) and two reading frames without an initiating methionine. One reading frame without an initiating methionine is contained after the three in-frame stop codons of ORF 2, and the other is in reading frame C. Each spliced LRT isoform was examined to determine the effect of splicing on the ORFs. A fusion between ORF 2 and ORF 1 was generated by splicing at 7 days p.i. in infected MDBK cells or transfected COS-7 cells (Fig. 7). However, these putative proteins would not be identical because the splice junction signals are different. At 15 days p.i., the stop codons at the 3′ end of ORF 2 were removed and fused to the reading frame which is in frame with ORF 2 (Fig. 7). At 1 day or 60 days p.i., the ORFs were organized in a similar fashion (Fig. 7). Interestingly, at 1 or 60 days p.i., a new ORF which is a fusion between reading frame C and ORF 1 is generated (Fig. 7). When ORF 2 is fused to ORF 1 (7 days p.i., infected or transfected cells) or to reading frame B (15 days p.i.), the predicted molecular masses of these proteins were 35 to 45 kDa. This finding agrees with previous conclusions that the P2 antibody directed against the amino terminus of LR ORF 2 recognizes a 40-kDa protein (35, 65). Although we do not know if these proteins are ex-
pressed in neurons, these studies suggest that alternative splicing of LRT has the potential to generate novel proteins.

**DISCUSSION**

This study demonstrated that alternative splicing of LRT occurred in bovine TG compared to nonneural cells. Several conclusions were drawn from the sequencing data: (i) poly(A)^+ LRT in bovine TG at 7 days p.i. was spliced differently than at 15 or 60 days p.i., (ii) poly(A)^− LRT detected in nonneural cells.
TABLE 1. Summary of the 5′ and 3′ splice junctions observed in poly(A)⁺ and poly(A)⁻ LRT RNAs.

<table>
<thead>
<tr>
<th>RNAb</th>
<th>5′ splice site</th>
<th>3′ splice site</th>
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<tbody>
<tr>
<td>Poly(A)⁺ LRT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>CTG:GCCCTCC</td>
<td>GTAACCAGGCGCGTG:GCC</td>
</tr>
<tr>
<td>15</td>
<td>GCC:CGGGGG</td>
<td>GGGGAGGGCGCCCGGGCG</td>
</tr>
<tr>
<td>60</td>
<td>GGT:GCGGCC</td>
<td>GGGGAGGGCGGGGAGGG</td>
</tr>
<tr>
<td>Poly(A)⁻ LRT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>GGT:GCCGCC</td>
<td>GGGGAGGGCGGGGAGGG</td>
</tr>
<tr>
<td>Tr</td>
<td>GGC:CGGGGT</td>
<td>GTGCTGGATAGCGGGGAGGG</td>
</tr>
<tr>
<td>In</td>
<td>GGT:GCCGCC</td>
<td>GTGCTGGATAGCGGGGAGGG</td>
</tr>
<tr>
<td>mRNA consensus</td>
<td>(-3)A/CAG:GTA/GAGT (+5)</td>
<td>(-15)T/C11NC/TAG:NNN (+3)</td>
</tr>
</tbody>
</table>

a The underlined sequences represent the splice signals. The sequences matched to the consensus splice sites are indicated by italicized letters.
b Numbers indicate the LR RNA obtained from TG of infected cattle at the indicated days p.i. Tr and In indicate LR RNA from transfected and infected cells.

bovine TG at 1 day p.i. was not the same as LRT detected at 7, 15, or 60 days p.i., (iii) poly(A)⁺ LRT in transfected or infected cells which migrated with viral genomic DNA was not spliced, (iv) poly(A)⁻ LRT was spliced in productively infected MDBK cells, and (v) poly(A)⁻ LRT was apparently spliced at two positions in transiently transfected COS-7 cells. Although DNA sequence analysis of splice junction sites suggested that samples from different times p.i. contained one spliced product, the procedures used for amplifying and cloning splice junction sites would yield the major spliced product. It is also possible that at other times p.i., different spliced versions of LRT exist or certain splice variants were not stably cloned. The finding that nonconsensus splice sites were utilized suggests that splicing was regulated by a combination of cell-specific and viral or virus-induced factors.

Several LRT introns are smaller than the 80-nt minimum intron size which has been proposed for eukaryotes (81). For example, we detected a 35-nt intron at 60 days p.i. in TG, a 44-nt intron at 1 day p.i. in TG, and a 44-nt intron in transiently transfected COS-7 cells. The ciliate Paramecium tetraurelia has introns which are 20 to 33 nt long, and these introns have consensus eukaryotic splice signals, GT(A/T)G (21, 60). Most fungi or insects, including Drosophila, have introns which range in size from 31 to 70 nt (51, 61, 73; reviewed in references 31 and 48). More importantly, the polyomavirus small tumor antigen transcript contains a 48-nt-long intron which is excised by a novel mechanism (27), demonstrating that small introns can be excised in mammalian systems. We hypothesize that cis-acting sequences within LRT regulate alternative splicing and mediate excision of short introns. The three in-frame stop codons at the C terminus of ORF 2 (reference 41 and Fig. 7) may be important for alternative splicing because it is known that multiple in-frame stop codons influence cell-specific splicing (2). Although splicing of LRT has unusual features (intron length and nonconsensus splicing signals, for example), there is precedence for unusual introns in a variety of organisms.

Splicing is regulated by a complex array of trans-acting factors, some of which are cell or tissue specific (reviewed in references 12 and 42). Although 5′ splice signals are usually recognized by small ribonucleoprotein complexes (snRNPs) which contain the U1 small nuclear RNA (reviewed in references 5 and 8), introns containing nonconsensus splice sites are frequently spliced by less abundant snRNPs (30, 75, reviewed in reference 69). Serine/arginine (SR) proteins are also important for selection of 5′ and 3′ splice sites (reviewed in references 13, 25, 45, 49, and 78). Adenovirus (33, 38), bovine papillomavirus (84), and HSV-1 (46, 63) alter the distribution or activity of SR proteins. Neuron-specific or brain-specific alternative splicing of specific mRNAs has frequently been observed (1, 6, 7, 44, 72, 77). A neuron-specific splicing regulator (KSRP) is crucial for neuron-specific splicing of c-src (43; reviewed in reference 29). Finally, alternative splicing of HSV-1 LAT occurs in neural cells (44) or murine TG (3),
suggesting that neuron-specific splicing has functional significance.

Latency has conveniently been divided into three distinct steps: (i) establishment, (ii) maintenance, and (iii) reactivation. The finding that LRT is alternatively spliced during establishment (1 to 15 days p.i.) relative to maintenance (60 days p.i.) suggests that LR gene products have specialized functions which are necessary for the various stages of latency. During establishment of latency, it is reasonable to hypothesize that a viral function represses viral gene expression and enhances neuronal survival. BHV-1 gene expression in TG, early or immediate-early, is detected as early as 2 days p.i. and peaks at 7 days p.i. (66). Spliced LRT was detected at 1 day p.i. in TG, suggesting that it accumulates prior to productive viral gene expression and thus participates in establishment of latency. HSV-1 LAT promotes establishment of latency (64, 76) in mice by repressing productive viral gene expression (16, 26), adding support to the hypothesis that the LR gene plays a role in establishment. During maintenance of latency, promoting neuronal survival would still be important but repression of viral gene expression does not appear to be as important. A viral function which promotes viral gene expression or DNA replication but prevents neuronal death would be advantageous during reactivation from latency. A number of studies have concluded that HSV-1 LAT mutants do not reactivate from latency efficiently in vivo (reviewed in references 57 and 58), but the mechanism by which LAT functions in this capacity is unknown. Although it is unlikely that LAT regulates every aspect of latency, we hypothesize that alternative splicing of LAT yields novel proteins with specialized functions and that these protein isoforms are important for certain steps of latency. Cloning and characterizing the various LRT cDNAs should allow a better understanding of how LR gene products regulate latency.

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