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The Latency-Associated Promoter of Herpes Simplex Virus Type 1 Requires a Region Downstream of the Transcription Start Site for Long-Term Expression during Latency

J. R. LOKENSGARD,† HERVE BERTHOMME, AND LAWRENCE T. FELDMAN*

Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, California 90024

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The latency-associated transcript (LAT) promoter of herpes simplex virus type 1 (HSV-1) is unique among the many promoters on the viral genome in that it remains active during the latent state. We have previously shown that a DNA fragment comprising the LAT promoter element through the cap site, when moved from the LAT locus to the glycoprotein C gene, is capable of only short-term expression. These and other data suggested that an HSV DNA element from the repeat region, not included in the LAT promoter itself, might be needed to preserve long-term expression. Based on a number of recombinant viruses, we narrowed our search for this putative element to a region 3′ of the LAT transcription start site. In the present study, we have shown that a 1.1-kb DNA fragment containing the putative long-term expression element (LTE) is able to restore latent-phase gene expression to the LAT promoter. The element appeared to function best when it was placed in its natural location, which is 3′ of the LAT promoter; however, partial function was obtained when the LTE was inserted upstream of the LAT promoter in the reverse direction. These data indicate that the LAT promoter region is more complex than originally anticipated and that in addition to requiring both a core promoter and neuronal transcription factor binding sites, the promoter requires a specific region of DNA to prevent its shutoff during a latent infection.

A fundamental aspect of herpes simplex virus type 1 (HSV-1) latency in the peripheral nervous system is the virtual absence of gene expression from the many lytic-cycle promoters. Viral RNA, at the level detectable by in situ hybridization, is found only in a region of the long repeats (4, 18). The RNA generating this intense nuclear signal was termed the latency-associated transcript (LAT) (3, 16, 17, 19, 20). The most abundant of these RNAs is actually a stable intron (6, 9), and the promoter for the entire transcription unit is located almost 700 bases upstream of the intron (1, 7, 12, 15, 22, 23). An interesting question, then, is why the LAT promoter remains active during latency whereas the 70 or more lytic-cycle promoters are inactive.

In an attempt to identify the functional parts of the LAT promoter, we placed over 800 bases of this promoter in front of the *lacZ* gene and inserted this construct into the virus at the glycoprotein C (gC) gene. This upstream LAT promoter construct allows the production of β-galactosidase (β-gal) activity, as evidenced by the neurons staining blue with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (with X-Gal). However, the activity diminished over the course of 3 weeks. We investigated this shutoff of the LAT promoter and found that it occurred in all of the constructs inserted at the gC gene (14). These results suggested that there was not a completely functional LAT promoter in the 800 bases that were moved to the gC gene. Other investigators reported similar findings (5, 10).

We had reported earlier that the long terminal repeat (LTR) of Moloney murine leukemia virus remained active when placed downstream of the LAT promoter in the repeat region. We subsequently showed that the LTR did not act by itself but required DNA elements from the upstream LAT promoter to function (13). This led us to hypothesize that there are two functional elements to latent gene transcription, one being neuronal-specific regions which match transcription factor binding sites to transcription factors present in the dorsal root ganglia (8) and the second being a region of DNA which is required to keep the promoter active.

A simple deduction that can be made from our hypothesis is that the LAT region must contain a region, similar in function to the LTR, that protects the upstream LAT promoter region from shutoff. A comparison of the structures and functions of many of the recombinant viruses we had made led us to conclude that this region must lie downstream of the LAT transcription start site. To test this idea, we added a 1.1-kb DNA fragment, from just downstream of the start site through the first third of the LAT intron, to the LAT promoter-*lacZ* constructs at the gC gene. The data show that this fragment is sufficient to keep the LAT promoter active during latency.

MATERIALS AND METHODS

Viral recombinants. The recombinant viruses utilized in this study are displayed in Fig. 1. Construction of the LAT virus has been previously described (8). It is 908 bp in length and extends from a *Sma*I site in the promoter to a *Sac*II site approximately 40 bases 3′ of the transcription start site. The downstream region is a 1.1-kb DNA fragment extending from a downstream *Pst*I site, 20 bases 3′ of the *Sac*II site, to a *Bst*EII site approximately one-third of the way into the LAT intron. The virus +66(f) contains the same LAT promoter as the LAT virus, with the downstream 1.1-kb region inserted in the forward direction at the *Sac*II site. The virus +66(r) has the same fragment inserted in the direction opposite from its orientation in the wild-type virus at the normal LAT locus. The virus −863(r) has this downstream region inserted 5′ of the LAT promoter region and in the opposite direction.

Animal inoculation. Six-week-old female Swiss Webster or C57 black mice were anesthetized by intraperitoneal injection of 15% pentobarbitol and inoculated via the footpad with 10^7 PFU of each recombinant virus (per mouse) as previously described (8).

Tissue preparation and histochemical staining. Animals were sacrificed at 4 days (acute phase) or 28 days (latent phase) postinfection (p.i.) by inhalation of halothane and perfused through the left ventricle with phosphate-buffered saline followed by a fixative solution consisting of 2% paraformaldehyde and 0.2%

* Corresponding author. Phone: (310) 206-1014. Fax: (310) 206-3865. E-mail: LFELDMAN@MICROIMMUN.MEDSCH.UCLA.EDU.

† Present address: Minneapolis Medical Research Foundation, Minneapolis, MN 55404.

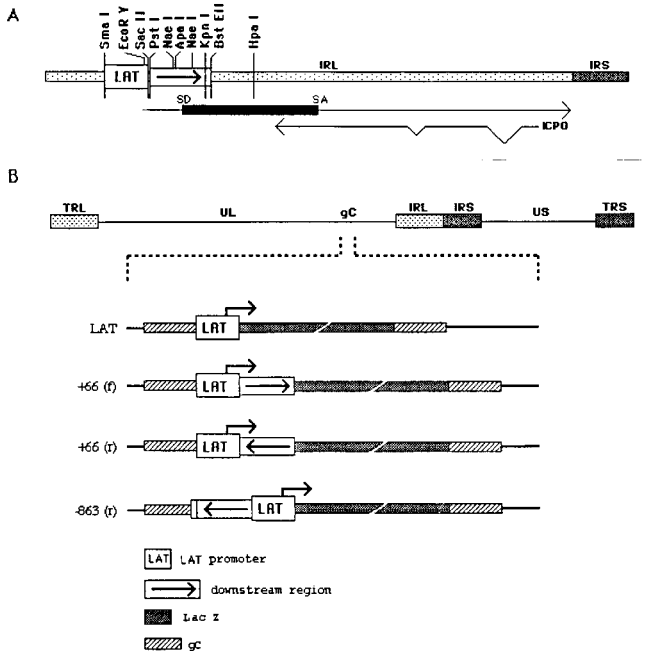

FIG. 1. (A) The region of the LAT transcription unit. The DNA fragment containing the LTE is marked by an arrow in the open box adjacent to the LAT promoter. The LAT intron is marked as a dark band between the splice donor (SD) and splice acceptor (SA) sites. IRL, internal repeat long (long inverted repeat); IRS, internal repeat short (short inverted repeat). (B) Structures of the recombinant viruses. All constructs driving the *Escherichia coli lacZ* reporter gene were inserted into HSV by homologous recombination at the gC locus. LAT, latency-associated transcript promoter (908-bp *Sma*I-*Sac*II fragment); +66(f), LAT promoter plus the downstream fragment (1,114-bp *Pst*I-*Kpn*I fragment) inserted in the forward orientation at position +66 relative to the LAT transcription start site; +66(r), LAT promoter plus downstream fragment inserted in the reverse orientation at position +66; −863(r), LAT promoter plus downstream fragment (1,225-bp *Pst*I-*Bst*EII fragment) inserted in the reverse orientation at position −863 relative to the LAT transcription start site. TRL, terminal repeat long (long tandem repeat); TRS, terminal repeat short (short tandem repeat); UL, unique long region; US, unique short region.

glutaraldehyde in phosphate-buffered saline. Their dorsal root ganglia (L3 to S1) were microdissected and subjected to whole-mount histochemical staining with X-Gal (Sigma) as previously described (8). Additional ganglia from unfixed mice were snap frozen for RNA extraction and RNase protection assays.

RNA isolation and RNase protection assay. Total RNA was isolated from homogenates of pooled murine dorsal root ganglia by using the GLASSMAX RNA microisolation system (Life Technologies, Gaithersburg, Md.). RNase protection assays were performed with the RPA II kit (Ambion, Austin, Tex.) according to the manufacturer's instructions. Briefly, 3 μg of ganglionic RNA was ethanol precipitated along with 1.5×10^6 cpm of full-length, gel-purified antisense *lacZ* probe and 5×10^5 cpm of purified antisense LAT RNA probe. The RNA pellets were resuspended in 20 μl of hybridization buffer (solution A), heated to 90°C for 3 min, and incubated overnight at 45°C. Following hybridization, the remaining single-stranded RNA probe and unhybridized sample RNA were removed by digestion with a 1:100 dilution of an RNase A-RNase T_1 mixture (solution R); this was followed by RNase inactivation, precipitation, and resuspension in gel loading buffer. Samples were separated on denaturing 5% acrylamide gels (19:1 acrylamide/bisacrylamide) and autoradiographed. Linearized plasmid templates for use as RNA markers were purchased from Ambion.

RESULTS

Detection of *lacZ* and LAT transcripts during acute and latent phases of infection of murine dorsal root ganglia. Previous data from our laboratory showed that LAT promoter constructs placed in the unique long region at the gC gene would only support transcription for a limited period of time. Such constructs, driving the *lacZ* gene, were no longer positive for *lacZ* mRNA by 8 days p.i., and by 28 days p.i. the infected ganglia, stained with X-Gal, were no longer blue (14). These results, as well as those from other laboratories, suggested a shutoff of transcription during the first week of latency (5, 10). To determine why promoter constructs at the gC gene were shut off whereas those at the normal LAT locus in the long repeat remained active, we compared the structures of a number of recombinant viruses constructed in the laboratory. In several cases, we had cloned the LAT promoter as a DNA fragment from approximately positions −863 to +45 relative to the start of transcription and yet such promoters were inactive at the gC gene. Similarly, we had constructed KOS-62, in which the *lacZ* gene was placed downstream of the LAT promoter at the LAT locus but with the *Pst*I-to-*Hpa*I fragment deleted (Fig. 1). This virus also did not support long-term expression during latency. Because KOS-62 contained the entire normal repeat region upstream of the LAT start site, the lack of long-term expression from this virus suggested that it is the region downstream of the LAT transcription start site that is essential for long-term expression (Fig. 1). To test the idea that the region 3′ of the transcription start site, the downstream region, is required for long-term expression of the LAT promoter during latency, we added a 1.1-kb DNA fragment from this region to each of the LAT promoter constructs.

The control virus, designated here as LAT, contains the LAT promoter, from a *Sma*I site at position −863 to a *Sac*II site at position +45, attached to the *lacZ* gene and inserted into the virus at the gC gene (Fig. 1). In the recombinant virus designated +66(f), the 1.1-kb *Pst*I-to-*Bst*EII fragment was inserted between the LAT promoter and the *lacZ* gene. The LAT region in this virus is similar to that in the wild-type virus with the exception that the +66(f) virus does not contain the 21 bases between the *Sac*II and *Pst*I sites. The insertion of this DNA fragment in front of the *lacZ* mRNA's open reading frame results in a lack of functional β-gal protein. Murine dorsal root ganglia taken from animals infected with this virus are negative for blue neurons by X-Gal staining during both acute and latent infections (data not shown). Because the DNA was inserted upstream of the β-gal open reading frame in viruses with this construct, LAT promoter activity was assayed by RNase protection assays of the *lacZ* mRNA.

Figure 2A shows the results of an RNase protection assay for both *lacZ* and LAT transcripts. Total RNA was isolated from murine dorsal root ganglia at either 4 or 28 days p.i. The LAT intron was used in these experiments as an internal control for the extraction of RNA from the ganglia. Figure 2A shows that approximately equal amounts of LAT RNA were extracted in all four cases (lanes 6 to 9). The presence of a small LAT band at 100 bases may be due to a cryptic splice donor site (AGGT) present in the intron. At a 12-h exposure, the *lacZ* signals are extremely faint, due to the difference in RNA stability between the LAT intron and the *lacZ* mRNA, so the autoradiogram was exposed six times longer (Fig. 2C). In the LAT virus, the *lacZ* RNA is detected at day 4 but not at day 28 (Fig. 2C, cf. lanes 6 and 7). By contrast, the *lacZ* mRNA was detected at both 4 and 28 days p.i. in the mice infected with virus +66(f) (Fig. 2, lanes 8 and 9).

These data indicate that a DNA fragment of 1.1 kb in size is capable of at least partially restoring the function of continued transcription in latency to the upstream LAT promoter. This is seen from a comparison of lanes 7 and 9 of Fig. 2. However, it does not appear that full protection is achieved, since there is a stronger band at 380 bases in Fig. 2A, lane 8, than in lane 9 of that panel.

Effect of position of downstream region on latent promoter expression. To examine the effect of the position of the downstream region with respect to the LAT promoter, the 1.1-kb

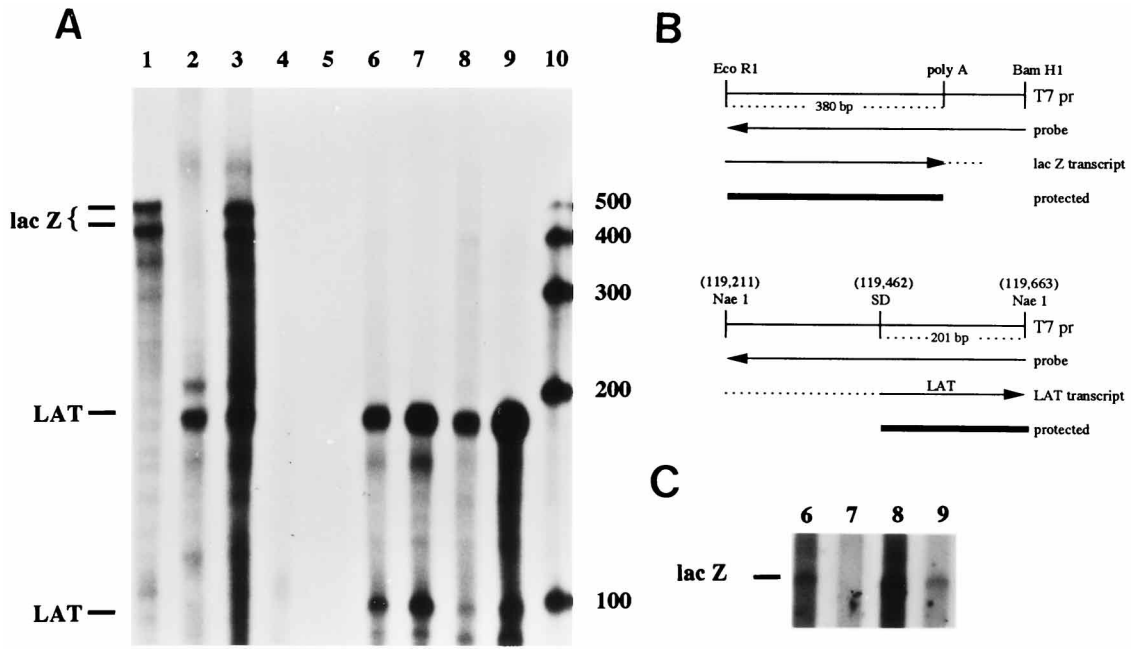

FIG. 2. Detection of *lacZ* and LAT transcripts during acute and latent infection of murine dorsal root ganglia. (A) RNase protection assays (12-h exposure). Lanes: 1 to 3, rabbit skin fibroblasts infected with the LAT-LTR virus and probed for *lacZ*, LAT, or both *lacZ* and LAT, respectively; 4, uninfected rabbit skin fibroblasts probed for both transcripts; 5, empty; 6 and 7, total RNA isolated from murine dorsal root ganglia infected with LAT at 4 or 28 days p.i., respectively; 8 and 9, +66(f) at 4 or 28 days p.i., respectively; 10, RNA markers. (B) Diagram of runoff transcripts utilized as probes and lengths of protected fragments. T7 pr, T7 promoter; SD, splice donor site of LAT intron. (C) Seventy-two-hour exposure of lanes 6 to 9 of panel A.

region was inserted into the LAT virus at a position 5′ of the LAT promoter (nucleotide −863). Perhaps because of the presence of a third copy of the LAT promoter and a third copy of the downstream region, we were not able to obtain a virus in which the downstream region was inserted in the forward direction. However, we were able to isolate and purify a recombinant virus in which the downstream region was inserted in the backwards or reverse direction, upstream of the LAT promoter. This virus was designated −863(r), and it has an advantage over virus +66(f) in that the insertion of the downstream region does not interfere with translation of the *lacZ* mRNA. In these infections, as discussed in the introduction, the LAT virus showed β-gal activity only at 4 days p.i., not at 28 days (Fig. 3a and 3b). By contrast, insertion of the downstream region at position −863 resulted in the retention of a small amount of β-gal activity (Fig. 3d). This activity remained present even at 40 days p.i. (data not shown). We have previously shown that the control virus loses β-gal activity by 28 days p.i.; in fact, we have made other viruses whose initial expression of β-gal was far greater than that of the LAT virus and yet lack this activity at 28 days p.i. (13). Therefore, the continued expression of β-gal activity in −863(r)-infected neurons is due to continued expression of the promoter, not to β-gal protein stability. In summary, these data indicate that the insertion of the long-term expression element (LTE) fragment in the direction opposite that of and upstream of the LAT promoter restores some long-term expression from the LAT promoter, although it is only partially effective at best.

Effect of orientation of downstream region on detection of latent *lacZ* transcripts. To examine the effect of the orientation of this downstream region on long-term expression, the 1.1-kb DNA fragment was inserted into wild-type virus at position +66 in the reverse orientation. Despite multiple attempts to visualize RNA, *lacZ* mRNA was never detected in a latent infection with this virus, +66(r) (Fig. 4). This result, and the low level of β-gal activity produced in infections with virus −863(r), led us to examine the level of *lacZ* mRNA in −863(r) infections. As with virus +66(r), multiple infections and assays failed to yield a detectable level of transcript under these conditions. It would appear that insertion of the 1.1-kb DNA fragment works better in the forward than in the reverse orientation, although even with insertion in the forward direction the level of mRNA made is barely detectable in our assays.

Mapping of LAT-*lacZ* initiation site during latent infection with virus +66(f). It has previously been suggested that there is a second promoter active in latency, termed LAP2 (11). This promoter is approximately 600 bases in size and comprises roughly the first half of the LTE DNA fragment we were analyzing. The original promoter, sometimes referred to as LAP1, is just upstream of these 600 bases. In our assays of the *lacZ* mRNA, we had previously used a probe for the 3′ end, as that end was common to all viruses in this study. To determine whether the latent RNA was initiated by LAP1 or LAP2, we examined the 5′ ends of the RNAs from mice acutely and latently infected with virus +66(f). In some ways this is an interesting experiment, since the viral RNA on either side of the LAT intron is normally degraded during an acute or latent infection. However, we hypothesized that by fusing the 5′ end of the LAT primary transcript to the *lacZ* gene, the fused mRNA might acquire a stability more similar to that of the *lacZ* mRNA. This might be because the fused RNA was 80% *lacZ* or because the 3′ ends of mRNAs are important factors in the RNA half-life. In any case, the fused RNA was more stable than the spliced product of the LAT primary transcript. As seen in Fig. 5, a band of 437 bases was observed for the RNA of basal root ganglia infected for either 4 or 28 days. This is the expected size for a fused RNA initiated by LAP1. Using this

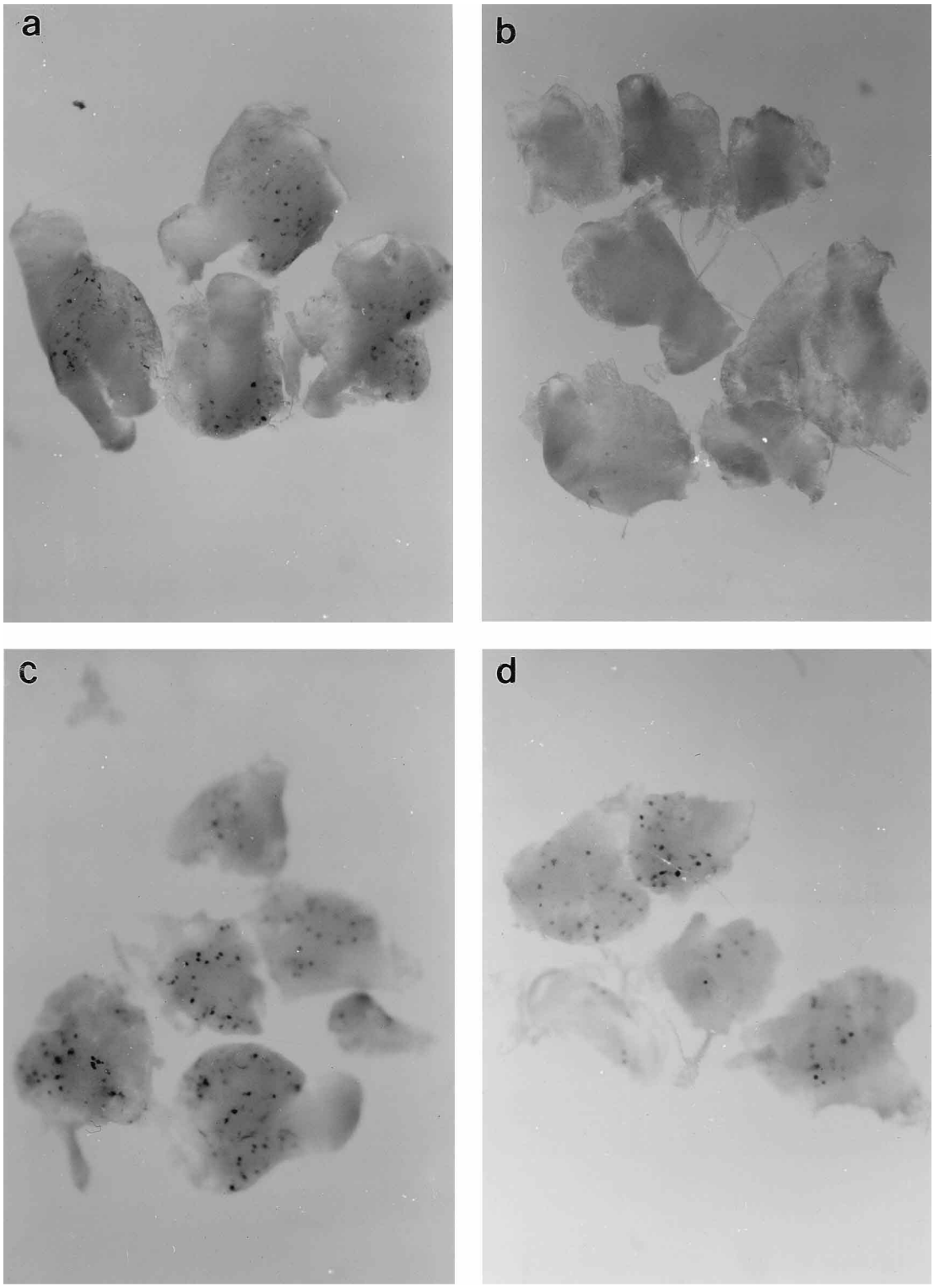

FIG. 3. Photomicrographs of whole-mount dorsal root ganglia. Mice were infected via footpad inoculation with 10^7 PFU of each recombinant virus. Their ganglia were removed at times representing the acute (4 days p.i.) and latent (28 days p.i.) phases of infection. Following excision, the ganglia were incubated at 37°C overnight with X-Gal (1 mg/ml), and neurons expressing β-gal activity from the viral genome stained blue. (A) LAT virus at 4 days p.i.; (B) LAT virus at 28 days p.i.; (C) virus −863(r) at 4 days p.i.; (D) virus −863(r) at 28 days p.i. Magnification, ×3.

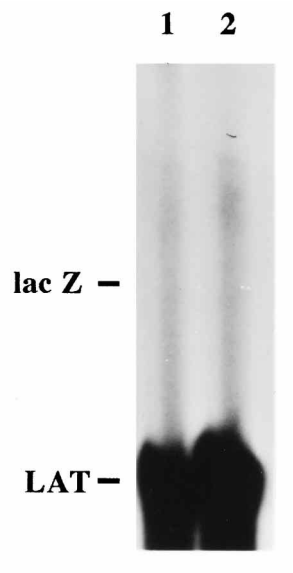

FIG. 4. Effect of orientation of downstream region on detection of latent *lacZ* transcripts. RNase protection assays were performed on total RNA from murine dorsal root ganglia infected with virus −863(r), isolated at 28 days p.i. (lane 1), or with virus +66(r), isolated at 28 days p.i. (lane 2).

particular probe, which differs from the LAT locus at the *Sac*II site (Fig. 1), we would expect to see a band at around 350 bases for LAT RNA coming from the LAT locus in the repeat region. Such a band was not observed, indicating that the fused RNA was more stable. There was a shorter band running at 280 bases; this RNA could represent a spliced version of the fused transcript, or it could have been an internal initiation or even a trapped degradation product of the primary transcript which had been stabilized by its fusion to the *lacZ* mRNA. As these possibilities extend beyond the focus of this work, this RNA was not further characterized.

Finally, it should be noted that the 437 bases of the fused LAT-*lacZ* mRNA comprise the 5′ end of the LAP2 promoter; it is therefore clear that the transcript we were assaying during latency was initiated from LAP1. This is in agreement with the recent studies of Chen et al. showing that LAP2 does not function as a promoter during latency (2).

DISCUSSION

In our previous work we showed that many promoters shut off in the first week of latency, including the LAT promoter attached to the *lacZ* gene (14). We also showed that addition of the LTR of Moloney murine leukemia virus to the LAT promoter enabled the LAT promoter to stay on during latency while not remaining active by itself (13). This suggested that the LTR performed some long-term expression function for the LAT promoter.

We also examined the structures and functions of a number of recombinant viruses that we have produced in our laboratory over the past 8 years. Only two viruses, the wild-type virus, which expresses LAT, and the β-globin virus, which expresses rabbit β-globin from the LAT promoter, continue to express RNA during latency (7). Both of these viruses contain the downstream LTE region near the promoter. In the case of rabbit β-globin, the LTE is 2 kb downstream of the LAT promoter. By contrast, the many viruses that we have constructed with insertions at the gC gene lack a proximal LTE, and they all shut off transcription during latency. Similarly, KOS-62, which has the *lacZ* gene inserted at the LAT locus, is not transcriptionally active during latency, as we had to delete the LTE from the *Pst*I site to the *Hpa*I in order to fit two copies of the *lacZ* gene into the virus. Thus, while KOS-62 has the *lacZ* gene inserted into the repeat region and has all the upstream DNA that the wild-type and β-globin viruses have, it does not continue to express *lacZ* mRNA during latency (14).

These facts strongly suggested to us that the region extending approximately from the *Pst*I site to the *Hpa*I site constituted a region required for long-term expression of the LAT promoter. For this reason, we dubbed this region the LTE. In the present work, we demonstrated that a 1.1-kb DNA fragment from the LAT transcription unit was able to restore long-term expression to the LAT promoter. This DNA fragment appeared to function better when it was placed in its natural position, which is downstream of the LAT transcription start site. It appeared to function less well when placed upstream of the LAT promoter, in the backwards direction.

The structure and function of RNA from the LAT locus have been the subject of considerable research in a number of laboratories. It is interesting that not a single mRNA structure has been identified from the entire 8.5-kb transcription unit. Furthermore, the DNA lying between the LAT promoter and the intron has been the subject of some debate. Glorioso and colleagues have referred to this region as a second LAT promoter, termed LAP2 (11). Recently they showed that this promoter is not active in latency and that the LAP1 promoter

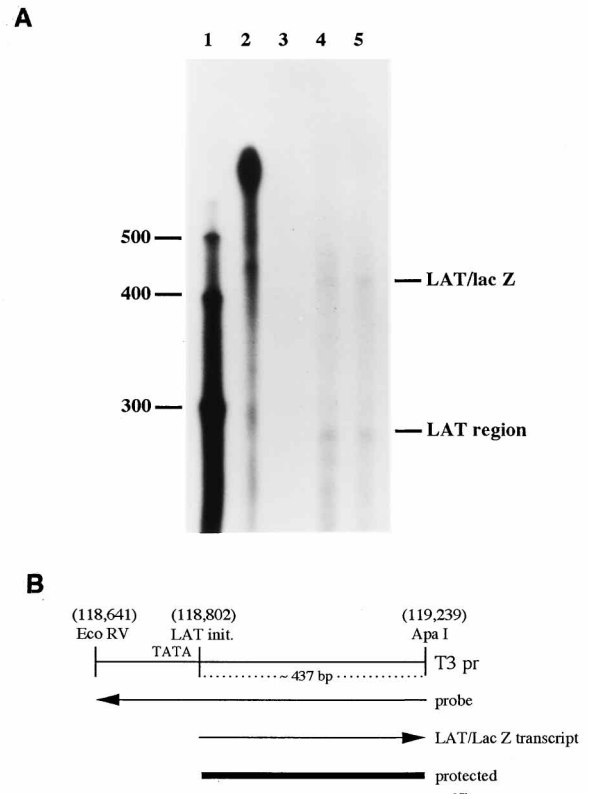

FIG. 5. Mapping of the LAT-*lacZ* initiation site during latent infection with virus +66(f). (A) Lanes: 1, RNA markers; 2, probe; 3, empty; 4 and 5, total RNA isolated from +66(f)-infected murine dorsal root ganglia at 4 or 28 days p.i., respectively. (B) Diagram of the runoff transcript utilized as a probe and length of the protected fragment. A protected fragment of 437 bp is expected. T3 pr, T3 promoter.

is essential for latent expression (2). Interestingly, in one experiment, in which they deleted LAP2 from the virus, they saw a two- to three-fold reduction in the level of LAT during latency and concluded that LAP2 contributed to the accumulation of LAT in a LAP1-dependent manner. Similarly, Yoshikawa et al. showed in HSV-2 that a virus deleted for the LAP2 region expressed less LAT intron during latency than the wild-type virus (21). Thus, both the HSV-1 and HSV-2 LAP2 deletions provide evidence that these sequences contribute to the highest level of activity of the LAT promoter during latency. As mentioned earlier, when these and other sequences were deleted from the LAT-*lacZ* construct KOS-62, there was no long-term expression of the *lacZ* gene. The results of all three of these genetic studies are in agreement with those of our current experiments, in which this region, when added back to the LAT promoter-*lacZ* constructs at the gC gene, showed an effect on long-term expression of the LAT promoter in the latent state.

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REFERENCES

1. **Batchelor, A. H., and P. O'Hare.** 1990. Regulation and cell-type-specific activity of a promoter located upstream of the latency-associated transcript of herpes simplex virus type 1. J. Virol. **64:**3269–3279.
2. **Chen, X., M. C. Schmidt, W. F. Goins, and J. C. Glorioso.** 1995. Two herpes simplex virus type 1 latency-active promoters differ in their contributions to latency-associated transcript expression during lytic and latent infections. J. Virol. **69:**7899–7908.
3. **Croen, K. D., J. M. Ostrove, L. J. Dragovic, J. E. Smialek, and S. E. Straus.** 1988. Latent herpes simplex virus in human trigeminal ganglia. Detection of an immediate-early gene "antisense" transcript by in situ hybridization. N. Engl. J. Med. **317:**1427–1432.
4. **Deatly, A. M., J. G. Spivack, E. Lavi, and N. W. Fraser.** 1987. RNA from an immediate early region of the type 1 herpes simplex virus genome is present in the trigeminal ganglia of latently infected mice. Proc. Natl. Acad. Sci. USA **84:**3204–3208.
5. **Deshmane, S. L., T. Valyi-Nagy, T. Block, J. Maggioncalden, J. H. Wolf, A. Dillner, and N. W. Fraser.** 1995. An HSV-1 containing the rat beta-glucuronidase cDNA inserted within the LAT gene is less efficient than the parental strain at establishing a transcriptionally active state during latency in neurons. Gene Ther. **2:**209–217.
6. **Devi-Rao, G. B., S. A. Goodart, L. M. Hecht, R. Rochford, M. K. Rice, and E. K. Wagner.** 1991. Relationship between polyadenylated and nonpolyadenylated herpes simplex virus type 1 latency-associated transcripts. J. Virol. **65:**2179–2190.
7. **Dobson, A. T., F. Sederati, G. Devi-Rao, W. M. Flanagan, M. J. Farrell, J. G. Stevens, E. K. Wagner, and L. T. Feldman.** 1989. Identification of the latency-associated transcript promoter by expression of rabbit beta-globin mRNA in mouse sensory nerve ganglia latently infected with a recombinant herpes simplex virus. J. Virol. **63:**3844–3851.
8. **Dobson, A. T., T. P. Margolis, W. A. Gomes, and L. T. Feldman.** 1995. In vivo deletion analysis of the herpes simplex virus type 1 latency-associated transcript promoter. J. Virol. **69:**2264–2270.
9. **Farrell, M. J., A. T. Dobson, and L. T. Feldman.** 1991. Herpes simplex virus latency-associated transcript is a stable intron. Proc. Natl. Acad. Sci. USA **88:**790–794.
10. **Fink, D. J., L. R. Sternberg, P. L. Weber, M. Mata, W. F. Goins, and J. C. Glorioso.** 1992. In vivo expression of β-galactosidase in hippocampal neurons by HSV-mediated gene transfer. Hum. Gene Ther. **3:**11–19.
11. **Goins, W. F., L. R. Sternberg, K. D. Croen, P. R. Krause, R. L. Hendricks, D. J. Fink, S. E. Straus, M. Levine, and J. C. Glorioso.** 1994. A novel latency-active promoter is contained within the herpes simplex virus type 1 U_L flanking repeats. J. Virol. **68:**2239–2252.
12. **Leib, D. A., K. C. Nadeau, S. A. Rundle, and P. A. Schaffer.** 1991. The promoter of the latency-associated transcripts of herpes simplex virus type 1 contains a functional cAMP-response element: role of the latency-associated transcripts and cAMP in reactivation of viral latency. Proc. Natl. Acad. Sci. USA **88:**48–52.
13. **Lokensgard, J. R., D. C. Bloom, A. T. Dobson, and L. T. Feldman.** 1994. Long-term promoter activity during herpes simplex virus latency. J. Virol. **68:**7148–7158.
14. **Margolis, T. P., D. C. Bloom, A. T. Dobson, L. T. Feldman, and J. G. Stevens.** 1993. Decreased reporter gene expression during latent infection with HSV LAT promoter constructs. Virology **197:**585–592.
15. **Mitchell, W. J., I. Steiner, S. M. Brown, A. R. MacLean, J. H. Subak-Sharpe, and N. W. Fraser.** 1990. A herpes simplex virus type 1 variant, deleted in the promoter region of the latency-associated transcripts, does not produce any detectable minor RNA species during latency in the mouse trigeminal ganglion. J. Gen. Virol. **71:**953–957.
16. **Rock, D. L., A. B. Nesburn, H. Ghiasi, J. Ong, T. L. Lewis, J. R. Lokensgard, and S. L. Wechsler.** 1987. Detection of latency-related viral RNAs in trigeminal ganglia of rabbits latently infected with herpes simplex virus type 1. J. Virol. **61:**3820–3826.
17. **Spivack, J. G., and N. W. Fraser.** 1987. Detection of herpes simplex virus type 1 transcripts during latent infection in mice. J. Virol. **61:**3841–3847.
18. **Stevens, J. G., E. K. Wagner, G. B. Devi-Rao, and L. T. Feldman.** 1987. RNA complementary to a herpesvirus alpha mRNA is prominent in latently infected neurons. Science **235:**1056–1059.
19. **Wagner, E. K., G. Devi-Rao, L. T. Feldman, A. T. Dobson, Y.-F. Zhang, W. M. Flanagan, and J. G. Stevens.** 1988. Physical characterization of the herpes simplex virus latency-associated transcript in neurons. J. Virol. **62:**1194–1202.
20. **Wechsler, S. L., A. B. Nesburn, R. Watson, S. M. Slanina, and H. Ghiasi.** 1988. Fine mapping of the latency-related gene of herpes simplex virus type 1: alternative splicing produces distinct latency-related RNAs containing open reading frames. J. Virol. **62:**4051–4058.
21. **Yoshikawa, T., L. R. Stanberry, N. Bourne, and P. R. Krause.** 1996. Downstream regulatory elements increase acute and latent herpes simplex virus type 2 latency-associated transcript expression but do not influence recurrence phenotype or establishment of latency. J. Virol. **70:**1535–1541.
22. **Zwaagstra, J., H. Ghiasi, A. B. Nesburn, and S. L. Wechsler.** 1989. In vitro promoter activity associated with the latency-associated transcript gene of herpes simplex virus type 1. J. Gen. Virol. **70:**2163–2169.
23. **Zwaagstra, J. C., H. Ghiasi, A. B. Nesburn, and S. L. Wechsler.** 1991. Identification of a major regulatory sequence in the latency associated transcript (LAT) promoter of herpes simplex virus type 1 (HSV-1). Virology **182:**287–297.
24. **Zwaagstra, J. C., H. Ghiasi, S. M. Slanina, A. B. Nesburn, S. C. Wheatley, K. Lillycrop, J. Wood, D. S. Latchman, K. Patel, and S. L. Wechsler.** 1990. Activity of herpes simplex virus type 1 latency-associated transcript (LAT) promoter in neuron-derived cells: evidence for neuron specificity and for a large LAT transcript. J. Virol. **64:**5019–5028.