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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

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The herpes simplex virus type 1 latency-associated transcript (LAT) is expressed as a major species in latently infected mouse neurons. Previous sequence analysis revealed no obvious promoter elements near the 5′ end of the LAT, but a TATA box and other potential promoter elements were found 700 base pairs upstream. A recombinant virus in which the rabbit beta-globin gene was inserted immediately downstream of the TATA box expressed globin mRNA and did not express the LAT. A second recombinant virus, in which this TATA box was removed, was negative for LAT expression in a latent infection. The location of the LAT promoter suggested that RNA upstream of the LAT was synthesized and degraded during latent-phase transcription. Low levels of this RNA were observed by in situ hybridization. In other experiments, RNA from a productive infection was used to detect a transcript extending from the LAT promoter to a polyadenylation signal approximately 8.5 kilobases downstream. These data suggest that the LAT may be processed from a larger transcription unit which begins distal to the TATA box 700 base pairs upstream of the LAT and extends to a polyadenylation signal almost 5 kilobases downstream of the 3′ end of the LAT.

Herpes simplex virus (HSV) can produce latent infections of the peripheral and central nervous systems of humans and experimental animals (J. G. Stevens, Microbiol. Rev., in press). Transcripts from only one region of the viral genome are readily detectable during such an infection, and these have been collectively referred to as the latency-associated transcripts (LATs) (1, 2, 5, 14, 15, 19, 20, 21, 25, 26). Two colinear forms of the LAT have been described, one is an apparently unspliced 2.1-kilobase (kb) transcript while the other contains an approximately 600-base intron (15, 24, 25, 27). Two laboratories have also described a third, slightly smaller species (16, 27). Interestingly, stable LAT is not polyadenylated and is largely confined to the nucleus of latently infected neurons.

The region surrounding the LAT has been sequenced in three strains of HSV type 1 (HSV-1): KOS(M), 17syn+, and F (12, 24, 28). Comparison of sequence data with primer extension and S1 nuclease analysis has positioned the 5′ end of the LAT within the sequence AGGT, which is a potential 5′ splice signal (13). The region just upstream of this site contains no obvious promoter elements such as TATA or CAAT box homologies, although such homologies and two potential SP1-binding sites are found approximately 700 bases further upstream of this point (12, 28) and have been noted by others (27, 28).

These data are consistent with one of two possible models. (i) The LAT is transcribed from an unusual promoter contiguous with its 5′ end, or (ii) the LAT is transcribed from a conventional promoter element, and the stable species observed are derived from an unstable primary transcript.

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MATERIALS AND METHODS

Establishment of latent infection in mice and processing of spinal ganglia. Six-week-old outbred Swiss-Webster mice (Simonson Laboratories, Gilroy, Calif.) were used in all experiments. They were inoculated in each rear foot with 10^8 PFU of HSV-1 strain KOS(M) or with recombinant viruses of this strain. The derivation of this virus strain and the methods used for inoculation have been described previously (20, 22). At 3 or more weeks later, after latent infections had been established in lumbosacral spinal ganglia (7, 18), ganglia were removed from the animals, quick frozen in liquid nitrogen, and either sectioned on a cryostat or stored at −70°C until used.

RNA isolation and fractionation. RNA was isolated from latently infected ganglia by the guanidinium isothiocyanate-hot phenol method as described previously (20). RNA from a productive infection of rabbit skin cells infected at a multiplicity of 10 PFU per cell at 6 h postinfection was isolated as described previously (20, 22). Poly(A)$^-$- and poly(A)$^+$-containing RNA was selected by oligo(dT)-cellulose (Collaborative Research, Inc., Waltham, Mass.) chromatography (3). RNA was size fractionated by electrophoresis with 1.2% agarose gels containing 6% formaldehyde as described previously (25); RNA was transferred by electrophoresis onto nylon membranes (GeneScreen; New England Nuclear Corp., Boston, Mass.).

Analysis of RNA. HSV-1 DNA fragments were uniformly labeled by random hexamer priming with [α-^{32}P]dCTP (3 Ci/mol; Amersham Corp., Arlington Heights, Ill.) (24). Synthetic oligomer probes were 5′-end labeled to a specific activity of 5×10^5 cpm/ng, using [γ-^{32}P]ATP (7 Ci/mol; ICN Biochemicals, Irvine, Calif.) and bacteriophage T4 kinase (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) as described by Maniatis et al. (10).

RNA transfer blots were hybridized in a volume of 5 ml containing 4×10^7 cpm (Cerenkov) of radiolabeled DNA in the presence of 50% formamide–0.4 M Na^+–0.1 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 8.0)–0.005 M EDTA–Denhardt solution containing 100 g of denatured calf thymus DNA per ml at 49°C for 40 h. Details and procedures for washing have been described previously (24). For hybridization with synthetic oligomer probes, blots with the same amount of radioactivity were incubated at 48°C in 6× SSC (1× is 0.15 M NaCl plus 0.015 M sodium citrate)–10× Denhardt solution–0.5% sodium dodecyl sulfate–50 mM Na_2HPO_4 (pH 6.5) for 16 h. Blots were washed as described previously (25). Primer extension experiments were carried out as described previously, using unlabeled primers and [α-^{32}P]dCTP in the extension mix (24). Extension products were fractionated on a denaturing 10% acrylamide gel containing 7 M urea.

RNase protection experiments. The basic methods described in *Current Protocols in Molecular Biology* (4) were used for RNase protection assay of RNA. The RNA probe was made by using a pGEM1 template with Promega Biotec T7 polymerase, following the instructions supplied. Probe was labeled by using 100 μCi of [^{32}P]UTP (800 Ci/mM; Amersham) per μg of template DNA and 15 U of enzyme. The template was prepared by cloning the 207-base-pair (bp) *Pst*I piece containing the LAT promoter sequence into pUC, determining the proper orientation by sequencing, and then cloning the entire *Eco*RI-*Hin*dIII fragment into pGEM. The template was linearized at the *Eag*I site to get a 113-base full-length transcript (73 bases of which is HSV-specific sequence and 40 bases of which is polylinker and the T7 start site) and some premature termination product. The full-length probe was fractionated on a 6% acrylamide gel–8 M urea sequencing gel and eluted for hybridization. A total of 10^6 cpm of purified RNA probe was hybridized with poly(A)$^+$ RNA from 2×10^6 infected cells (10 μg) or poly(A)$^-$ RNA from 10^6 infected cells (40 μg) in 50 μl of 80% formamide–40 mM PIPES [piperazine-*N*,*N'*-bis(2-ethanesulfonic acid)] (pH 6.4)–400 mM NaCl–1 mM EDTA at 56°C for 16 h. The mix was then digested by the addition of 300 μl of 40 μg of RNase A (Sigma Chemical Co., St. Louis, Mo.) per ml–2 μg of RNase T_1 (Sigma) per ml in 10 mM Tris (pH 7.5)–300 mM NaCl–5 mM EDTA for 30 min at 30°C. The digestion mix was then treated with 50 μg of proteinase K (Sigma) at 37°C for 15 min and phenol-chloroform extracted. RNase-resistant material was fractionated on a 12% acrylamide–8 M urea sequencing gel, using DNA size standards. Because RNA migrates at a different rate than single-stranded DNA, we used 273-, 113-, and 54-base RNA transcripts as calibration size markers.

In situ hybridization methods. Appropriate cloned DNA fragments were labeled by nick translation, using ^{35}S-labeled deoxynucleotides to specific activities of 10^8 cpm/μg of DNA (20). Each probe was used on cryostat-cut sections of lumbosacral spinal ganglia taken from latently infected mice. Slide preparations and RNA-specific hybridization reactions were modifications of those described by Haase et al. (6). After a 2-week exposure, the slides were developed, stained with Giemsa, and examined.

Construction of recombinant viruses. Rabbit beta-globin virus was made by inserting a copy of the rabbit beta-globin gene into the HSV-1 genome approximately 700 bases upstream of the 5' end of the LAT at an *Eag*I site 76 bases 5' of the *Pst*I site (Fig. 1). This positioned the beta-globin cap site 26 bases 3' of the potential TATA box. The location of this construction was confirmed by DNA sequencing; the

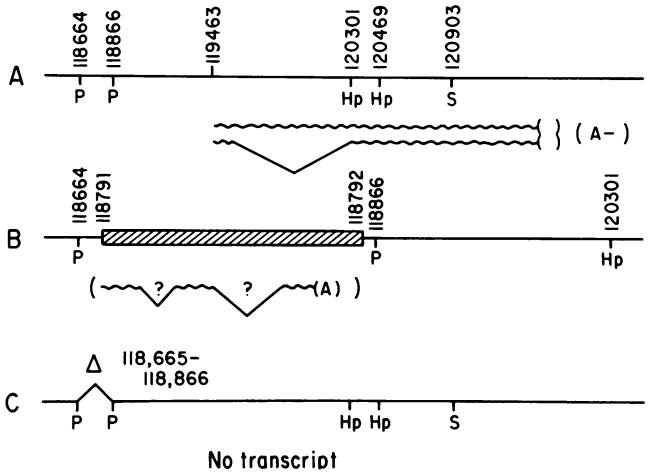

FIG. 1. Recombinant HSV-1 with altered ability to express the HSV-1 LAT during latent infection. The region corresponding to the HSV-1 internal long repeat (IR_l) is shown; these structures are also seen in the terminal long repeat (TR_l). (A) The positions of the spliced and unspliced poly(A)$^-$ nuclear stable LAT species are shown. Nucleotide numbers refer to the 17syn+ strain (11). Restriction sites are: P, *Pst*I; Hp, *Hpa*I; and S, *Sal*I. The previously reported 5' end of the LAT is at base 119,463. The TATA box homology discussed in the text is between bases 118,775 and 118,781. (B) Structure of the globin-containing recombinant virus. The rabbit beta-globin gene was cloned from a *Pvu*II site 10 bases 5' of the cap site through 300 bases 3' of its polyadenylation signal to a *Hin*dIII site within pBR322 (23). This fragment was inserted into the *Eag*I site at base 118,794. The expression of poly(A)$^+$ globin RNA during latency is indicated in parentheses. The correct splicing patterns were assumed from the fact that the size of the RNA comigrated with authentic globin mRNA (see text). (C) Structure of the *Pst*I deletion virus which expresses no transcript during the latent phase of infection.

sequence at the 5' insertion site was (HSV) TATA AAAGCGGGGGCGCGGC/CTGCTGCTTAC (globin). The globin gene contains three exons, a polyadenylation signal, and approximately 300 bases of DNA downstream of the polyadenylation signal (23). The beta-globin gene was inserted into the *Pst*I fragment cloned into pUC19, and then the *Pst*I fragment was cloned into a larger fragment encompassing much of the HSV-1 restriction fragment *Sal*I F. This clone was then used to make recombinant viruses by cotransfection with KOS(M) virion DNA as described previously (8). Viruses were screened in 96-well plates by hybridizing 50 μl of the supernatant with radioactively labeled globin DNA in a dot blot assay. This virus was plaque purified three times by this assay, and six plaques were analyzed by Southern blot analysis. Two viruses showed a complete isomerization of globin to both sides of the repeat region, and one of these was used for the experiments in this report.

The *Pst*I deletion virus (Fig. 1) was made by deletion of the 200-bp *Pst*I fragment from a subclone of HSV-1 restriction fragment *Sal*I F, and this DNA fragment was cotransfected with KOS(M) DNA which had a small *Bst*EII fragment deleted. Recombinant viruses were screened for rescue of this small deletion, and genome structures were confirmed by the absence of the *Pst*I fragment. By Southern blot analysis, this virus was also shown to contain the deletion in both long repeat regions and to have recovered the *Bst*EII fragment.

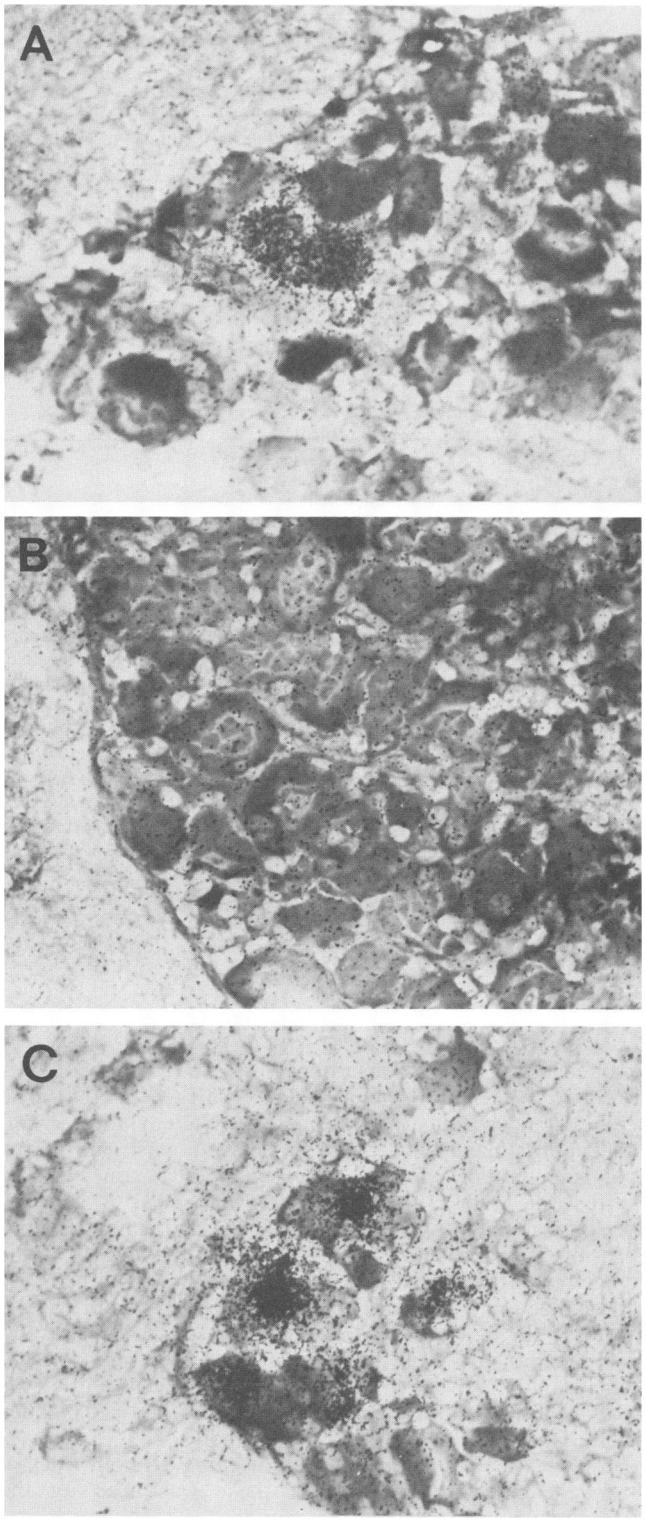

FIG. 2. Hybridization in situ of ^{35}S-labeled beta-globin and HSV-1 DNA probes to RNA in latently infected murine spinal ganglia. Probes with the map positions noted, prepared and hybridized to tissue sections as described in Materials and Methods, were used to detect virus-encoded RNA in ganglionic neurons. (A) Ganglia latently infected with the recombinant globin virus and probed with the *Pvu*II-*Hin*dIII fragment of the beta-globin plasmid. (B) Ganglia latently infected with the recombinant globin virus and probed with ATD 19, a 347-base fragment entirely within the stable LAT RNA sequences (119,628 to 119,975). (C) Ganglia latently infected with wild-type virus and probed with ATD 19.

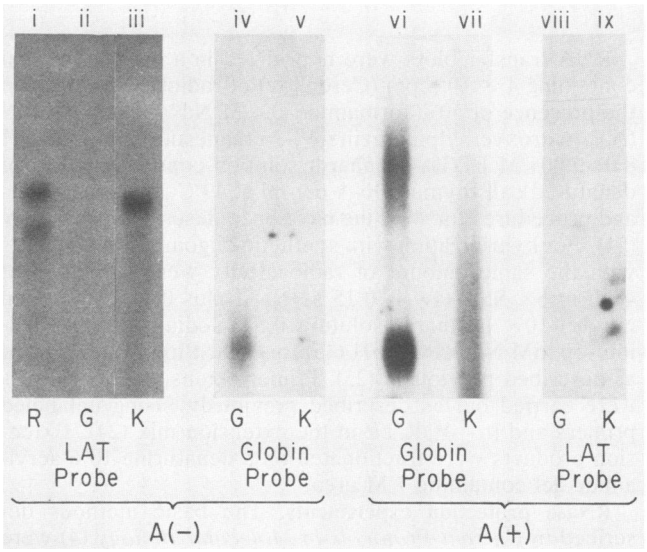

FIG. 3. Northern blot analysis of RNA isolated from mouse sensory ganglia latently infected with HSV-1 KOS(M) and the globin-containing recombinant HSV-1. Ganglia RNA was fractionated into poly(A)$^+$ and poly(A)$^-$ fractions, size fractionated, blotted, and hybridized with ^{32}P-labeled RNA homologous to bases 1 to 1200 of the sequence of the rabbit beta-globin gene (23; Globin Probe) or to bases 119,292 to 119,972 in HSV-1 (LAT Probe). These latter are bounded by an *Sph*I and a *Kpn*I site in the KOS(M) strain. Exposure was for 16 h at −70°C, using intensifying screens. Lanes: G, RNA from ganglia of mice latently infected with recombinant virus (lanes ii, iv, vi, and viii); K, RNA from mice latently infected with the KOS(M) strain of virus (lanes iii, v, vii, and ix); R, RNA from a ganglion of a rabbit latently infected with the McKrae strain of virus (lane i).

RESULTS

Latent-phase expression of poly(A)$^+$ globin RNA from a recombinant virus. The location of the HSV-1 LAT relative to the recombinant viruses used in this study is shown in Fig. 1. In the first recombinant virus, a genomic copy of the rabbit beta-globin gene was inserted immediately downstream of the TATA box. As shown in Fig. 1B, this insertion positioned the cap site of beta globin 26 bp downstream from the putative TATA box. If the TATA element is used to specify the start of transcription in a latent infection, this virus should initiate transcription near the beta-globin cap. This virus was used to establish latent infections in mouse ganglia by footpad inoculation. In situ hybridization of the latently infected ganglia revealed the presence of globin transcripts in the cytoplasm (Fig. 2A). No detectable hybridization was observed with the LAT probes (Fig. 2B).

These hybridization signals were confirmed by Northern (RNA) blot analyses of latently infected ganglia. A rabbit beta-globin transcript, approximately 600 bp in size, was observed in the poly(A)$^+$ fraction (Fig. 3, lane vi). This transcript comigrated with authentic rabbit beta-globin mRNA from rabbit reticulocytes (data not shown). Thus, the genomic copy of this gene was apparently correctly spliced, polyadenylated, and transported to the cytoplasm. A small amount of globin RNA was also seen in the poly(A)$^-$ fraction (lane iv); this could be due to inefficient fractionation or to the presence of some poly(A)$^-$ globin RNA. LAT expression was observed only in ganglia from mice latently infected with wild-type virus (lane iii); there was no LAT detectable in the latent infection with the globin-containing recombinant virus (lanes ii and viii). As reported previously,

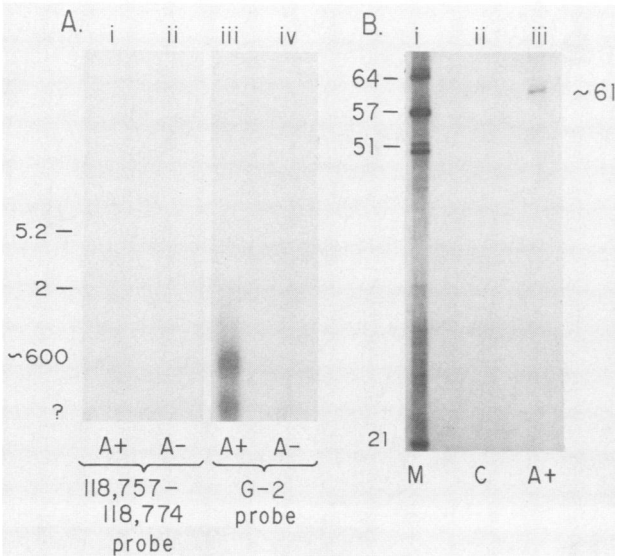

FIG. 4. Expression of poly(A)$^+$-containing globin RNA in cells productively infected with the globin-containing recombinant virus. (A) Northern blots of 5 μg of poly(A)$^+$ and 40 μg of poly(A)$^-$ RNA hybridized with an oligonucleotide homologous to HSV-1 sequences 5′ of the globin insert (lanes i and ii) or with a probe homologous to bases 66 to 85 of the published rabbit globin sequence. The 600-base globin mRNA-sized transcript is shown, and the location of a smaller, aberrantly processed transcript is indicated (?) (lanes iii and iv). Exposure was for 8 h at −70°C with intensifying screens. (B) Primer extension. The sizes of extension products, using a primer homologous to bases 40 to 61 of the globin sequence and poly(A)$^+$ RNA from cultured cells productively infected with the recombinant globin containing virus, are shown in lane iii. A lane containing material obtained by using uninfected cell RNA (lane ii) is also shown. The size markers (lane i) are derived from *Hae*III-digested pBR322 DNA which was then end labeled by the addition of kinase.

no poly(A)$^+$ RNA homologous to the LAT probe was seen from such latent infections (lane ix). We do not know the source of the higher-molecular-weight hybridization in lane vi, it may be unspliced beta globin.

Two experiments with poly(A)$^+$ and poly(A)$^-$ RNA from cells productively infected with the globin-containing recombinant virus were performed to confirm that transcription initiated near the expected cap site. First, Northern blots of RNA were hybridized with oligonucleotide probes complementary to a region 32 bases upstream of the site of the globin gene insertion and to two probes complementary to globin mRNA (probe G-1 covered bases 40 to 61 and probe G-2 covered bases 66 to 85 of the published sequence [23]). Examples of data are shown in Fig. 4A, in which no hybridization was seen with the upstream HSV-1-specific probe (lanes i and ii), whereas a poly(A)$^+$ RNA migrating with a rate corresponding to that of 600 bases was seen with either probe G-1 (not shown) or G-2 (lane iii). It was clear that not all the globin RNA was correctly processed during productive infection since variable amounts of smaller poly(A)$^+$ species were also seen in productive infection of cultured cells but not in latently infected ganglia. A primer complementary to a region 30 bases 3′ of the insertion site did not hybridize to the globin RNA (data not shown). These data indicated that the globin transcript initiated within the region of −32 and +40 bases of the nominal cap site and is terminated within the inserted sequences.

Primer extension experiments were then carried out by using primer G-1 to more precisely locate the transcription

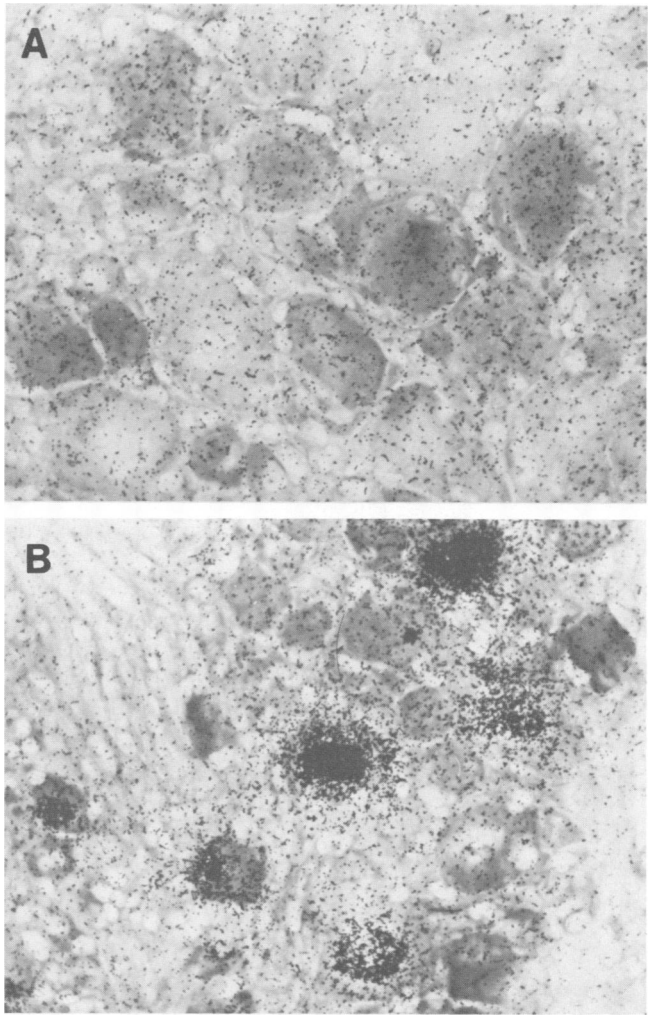

FIG. 5. Hybridization in situ of ^{35}S-labeled HSV-1 DNA to RNA from murine spinal ganglia latently infected with the *Pst*I deletion recombinant virus and with wild-type virus. (A) Ganglia latently infected with the *Pst*I deletion virus and probed with ATD 19. (B) Ganglia latently infected with HSV-1 strain KOS(M) and probed with ATD 19.

start of the poly(A)$^+$ globin RNA expressed in productively infected cultured cells. Extension of the primer to the nominal globin cap would produce an extension product 61 bases in length. As shown in Fig. 4B, an RNA product of this size was obtained. Therefore, the poly(A)$^+$ globin mRNA expressed during a productive infection starts at the globin cap site.

To confirm that the TATA element 688 bp upstream of the stable LAT RNA was needed for transcription of the LAT, a second recombinant virus was constructed. In this virus, the TATA box and approximately 100 bp on either side were deleted (Fig. 1C). This virus was used to establish latent infections in mice. Transcription of the LAT was reduced to background levels in this virus as observed by in situ hybridization (Fig. 5A and B). This result confirms that the LAT transcript requires an element from this region for maximal transcription rate.

Detection of RNA proximal to the TATA box. To search for evidence of unstable LAT RNA between the TATA box and the 5′ end of the stable LAT, two additional DNA probes

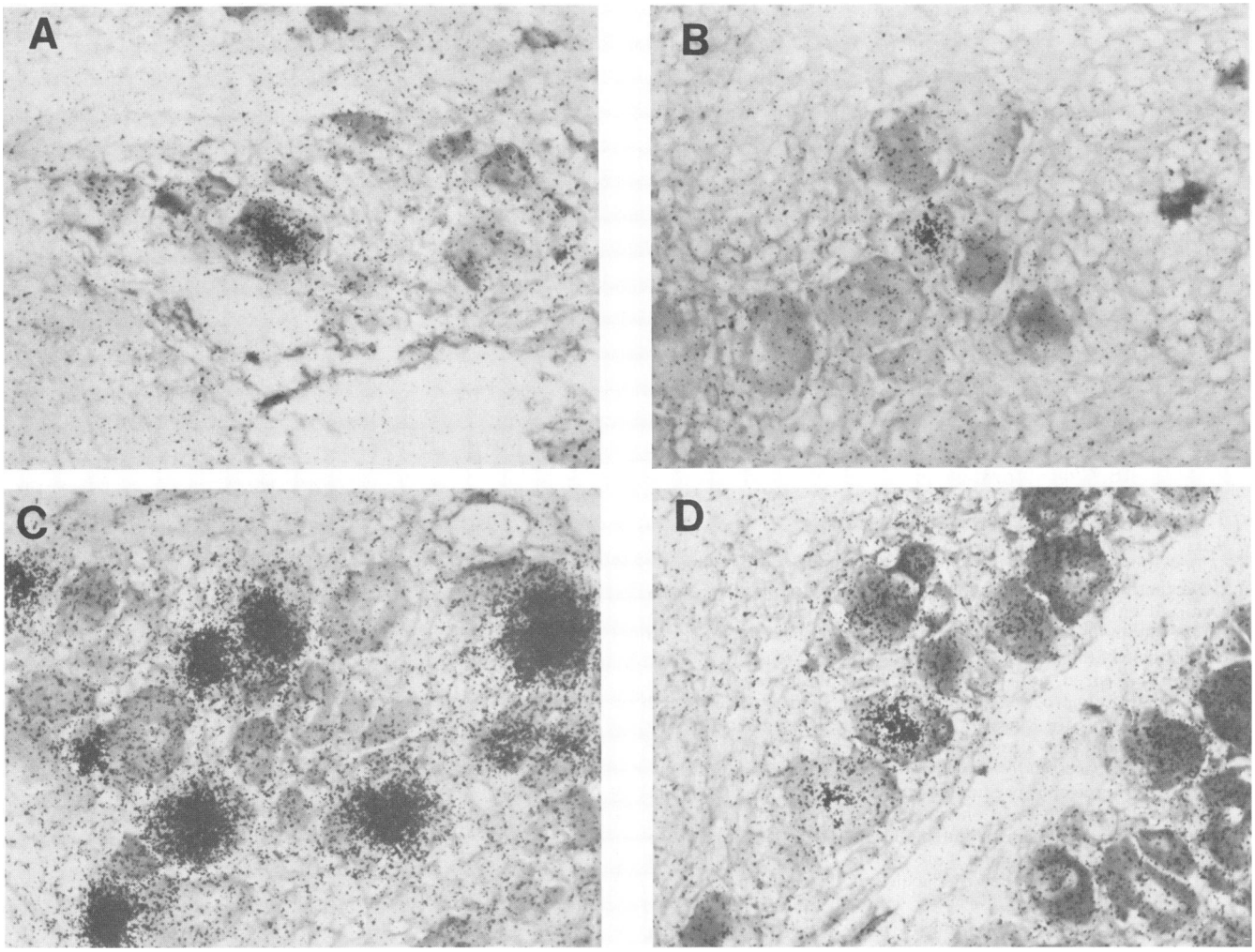

FIG. 6. Hybridization in situ of ^{35}S-labeled HSV-1 DNA fragments to RNA in ganglia latently infected with wild-type HSV-1 strain KOS(M). (A) Probe *Pst*I-*Pst*I (bases 118,665 to 118,866), (B) probe ATD 17 from *Pst*I-*Sph*I (bases 118,866 to 119,269), (C) probe ATD 19 (bases 119,628 to 119,975), and (D) probe *Sph*I-*Sac*I (bases 124,465 to 125,046).

were made and used for in situ hybridization of latently infected ganglia. Hybridization with the 200-bp *Pst*I fragment was weakly positive (Fig. 6A). This region contains the TATA box and 90 bases of RNA 3′ of the expected cap. Hybridization with a probe for the next 420 bases (*Pst*I to *Sph*I) was largely negative, although an occasional positive neuron could be observed (Fig. 6B). These regions were compared with the LAT region which is very strongly positive (Fig. 6C) and with the region downstream of the LAT (*Sph*I to *Sst*I) which was also positive (Fig. 6D). While it is difficult to quantitate the level of transcripts in these three regions, the upstream 700-base region has approximately fivefold fewer sites of hybridization than the LAT and a signal intensity perhaps 1/10 to 1/20 that of the LAT. The downstream region has perhaps twofold fewer sites than the LAT, with a signal intensity about one-fourth that of the LAT. Thus, the viral RNAs from different regions surrounding the LAT appear to have very different RNA stabilities. The detection of viral RNA from the region upstream of the LAT in a latent infection is further evidence that the promoter element functioning in a latent infection is far upstream of the stable LAT RNA. Although this region has been scored as negative by Wechsler et al. (27), the possible presence of very faint signals from this region is mentioned in their discussion.

Detection of a large poly(A)$^+$ transcript which contains the LAT in productive infection. The preceding data demonstrate that LAT expression requires sequences at least 700 bases 5′ of the start site of the stable species. Such a situation suggests that a primary transcript is expressed from this point through the LAT and terminates somewhere 3′ of the stable species. We have previously described the presence of weak in situ hybridization signals extending past the 3′ end of the stable LAT RNA (2, 15, 24). In theory, the RNA polymerase should continue transcription through the LAT until it reaches a polyadenylation signal and T-rich attenuation sites (9). The nearest polyadenylation signal is approximately 8.5 kb downstream of the TATA box. This is across the joint region and just 55 bases to the left of the polyadenylation signal for the ICP4 gene on the opposite strand (11). Figure 6D shows an in situ hybridization with a probe in this region. Weak signals are obtained for probes extending beyond the 3′ end of the LAT and ending in the vicinity of the polyadenylation signal.

A transcript extending from near the TATA box to near the polyadenylation site noted on the sequence of HSV-1

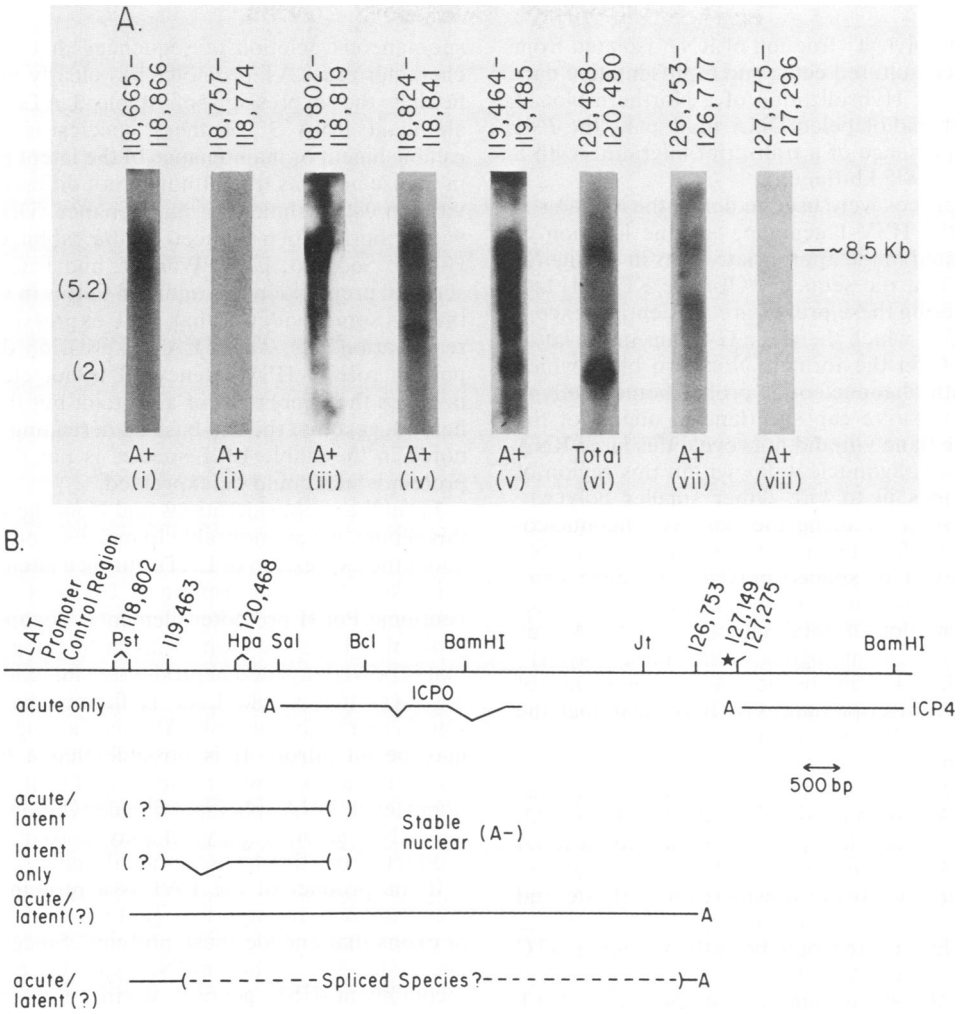

FIG. 7. Expression of a large poly(A)$^+$ transcript containing HSV-1 LAT during productive infection. (A) Limits of the transcript. Poly(A)$^+$ RNA (5 μg) from productively infected cells was fractionated, blotted, and hybridized with the probes shown above the appropriate lanes. Lane i was hybridized with labeled *Pst*I fragment while the others were hybridized with synthetic oligonucleotides. Lane vi contained 40 μg of total infected-cell RNA in order to estimate the relative abundance of the 8.5-kb transcript. (B) Proposed scheme for the expression of the LAT from the primary transcript. The transcripts identified in the present report and previously are shown. The existence of spliced poly(A)$^+$ species is inferred from the complexity of poly(A)$^+$ transcripts seen with the hybridization probes used. Uncertainty concerning the relationship between the 5′ end of stable nuclear poly(A)$^-$ LAT and the poly(A)$^+$ primary transcript is indicated. The location of oligonucleotide probes hybridizing to the primary transcript, the polyadenylation signal at the 3′ end of ICP4, and the location of the oligonucleotide 3′ of this, which does not hybridize to the primary transcript, are noted by the sequence numbers. (C) RNase protection analysis of the 5′ end of the long poly(A)$^+$ transcript. As described in the experimental section, RNase-resistant material formed by hybridization of the 73-base RNA probe that spans the *Eag*I-to-*Pst*I sites of HSV-1 and nonspecific linker RNA was hybridized with poly(A)$^+$ and poly(A)$^-$ RNA from infected cultured cells and was fractionated on a denaturing acrylamide gel. Lane i contains DNA size markers from *Hae*III-digested pBR322. The migration of two RNA size markers of 113 and 54 bases is different from these DNA markers and is shown in parentheses and indicated as RNA-M. The position of the major protected species from poly(A)$^+$ RNA (lane iii) corresponds to about 63 bases. Lane v shows the material obtained when the probe was hybridized with 50 μg of total RNA from uninfected rabbit skin cells.

was detected in the poly(A)$^+$ fraction of RNA isolated from productively infected cultured cells, and representative data are shown in Fig. 7A. Hybridization of a Northern blot of poly(A)$^+$ RNA with radiolabeled DNA that spans the *Pst*I sites indicates the presence of a transcript migrating with a size corresponding to 8.5 kb (lane i).

Oligonucleotide probes were used to define the location of this transcript on the HSV-1 genome, and the location of each probe is indicated in the appropriate lanes in the figure. It should be noted that the sequences for the KOS(M) and 17syn+ strains covering these probes are all identical except for position 118,807 in which there is a G-C pair in the latter sequence and a C-G in the former. Northern blots which were hybridized with oligonucleotide probes homologous to a region 5′ of the putative cap site (lane ii) and 3′ of the polyadenylation site (lane viii) did not reveal this large RNA species; however, all oligonucleotides within this region of the genome did (lanes iii to vii). Other smaller poly(A)$^+$ species were also seen by using the positive oligonucleotides, which suggests that this long transcript was variably processed to a number of spliced poly(A)$^+$ species; however, this finding was not investigated further at this time. Finally, a Northern blot of total infected-cell RNA was hybridized with a probe homologous to the stable poly(A)$^-$ LAT species in order to estimate the relative abundance of the large poly(A)$^+$ transcript (lane vi). It is clear that the transcript is significantly less abundant than is LAT in productive infection.

The precise 5′ end for transcription of the LAT was determined for RNA from a productive infection with wild-type virus. RNase protection experiments showed an RNA product of 63 to 64 bp, using a probe from the *Eag*I site upstream of the cap site to the downstream *Pst*I site and poly(A)$^+$ RNA (Fig. 7C). Smaller bands may reflect cryptic start sites. A possible cap site would be in the sequence ATC 24 bp downstream of the TATA box (11).

Taken together, the data suggest that the stable LAT transcript which is detected in latently infected neurons is derived from an 8.5-kb transcription unit (Fig. 7B). From this transcription unit a number of different nuclear RNA species have been observed. These species are present in different concentrations within the nucleus.

DISCUSSION

The present communication demonstrates that insertion of the rabbit beta-globin gene into HSV-1 immediately downstream of a potential Pol II promoter sequence 700 bases 5′ of the start of the stable nuclear poly(A)$^-$ LAT results in the expression of cytoplasmic poly(A)$^+$ globin RNA in latently infected neurons. This insertion results in the loss of all detectable LAT expression in the latent phase of infection. In a productive infection, this mRNA starts at the normal mRNA cap site. Our results indicate that stable LAT is derived from a primary transcription unit starting approximately 700 bases 5′ of its previously reported start. In further experiments, we have confirmed the existence of the primary transcript and shown that at least some transcription extends 8.5 kb from the start to a polyadenylation signal near the 3′ end of ICP4. Although we have been unable to detect such a transcript in Northern blots of acute or latently infected neurons, the weak in situ hybridization signals seen with probes spanning the LAT and its flanking sequences reported by us and others suggests that this transcription unit operates in neuronal infection with HSV-1 (2, 15, 24).

Previous work with an HSV variant (X10-13) which has a spontaneous deletion of sequences that include the newly characterized LAT promoter has clearly demonstrated that neither the expression of stable LATs nor of the less abundant RNA 3′ of these species is required for the establishment or maintenance of the latent phase of infection in mouse neurons (8). Although not different from wild-type virus in establishment or maintenance, virus X10-13 reactivates poorly when induced in the rabbit eye model (T. J. Hill, F. Sederati, E. K. Wagner, and J. G. Stevens, manuscript in preparation). In addition to this in vivo reactivation, there is some evidence that LAT expression affects in vitro reactivation (17). Thus, LAT expression does have an important role in HSV latency. It is not clear that this role involves the expression of a protein, but the data presented here suggest that the 900-base open reading frame previously noted in the stable LAT species is not the only candidate protein which could be expressed.

From these considerations and from the data presented in this report, we are now able to make several new statements about the expression of LATs during a latent infection. First, it is likely that transcription of LATs is regulated by conventional Pol II promoter elements. Second, stable LAT is probably processed from a much larger primary transcript that is polyadenylated in productive infection at least. Third, the facts that stable LAT is flanked by potential splice signals and is nuclear poly(A)$^-$ species suggest that the LAT may be an intron. It is possible that a large part of the regulation of this transcriptional unit is at the posttranscriptional level. RNA splicing, polyadenylation, RNA transport, and RNA stability may all play a role in regulating the products from this transcriptional unit.

If the product of the LAT is a protein or a number of proteins, then an important goal becomes the identification of exons that encode these proteins. Since stable, cytoplasmic, poly(A)$^+$ globin mRNA can be expressed from a recombinant HSV genome during a latent infection, the instability of LAT mRNAs must be due to the properties of the RNA expressed. Thus, it may be possible to devise methods of stabilizing such a species. We are currently investigating such a possibility.

Finally, there is considerable interest in using HSV-1 as a vector for gene transfer to neurons. The beta-globin virus used in these experiments fairly faithfully expresses a foreign gene product stably in neurons in vivo. The lack of expression of other lytic genes during latency and the stability of HSV-1 in latent neurons may make this an attractive system.

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