JOURNAL OF VIROLOGY, Oct. 1991, p. 5260–5271
0022-538X/91/105260-12$02.00/0

Vol. 65, No. 10

Cloning of the Latency Gene and the Early Protein 0 Gene of Pseudorabies Virus

ANDREW K. CHEUNG

Virology Swine Research Unit, National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa 50010

Received 1 March 1991/Accepted 30 June 1991

A collection of overlapping cDNA clones encoding the latency transcript of pseudorabies virus and the DNA nucleotide sequence of the latency gene has been obtained. The transcript is spliced with 4.6 kb of intervening sequences. This mRNA, designated the large latency transcript, is 8.5 kb. It is polyadenylated and contains a large open reading frame capable of coding for a 200-kDa polypeptide. The direction of transcription is antiparallel to that of the immediate-early gene IE180 and a newly identified early gene, EP0. The latency transcript overlaps the entire IE180 gene and most of the EP0 gene. The EP0 mRNA is 1.75 kb and polyadenylated. The deduced amino acid sequence revealed the presence of cysteine-rich zinc finger domain similar to that of the immediate-early gene ICP0 of herpes simplex virus type 1 and the gene 61 polypeptide of varicella-zoster virus. On the basis of the biological functions, conserved protein domains, and unique spatial arrangements of the homologous polypeptides (IE180 versus ICP4 and EP0 versus ICP0) between pseudorabies virus and herpes simplex virus type 1, it is predicted that a homologous protein domain is also encoded by the 8.5-kb large latency transcripts of these two viruses.

Pseudorabies virus (PRV) and herpes simplex virus type 1 (HSV-1) are members of the alphaherpesvirus subfamily (3, 38), and they are quite similar in genomic structure and organization. On the one hand, many gene homologs have been reported between the two viruses; on the other hand, some genes present in HSV-1 are not present in PRV. There are five HSV-1 immediate-early genes (infected cell polypeptide 0 [ICP0], ICP4, ICP22, ICP27, and ICP47) and only one PRV immediate-early gene (IE180). Analysis of the DNA and deduced amino acid sequences showed that HSV-1 ICP4 and PRV IE180 share extensive homology at two specific regions of the polypeptide (7, 8, 50). Biologically, these two viruses also exhibit many common characteristics, one of which is their ability to establish latency in their respective hosts.

PRV can establish a latent infection in swine following a primary infection (4, 11, 39). The recovered animals do not shed virus; however, periodically, free virus can be reactivated from these carriers spontaneously or after induction with external stimuli (4, 11, 48, 53). The reactivated virus becomes the source of transmission to other animals. This latency and reactivation process is regarded by most researchers to be an obstacle to the control and eventual eradication of PRV. During herpesvirus latency, the viral DNA persists preferentially in the nervous tissues of the host animal, and gene expression is limited to a restricted region of the virus genome. Viral RNAs known as latency-associated transcripts (LATs) or latency-related RNAs present in latently infected tissues have been reported for HSV (12, 14, 17, 21, 37, 43–45, 51, 52), bovine herpesvirus (36), varicella-zoster virus (10, 47), and PRV (6, 22, 23).

Among the RNAs expressed during latency, HSV-1 is the best-studied system to date. In this report, I have divided these transcripts into two groups as defined below. The HSV-1 LATs, 2 kb or less, are transcribed in the opposite sense with respect to ICP0, and they overlap the 3′ end of the ICP0 mRNA. They are not polyadenylated at the 3′ end, and a protein product encoded by the LATs has not been identified. Recent reports suggested that there may be a polyadenylated 8.5-kb LAT (13, 55); however, the exact nature of this transcript has not been fully elucidated. This RNA, designated the large latency transcript (LLT), has been proposed to overlap the entire ICP0 in the opposite orientation but does not overlap ICP4. It has been suggested that the LATs are stable introns derived from the 8.5-kb LLT.

The LATs of PRV were first localized to the 3′ end of the immediate-early gene IE180 (6), a homolog of HSV-1 ICP4 and not ICP0. They are transcribed in the antiparallel orientation with respect to IE180. Recent reports (22, 33) indicated that PRV LATs are encoded by DNA sequences that extend over 14 kb of the viral genome. Several RNA species (0.95, 2.0, and 5.0 kb) have been reported, and apparently, contradictory results have been obtained regarding the poly(A) nature of the LATs. This work demonstrates the presence of an 8.5-kb PRV-specific poly(A) RNA species in the trigeminal ganglia of a latently infected swine. This RNA, referred to here as PRV LLT, has an open reading frame (ORF) capable of encoding a 200-kDa protein. During the course of this work, a PRV early polypeptide homologous to the HSV-1 ICP0, designated early protein 0 (EP0), was identified. The gene for PRV EP0 was localized, and the DNA sequence was determined. In addition, a prediction was made suggesting the presence of a homologous protein domain encoded by the LLTs of both PRV and HSV-1.

MATERIALS AND METHODS

Virus and cell culture. The Indiana-Funkhauser or Becker strain of PRV (PRV-InFh or PRV-Becker) was grown on Madin-Darby bovine kidney cells cultivated in Eagle minimum essential medium supplemented with 10% fetal bovine serum (32).

RNA preparations. The isolation of total cellular RNAs and the selection of poly(A) RNAs have been previously described (6).

DNA and RNA hybridization. After electrophoresis, the DNAs and RNAs were transferred to nitrocellulose or nylon

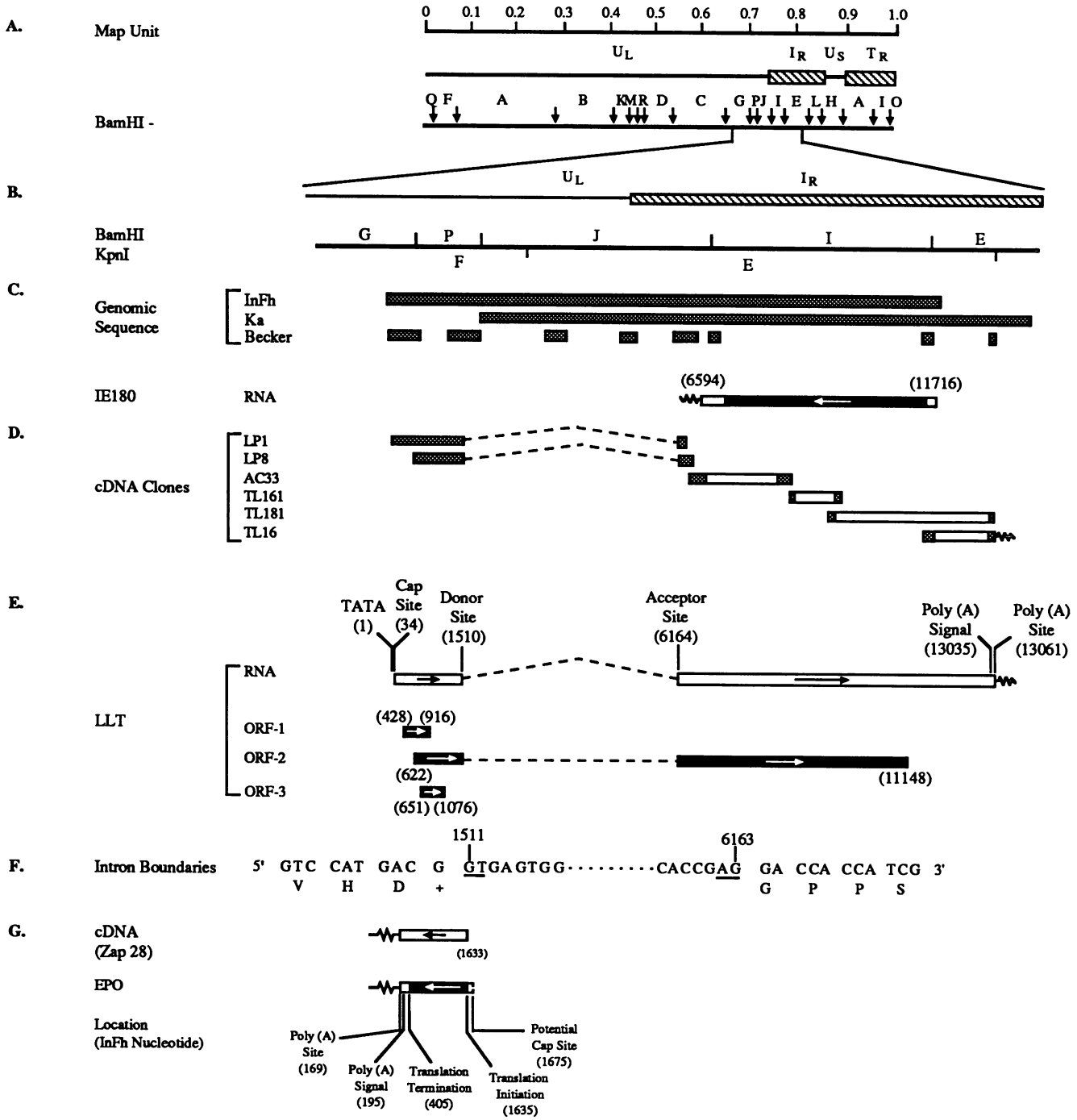

FIG. 1. (A) Schematic diagram of the PRV genome and *Bam*HI restriction enzyme map. The genome is organized into the unique long (U_L), internal repeat (I_R), unique short (U_S), and terminal repeat (T_R) sequences. (B) Expanded diagram of *Bam*HI-G, -P, -J, -I, and -E and *Kpn*I-F and -E restriction fragments in this region. (C) Available genomic DNA nucleotide sequences of three different strains of PRV (InFh, Ka, and Becker) and the location of PRV IE180. The direction of IE180 transcription is indicated by an arrow (leftward), and the poly(A) tail is indicated by a squiggle. Shaded areas represent the coding sequence. (D) Six overlapping cDNA clones. The cDNA library was constructed with total cellular RNAs from the trigeminal ganglia of a latently infected swine. The standard method with oligo(dT) primer was used for cDNA synthesis (18), and the cDNA was cloned into the lambda gt10 vector system (20). Nick-translated probes derived from *Bam*HI-P, -J, and -I were used to screen for PRV-specific clones. DNA inserts were excised and subcloned into Bluescript plasmids (Stratagene), and the DNA sequences were determined by the dideoxy-chain termination method (41). Areas for which nucleotide sequences have been determined are stippled. Dotted lines indicate splicing. (E) The LLT. Points of interest are indicated by the first nucleotide of the element. The direction of transcription is rightward, with a poly(A) tail at the 3′ end. Three possible ORFs are shaded; the coordinates for the coding sequences (based on PRV-InFh and -Ka) are also indicated. (F) Intron boundaries. The nucleotide sequence and deduced amino acid residues (in single-letter code) in the vicinity of the splice junctions are shown. The consensus dinucleotides present at the intron boundaries are underlined. Nucleotide 1510 (+), together with nucleotides 6164 and 6165, codes for the glycine residue. (G) Localization of a cDNA clone (Zap28) and its corresponding EP0 transcript. Arrows indicate the direction of transcription, dotted lines indicate splicing, and squiggles indicate poly(A) tracks. Shaded areas indicate ORFs.

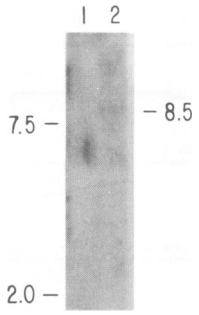

FIG. 2. Northern blot analysis. Equal amounts (15 μg per lane) of RNA from the trigeminal ganglia of a normal (lane 1) and a latently infected (lane 2) swine were denatured with glyoxal and dimethyl sulfoxide (26). After electrophoresis in a 1% agarose gel, the RNAs were transferred to and immobilized on a GeneScreen membrane. Hybridization was carried out at 60°C with a nick-translated *Bam*HI-P cloned DNA fragment. After hybridization, the blot was dried and exposed to X-ray film for 6 days at −80°C. Sizes of the markers in kilobases are indicated on the left, and the detected RNA species is indicated on the right.

membranes in 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). Hybridization was carried out at 60°C with nick-translated probes for 18 to 24 h in the presence of 5× SSC–5× Denhardt solution (1× Denhardt solution is 0.2% each bovine serum albumin, Ficoll, and polyvinylpyrrolidone). The blots were washed at 60°C with SSC solutions (2×, 1×, and 0.1× SSC) for 1 h each.

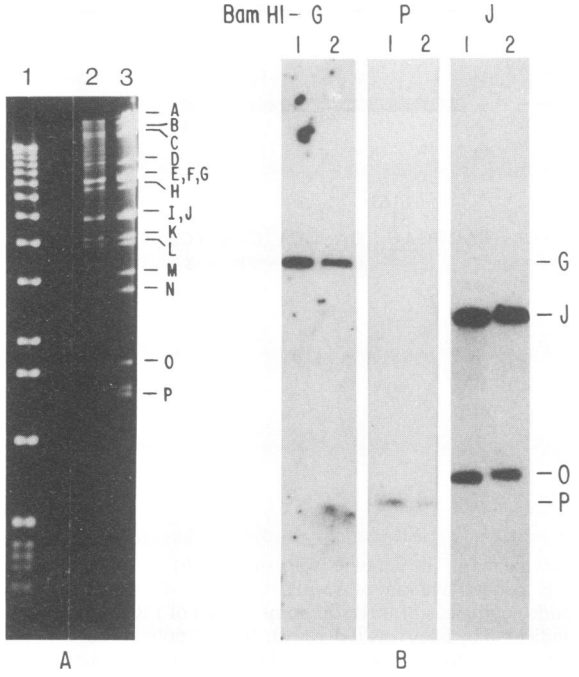

FIG. 3. Genomic organization of PRV-InFh and -Becker. (A) *Bam*HI restriction enzyme digestions of PRV-InFh (lane 1) and -Becker (lane 2) were analyzed on a 0.8% agarose gel. Organizations of the *Bam*HI fragments are shown in Fig. 1A. Lane 1 contains molecular weight markers. (B) Assay in which the digested DNA fragments were transferred to nylon membrane and probed with ^{32}P-labeled, nick-translated, cloned *Bam*HI-G, -P, and -J fragments derived from PRV-InFh. Lanes: 1, PRV-InFh DNA; 2, PRV-Becker DNA.

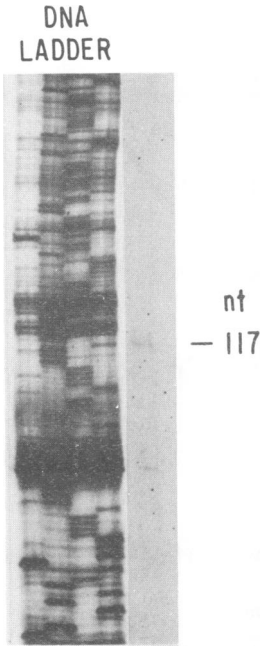

FIG. 4. LLT 5′ end. A 19-mer oligonucleotide (5′-TCTTCTATG GCTGAGGGAG-3′) complementary to the DNA sequence from nucleotides 150 to 132 (Fig. 6) was ^{32}P labeled at the 5′ end (23) and used in a primer extension experiment with oligo(dT)-selected RNAs from 15 μg of total trigeminal ganglia RNAs of a latently infected pig. The sample was heat denatured and analyzed on a 6% polyacrylamide sequencing gel. The gel was dried and exposed to X-ray film for 2 weeks at −80°C. The size of the product was determined from a known sequencing ladder.

Primer extension. The reaction (50 μl) was carried out at 42°C for 30 min in the presence of 50 mM Tris-HCl (pH 7.6), 70 mM KCl, 10 mM $MgCl_2$, 1 mM each deoxynucleoside triphosphate, 4 mM dithiothreitol, 25 U of RNase inhibitor, and 50 U of avian myeloblastosis virus reverse transcriptase as described previously (2).

Nucleotide sequence accession numbers. The DNA sequence data in this report have been submitted to the GenBank data base under accession numbers M57505 (PRV LLT) and M57504 (EP0).

RESULTS

cDNA libraries. Oligo(dT) was used to prime total cellular RNAs isolated from the trigeminal ganglia of swine previously shown to be latently infected with PRV-Becker (6). A cDNA library in the lambda gt10 vector system (20) was constructed. To ensure that this cDNA library contained clones derived from PRV transcripts expressed during latency and not during a productive infection, this library was analyzed in parallel with a second oligo(dT)-primed cDNA library constructed with oligo(dT)-selected RNAs (1, 9) isolated from Madin-Darby bovine kidney tissue culture cells infected with PRV-InFh (32). Equivalent amounts of poly(A) RNAs from 2, 4, 6, 8, 10, and 12 h postinfection and a 2-h-infected, cycloheximide-treated culture were mixed and used to generate a cDNA library, in the lambda ZapII vector system (Stratagene), representative of the entire PRV reproductive cycle.

Identification of an LLT. Both the latency and productive infection cDNA libraries were screened concurrently, in parallel, with ^{32}P-labeled nick-translated probes (35) of cloned PRV-InFh fragments, *Bam*HI-P, -J, or -I. Recombinant clones representing 99% of each library were screened. More than 50 clones from each library were isolated and partially characterized. Recombinant clones representing a specific transcript are unique to each library. The spliced mRNA presented in Fig. 1 was found only in the library constructed from latently infected trigeminal ganglia RNAs. Among the 50 cDNA clones that have been isolated, purified, and characterized, 6 overlapping clones are shown (Fig. 1D). They covered the entire length of an 8.4-kb RNA, with one of the clones (TL16) carrying a poly(A) tail of 14 A residues. Northern (RNA) blot analysis showed that a very low abundance RNA species of 8.5 kb could also be detected in the trigeminal ganglia of a pig latently infected with PRV-Becker (Fig. 2). To distinguish this polyadenylated 8.5-kb transcript from the smaller, nonpolyadenylated LATs (see Discussion), it is designated the LLT.

Genome organization and RNA splicing. In the initial characterization of cDNA clones LP1 and LP8, Southern blot experiments (42) showed that the insert DNAs hybridized to two discontinuous regions of the PRV-InFh genome: *Bam*HI-P and the right end portion of *Bam*HI-J (data not shown). To ensure that the joining of these two noncontiguous DNA sequences in LP1 and LP8 was the result of RNA splicing and not DNA rearrangement, the PRV-Becker genome was examined and compared with the PRV-InFh genome. Restriction enzyme digestion with *Bam*HI showed similar DNA fragments (Fig. 3A). Southern blot analysis with cloned PRV-InFh *Bam*HI-G, -P, and -J probes showed similar results (Fig. 3B), including hybridization of the *Bam*HI-J probe to the repeated sequences present in *Bam*HI-O. DNA sequence analysis at strategic sites (Fig. 1C) further confirmed that the genomes of PRV-Becker and -InFh are organized in identical fashion.

The nucleotide sequences of LP1 and LP8 were determined and compared with the known DNA sequences of PRV-InFh, the Ka strain of PRV (PRV-Ka), and PRV-Becker (7, 8, 49, 50). It was evident that LP1 and LP8 were derived from spliced RNAs. The intron boundaries are flanked by the consensus dinucleotides (5′-GT----AG-3′) at the donor and acceptor sites of the splice junction (Fig. 1F). Further analysis showed that LP1 contains DNA sequences close to the 5′ end of the transcript (see Fig. 6). Limited DNA sequence analysis of the PRV-Becker genome and clone TL16 showed that TL16 has a poly(A) tail. The rest of LLT is encoded by three overlapping cDNA clones: AC33, TL161, and TL181. The terminal nucleotide sequences of all of the cDNA clones were determined, and the predicted sizes of the DNA inserts correspond to the sizes obtained from agarose gel estimates. The results are schematically outlined in Fig. 1E. The DNA sequences of PRV-InFh *Bam*HI-G, -J, and -I and PRV-Ka *Bam*HI-E were used as references.

Localization of the termini of LLT. To investigate the 5′ end of the LLT, the LP1 nucleotide sequence was aligned with PRV-InFh and -Becker genomic sequences. It was noted that LP1 starts 135 nucleotides downstream of a TATATA sequence (see Fig. 6). The transcription initiation site of LLT was determined by primer extension with a 19-mer oligonucleotide (5′-TCTTCTATGGCTGAGGGAG-3′) complementary to the DNA nucleotides from 150 to 132. The extended product was 117 nucleotides (Fig. 4), which placed the LLT cap site 34 nucleotides downstream of the TATATA sequence present in the *Bam*HI-G fragment (Fig. 1E and Fig. 6). A smaller primer extension product was also observed 57 nucleotides 3′ of this TATATA sequence (Fig. 4). Since this product extended past the TATA sequence at nucleotide 180 (Fig. 6), it is considered an incomplete product that failed to reach 117 nucleotides in the reaction.

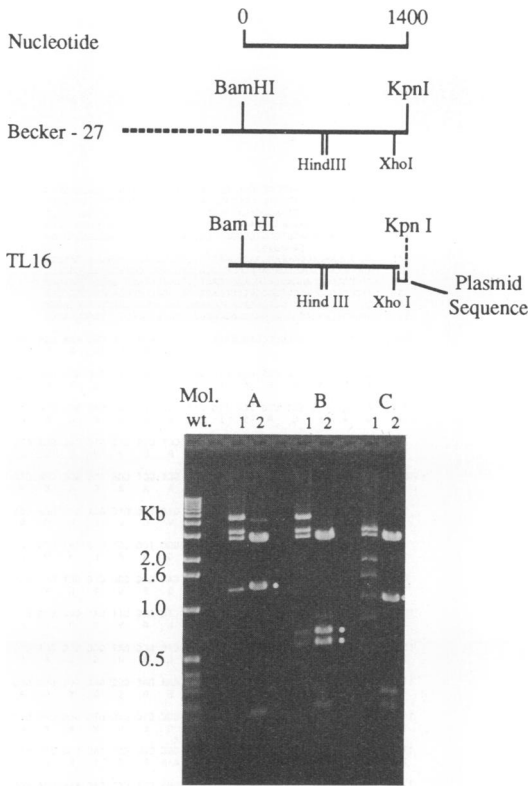

FIG. 5. Evidence that the 3′ end of LLT is colinear with the genomic sequence. Restriction enzyme analyses were carried out on genomic (Becker-27) and cDNA (TL16) sequences. Becker-27 DNA (lanes 1) or TL16 DNA (lanes 2) was digested with *Bam*HI and *Kpn*I to give the *Bam*HI-*Kpn*I fragment (A). The digested products of panel A were further cleaved with either *Hin*dIII (B) or *Xho*I (C) and analyzed on a 1% agarose gel. Fragments of similar size present in both Becker-27 and TL16 DNAs are indicated by dots. Sizes of molecular weight markers are shown on the left.

The LLT 3′ terminus was determined from cDNA clone TL16, which contains 14 A bases after nucleotide 13061 in the *Bam*HI-E fragment of the PRV-Ka genome (Fig. 1E). DNA sequence analysis on both PRV-Becker genomic and cDNA clones showed that a consensus polyadenylation signal (AATAAA) was present 27 nucleotides upstream of the actual poly(A) addition site. The PRV-specific sequence present in TL16 and PRV-Becker *Bam*HI-E was further examined by restriction enzyme analysis. Becker-27 is a *Kpn*I-E genomic clone that contains part of *Bam*HI-J, all of *Bam*HI-I, and a portion of *Bam*HI-E (Fig. 1B). The *Bam*HI-E-specific sequences were excised from Becker-27 and TL16 with *Bam*HI and *Kpn*I and analyzed with additional restriction enzymes. The results showed that DNA fragments of identical size could be generated from both the genomic and cDNA clones (Fig. 5), indicating that the 3′ portion of LLT is colinear with the viral genome for at least 1,400 nucleotides.

Sequence analysis of the latency gene. The composite

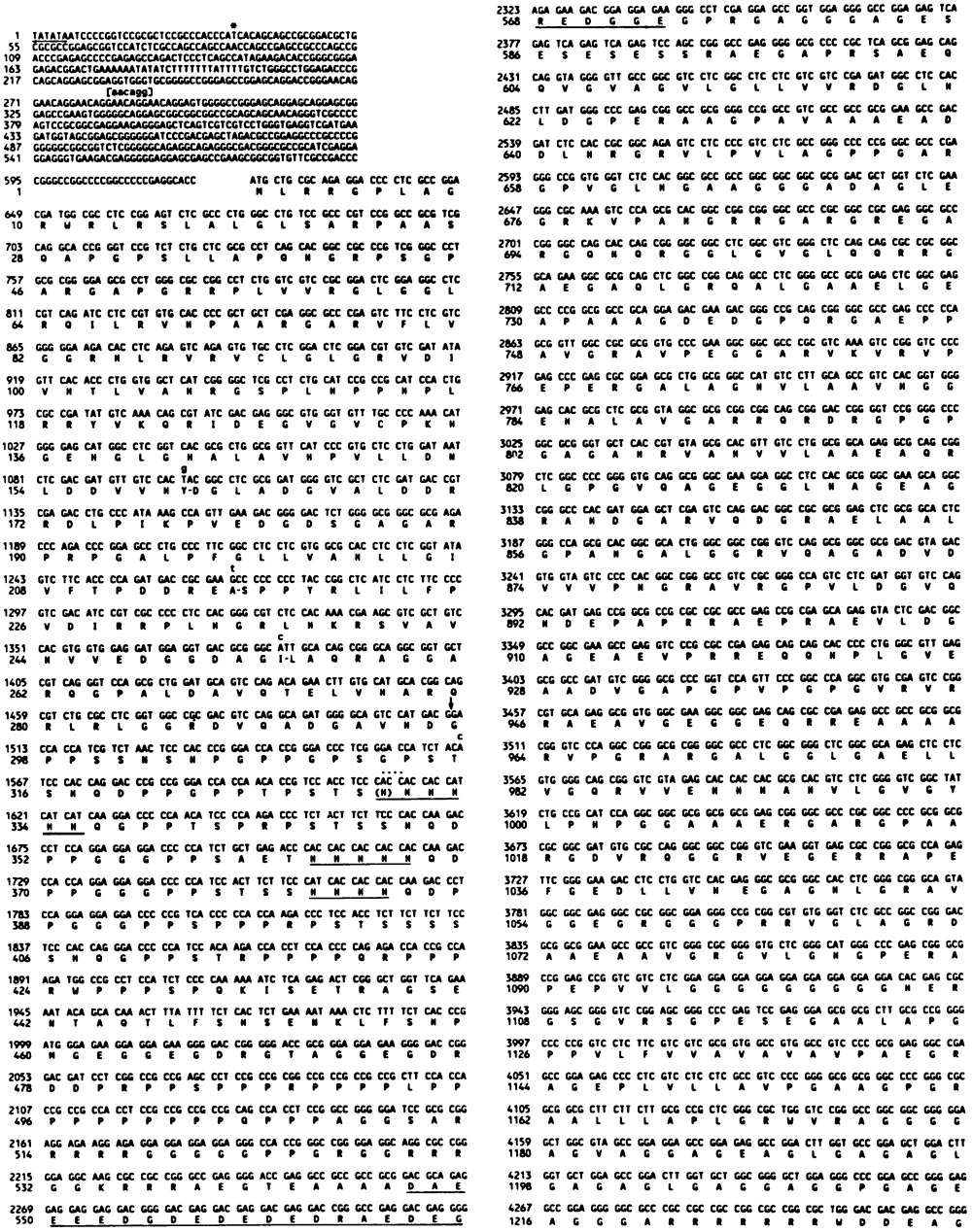

FIG. 6. Nucleotide sequence of the complete PRV LLT and predicted amino acid sequence of ORF-2. The basic sequence is derived from PRV-InFh (from nucleotides 1 to 7013) and PRV-Ka (from nucleotides 7014 to 8425). PRV-Becker nucleotides that differ from the prototype are indicated in small letters above the basic sequence, the corresponding amino acid residue changes are presented at the third position of the codon, deletions are indicated by parentheses with dots on top, and insertions are indicated above the basic sequence in brackets. Nucleotide coordinates after the splice junction at nucleotide 1511 apply only to LLT. DNA nucleotide sequences of the TATA box and the poly(A) signal and the amino acid sequences of the histidine-rich, acidic residue-rich, and cysteine-rich regions of the polypeptide are underlined. The RNA cap site, poly(A) addition site, and termination codon are indicated by asterisks.

FIG. 6—*Continued*.

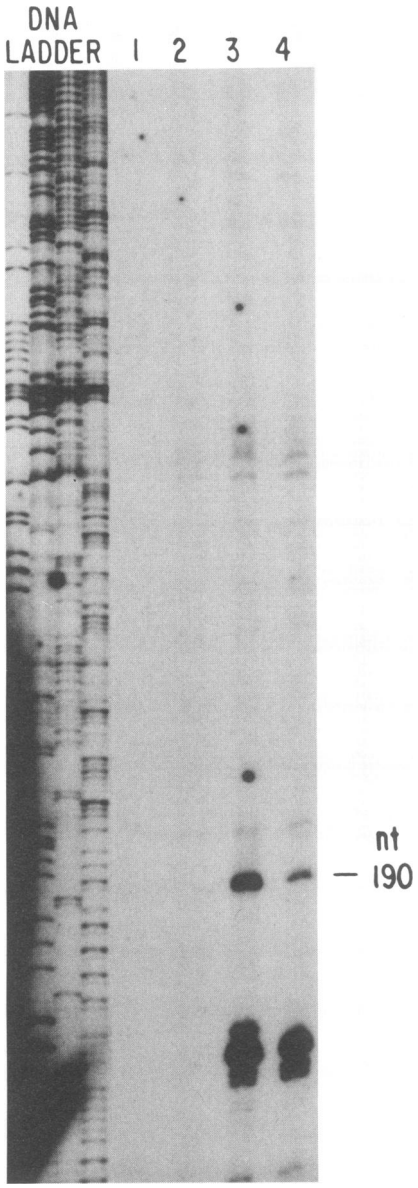

FIG. 7. EP0 5′ end. A 17-mer oligonucleotide (5′-AGGCAGATG GGGCAGTC-3′) complementary to the DNA sequences from 1486 to 1503 (Fig. 3) was ^{32}P labeled at the 5′ end (23) and used in a primer extension experiment with oligo(dT)-selected RNAs (10 μg each) from uninfected (lane 2), PRV-InFh-infected (4 h) (lane 3), and PRV-Becker-infected (4 h) (lane 4) Madin-Darby bovine kidney cell cultures. Lane 1 contained only the input labeled primer. The dried gel was exposed to X-ray film for 12 h at −80°C. Sizes of the extended products were determined from a known DNA sequencing ladder.

PRV-InFh/Ka LLT is 8,404 nucleotides plus a poly(A) tail (Fig. 6). The transcription initiation site is 34 nucleotides downstream from a TATATA sequence. Another TATA sequence was also noted at nucleotide 180. The fact that LP1 starts at nucleotide 145, which is 5′ of the second TATA sequence, indicates that the TATA sequence at nucleotide 1 is functional. Whether the TATA sequence at nucleotide 180 is also functional has not been determined. Clones that start 3′ of both TATA sequences (e.g., LP8, which starts at nucleotide 615) could be the result of partial cDNA cloning. Minor variations between the composite prototype and PRV-Becker DNA sequences that have been determined include four single base changes (three of which resulted in amino acid residue changes), two three-nucleotide deletions (one in ORF-2 at nucleotide 1613 and one in the 3′ noncoding region at nucleotide 8374), and a six-nucleotide insertion in the 5′ noncoding region at nucleotide 283. The mRNA splice junction occurs after nucleotide 1510. The polyadenylation signal AATAAA is located at nucleotide 8382, and the poly(A) addition site is after nucleotide 8408. Another AATAAA sequence was also noted at nucleotide 1978 of PRV-InFh and -Becker, but this sequence has mutated to AACAAA in the PRV-Ka genome (7–9, 49, 50). On the basis of this information and the data compiled from the cDNA clones, it was concluded that this AATAAA sequence did not function as a polyadenylation signal for the synthesis of PRV LLT.

At the 5′ end of LLT, three AUG codons at nucleotides 428, 622, and 651 (one in each of the three ORFs) could yield a potential polypeptide of 17, 200, and 15-kDa, respectively (Fig. 6). Although the initiation codon at nucleotide 622 is the third one encountered from the 5′ end (the first two at nucleotides 428 and 434 are in the same ORF), this ORF-2 arrangement confers on PRV LLT the general features that best resemble a conventional eukaryotic mRNA. With ORF-2, the 5′ and 3′ noncoding regions of LLT are 621 and 1,940 nucleotides, respectively. The ORF-2 deduced amino acid sequence is presented because homology was detected in the amino acid sequence of the predicted HSV-1 LLT (see below).

ORF-2, from nucleotides 622 to 6495, is capable of encoding a predicted 1,958-amino-acid polypeptide of 200 kDa. Nucleotide variations between PRV-InFh and -Becker that resulted in amino acid residue changes occurred at residues 160, 216, and 254. The deleted codon of PRV-Becker at nucleotide 1613 resulted in one less histidine residue among a series of six (residues 330 to 335). This cluster of histidine residues is one of three clusters present in this region of the molecule; the other two clusters occur at residues 363 and 381. A highly acidic region is noted from residues 547 to 573. Toward the 3′ end, from residues 1830 to 1840 there is a cysteine-rich region Cys-X_2-Cys-X_3-Cys-X_2-Cys, where X can be any amino acid residue. This sequence arrangement resembles the zinc finger motif of many nucleic acid-binding proteins (5).

Localization of EP0. The cDNA library constructed in the lambda ZapII vector system with RNAs isolated from Madin-Darby bovine kidney cells infected with PRV-InFh was screened with nick-translated (35), cloned PRV-InFh *Bam*HI-P, -J, or -I DNA or LP8 cloned DNA (Fig. 1). Several independent cDNA clones with poly(A) tails were identified with *Bam*HI-P or LP8 cloned DNA. The clone with the largest EP0 DNA insert was Zap28. The nucleotide sequence of Zap28 was determined and aligned with the genomic sequence (Fig. 1G). In Zap28, a poly(A) track was observed 26 nucleotides downstream from a polyadenylation signal (AATAAA), which indicates that EP0 is transcribed in the same orientation as IE180 but in the opposite polarity with respect to PRV LLT. Zap28 starts at nucleotide 1633, 2 nucleotides short of the proposed initiation codon, and ends with 18 A nucleotides.

Mapping the cap site of the EP0 mRNA. To locate the 5′ end of the EP0 transcript, a 17-mer oligonucleotide (5′-AGGCAGATGGGGCAGTC-3′) from nucleotides 1486 to 1503 was used in a prime extension experiment. Oligo(dT)-selected RNAs from cultures infected with PRV-InFh or

A

```
1    GGATCCGCAGCGCCGCTTTCAGACCCAGGAGCCGTCGACCCACCGCGGGAGGGC
55   CCGCTTCCCACGACGGCGCGCCCGGGCCATCGTCCCGGGACGGCCCGAGGGGGC
109  GGGGGGAGCCCCGACGGGGCGGGCGGAAGGGGGCGTGGACGCCCCGGGCGAAGA
163  CAAACAAAGGGGCCGGGCACCCGGTTAAAAAACGGGGCCCTCGACACC          ATG GGC
1                                                              M   G
217  TGC ACG GTC TCT CGG AGA CGG ACG ACC ACC GCC GAG GCT TCC AGC GCC TGG GGG
3    C   T   V   S   R   R   R   T   T   T   A   E   A   S   S   A   W   G
271  ATC TTT GGC TTC TAC CGC CCC AGA AGC CCC TCG CCA CCG CCG CAG CGC CTG TCA
21   I   F   G   F   Y   R   P   R   S   P   S   P   P   P   Q   R   L   S
325  CTG CCA CTC ACC GTC ATG GAC TGC CCC ATC TGC CTG GAC GTC GCG GCC ACC GAG
39   L   P   L   T   V   M   D   C   P   I   C   L   D   V   A   A   T   E
379  GCG CAG ACG CTG CCG TGC ATG CAC AAG TTC TGT CTG GAC TGC ATC CAG CGC TGG
57   A   Q   T   L   P   C   M   H   K   F   C   L   D   C   I   Q   R   W
433  ACC CTG ACG AGC ACC GCC TGC CCG CTG TGC AAG GCC CGC GTC ACC TCC ATC CTC
75   T   L   T   S   T   A   C   P   L   C   K   A   R   V   T   S   I   L
487  CAC CAC GTG GAC AGC GAC GCT TCG TTT GTG GAG ACG CCC GTG GAG GGG GCG ACG
93   H   H   V   D   S   D   A   S   F   V   E   T   P   V   E   G   A   T
541  GAT GTC GAC GGG GAA GAG GAT GAG CCG GTA GGG GGG GGA TTC GCG GTC ATC TGG
111  D   V   D   G   E   E   D   E   P   V   G   G   G   F   A   V   I   W
595  GGT GAA GAC TAT ACC GAG GAG GTG CGC CAC GAG GAG GCC GAA GGG CAG GGC TCC
129  G   E   D   Y   T   E   E   V   R   H   E   E   A   E   G   Q   G   S
649  GGG TCT GGG TCT CGC GCC CGC CCC AGA GTC CCC GTC TTC AAC TGG CTT TAT GGG
147  G   S   G   S   R   A   R   P   R   V   P   V   F   N   W   L   Y   G
703  CAG GTC TCG ACG GTC ATC GAG AGC GAC CCC ATC CGC GAG GCC GTC GTG GAC AAC
165  Q   V   S   T   V   I   E   S   D   P   I   R   E   A   V   V   D   N
757  ATC GTC GAG ATT ATC CAG GAG CAC GGG ATG AAC CGC CAG CGC GTG ACC GAG GCC
183  I   V   E   I   I   Q   E   H   G   M   N   R   Q   R   V   T   E   A
811  ATG CTC CCC ATG TTT GGG GCA AAC ACC CAC GCC CTC GTC GAT ACG CTG TTT GAC
201  M   L   P   M   F   G   A   N   T   H   A   L   V   D   T   L   F   D
865  ATA TCG GCG CAG TGG ATG CGG CGG ATG CAG AGG CGA GCC CCG ATG AGC CAC CAG
219  I   S   A   Q   W   M   R   R   M   Q   R   R   A   P   M   S   H   Q
919  GGT GTG AAC TAT ATC GAC ACG TCC GAG TCC GAG GCA CAC TCT GAC TCT GAG GTG
237  G   V   N   Y   I   D   T   S   E   S   E   A   H   S   D   S   E   V
973  TCT TCC CCC GAC GAG GAA GAC TCG GGC GCC TCG AGC AGC GGG GTG CAC ACG GAG
255  S   S   P   D   E   E   D   S   G   A   S   S   S   G   V   H   T   E
1027 GAT CTG ACG GAG GCC TCC GAG TCC GCG GAC GAC CAG AGG CCG GCG CCC AGG CGC
273  D   L   T   E   A   S   E   S   A   D   D   Q   R   P   A   P   R   R
1081 TCC CCG CGC AGG GCC CGA CGG GCG GCC GTG CTG AGG CGC GAG CAG AGA CGG ACC
291  S   P   R   R   A   R   R   A   A   V   L   R   R   E   Q   R   R   T
1135 CGG TGC CTG CGA CGC GGC CGG ACG GGC GGA CAG GCC CAG GGC GAG ACT CCG GAG
309  R   C   L   R   R   G   R   T   G   G   Q   A   Q   G   E   T   P   E
1189 GCG CCA TCG TCC GGC GAG GGG TCC TCT GCG CAG CAT GGT GCC TCG GGG GCC GGG
327  A   P   S   S   G   E   G   S   S   A   Q   H   G   A   S   G   A   G
1243 GCC GGC CCG GGG TCG GCG AAC ACC GCC GCT TCG GCT CGC TCC TCC CCC TCG TCT
345  A   G   P   G   S   A   N   T   A   A   S   A   R   S   S   P   S   S
1297 TCA CCC TCC TCC TCG ATG CGG CGC CCG TCG CCC TCT GCC TCT GCC CCC GAG ACC
363  S   P   S   S   S   M   R   R   P   S   P   S   A   S   A   P   E   T
1351 GCC GCC CCC CGG GGC GGG CCT CCG GCG TCT AGC TCG TCG GGA TCC CCC CGC TCC
381  A   A   P   R   G   G   P   P   A   S   S   S   S   G   S   P   R   S
1405 GCT ACC ATC TTC ATC GAC CTC ACC CAG GAC GAC GAC TGA     GCTCCCTCTTCCTCG
399  A   T   I   F   I   D   L   T   Q   D   D   D   *
1459 CCGCGGACTGGGGCGACCCTGTTGCTGCTGCGGCCGCCGCCGCTCCTGCCCCCA
1513 CTTCGGCTCCCGCTCCTGCTCCTGCTCCCGGCCCCACTCCTGTTCCTGTTCCTG
1567 TTCCTGTTCCTGTTCCTGTTCCCGGTCCTGCTCCGGCTCCCGGCCCCGCACCCA
1621 CCTCCGCTCCTGCTGCGGGTCTCCAGGCCCAGACAAAATAAAAAAAGATATATT
1675 TTTTCAGTC
```

B

```
          41                  61                  81                 101
EPO   LTVMDCPICLDVAATEAQ--TLPCMHKFCLDCIQRWTLTSTACPLCKARVTSILHHVDSDASFVETPVE
      * * * *       * **** ** *  *       **** *                  **   *
ICPO  DEGDVCAVCTDEIAPHLRCDTFPCMHRFCIPCMKTWHQLRNTCPLCNAKLVYLIVGVTPSGSFSTIPIV
          111                 131                 151                 171

          41                  61                  81                 101
EPO   LTVMDCPICLDVAATEAQTLPCMHKFCLDCIQRWTLTSTACPLCKARVTSILHHVDSDASFVETPVE
      * **       * ** * **  **  ** **  ****   * ****   ** *  *  *
61    ASDNTCTICMSTVSDLGKTMPCLHDFCFVCIRAWTSTSVQCPLCRCPVQSILHKIVSDTSYKEYEVH
          14                  24                  44                  64
```

FIG. 8. (A) Nucleotide and deduced amino acid sequences of EP0. The DNA nucleotide sequence was determined by the dideoxy-chain termination method (41). The first six nucleotides of this sequence constitute a *Bam*HI restriction site, which is located between *Bam*HI-P and *Bam*HI-J in Fig. 1; transcription is leftward, as indicated by the arrow. The potential cap site and the termination codon are indicated by asterisks. The cysteine-rich zinc finger motif and the polyadenylation signal are underlined. The actual poly(A) addition site is located at the last nucleotide of the sequence. (B) Comparison of the deduced homologous protein domain of PRV EP0 with the protein domains of HSV-1 ICP0 and varicella-zoster gene 61. The coordinates indicate the positions of the amino acid residues in their respective polypeptides. Gaps are introduced into the sequence (in dashes) for best alignment. Identical residues are indicated by asterisks between the sequences. Cysteine residues that are part of the zinc finger motif are overlined.

-Becker for 4 h were used. Several groups of extension products were detected (Fig. 7). Those larger than 190 bases were present in both PRV-infected and mock-infected cultures, and they are considered background noises. Those smaller than 190 bases are likely to be incompletely extended products. The 190-base bands were taken as fully extended products for both RNA samples and therefore placed the potential cap site at nucleotide 1675. DNA sequence analysis showed that the EP0 RNA is 1,513 nucleotides plus a poly(A) tail, in agreement with the RNA blotting results (see below). Based on these data, the 5′ and 3′ noncoding regions are 40 and 236 nucleotides, respectively. However, genomic sequence analysis did not reveal a TATA box in the immediate area. The closest TATA sequence is located at nucleotide 4789, 3,114 bases upstream of this potential cap site. Therefore, it is possible that this mRNA is spliced at the 5′ end.

Deduced amino acid sequence of EP0. The EP0 transcript has an ORF of 1,230 nucleotides, capable of coding for a 410-amino-acid residue polypeptide of 45 kDa. The DNA nucleotide sequence and its deduced amino acid sequence are presented in Fig. 8A. The most noticeable feature of the encoded protein product is a cysteine-rich region. The Cys-X_2-Cys-X_{16}-Cys-X_2-Cys-X_{10}-Cys configuration, from amino acid residues 46 to 84, mimics the zinc finger motif of many DNA-binding proteins (5). Computer search on the National Institutes of Health data base (Bethesda, Md.) for homologous sequences yielded two proteins: ICP0 of HSV-1 and the gene 61 product of varicella-zoster virus (31). In addition to the zinc finger motif, EP0, gene 61, and ICP0 share many identical amino acid residues in the same restricted region of the polypeptides (Fig. 8B).

Biosynthesis of the EP0 mRNA during a productive infection. Conventionally, herpesvirus genes are divided into

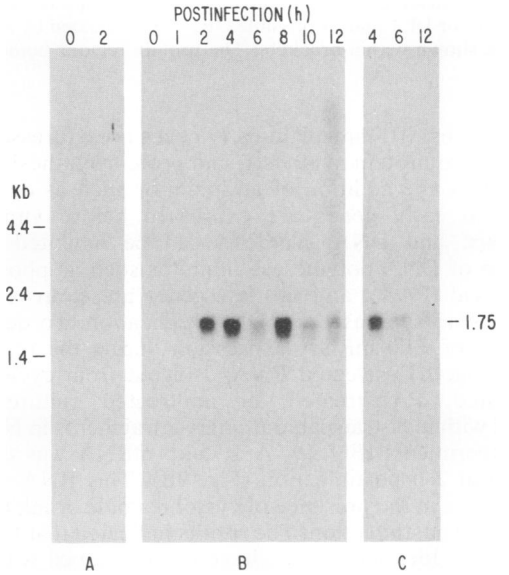

FIG. 9. Northern blot analysis. Equal amounts (10 μg each) of poly(A) RNAs from PRV-InFh-infected, cycloheximide-treated (A), untreated (B), and PAA-treated (C) cultures at the indicated times postinfection were prepared for Northern blot analysis. Nick-translated *Bam*HI-P probes were used and exposed to X-ray film for 18 h at −80°C. Positions of molecular weight markers are indicated on the left; the position of the detected EP0 RNA is indicated on the right.

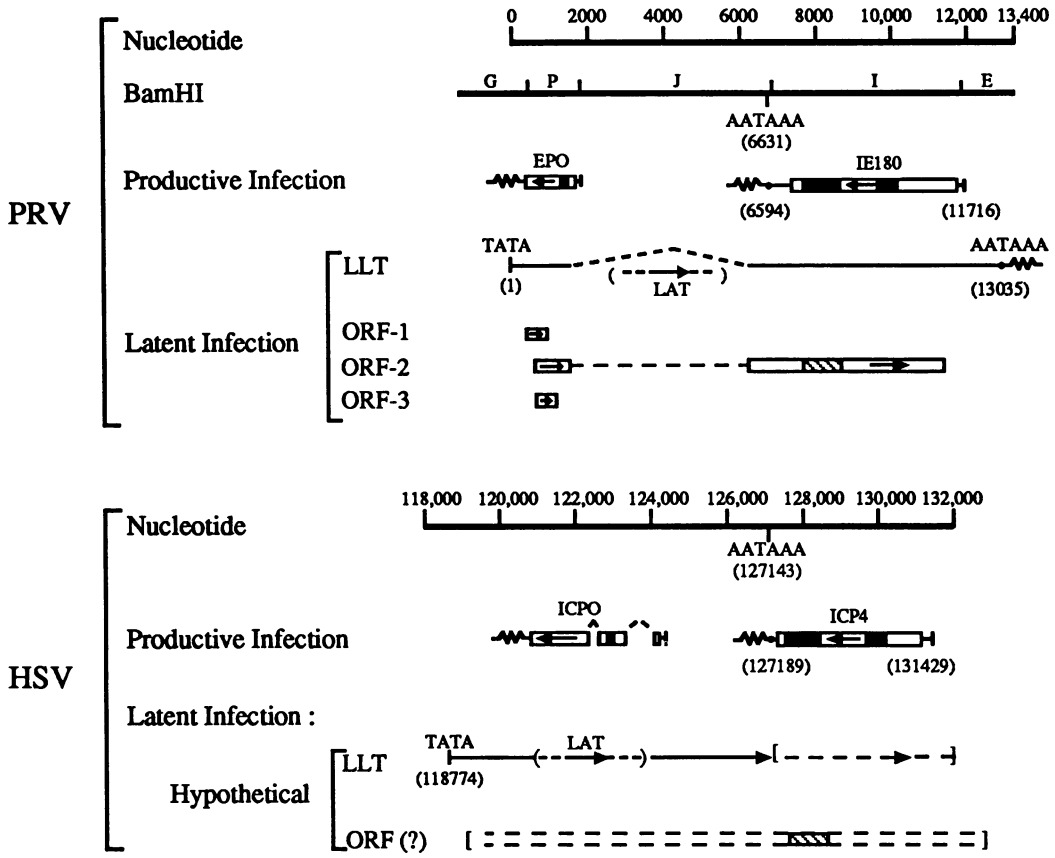

FIG. 10. PRV and HSV-1 transcripts during a latent or productive infection. The coordinates for PRV are indicated by *Bam*HI fragments (sizes are indicated in nucleotides), and the coordinates for HSV are indicated by the nucleotide positions of the genome (24, 25). Polyadenylated RNAs are denoted by squiggles, and the numbers in parentheses represent coordinates for the cap site or poly(A) signal, which is indicated by a dot. Transcripts synthesized during a productive infection for PRV are EP0 and IE180; those for HSV are ICP0 and ICP4. The conserved protein domains present in EP0 versus ICP0 and IE180 versus ICP4 are shaded. The directions of transcription for PRV LATs and for LLT and its possible ORFs are indicated by arrows. Splicing is indicated by dotted lines. The proposed 3′ end and ORF of HSV LLT are shown within brackets. The protein regions homologous between the ORFs of PRV LLT and HSV LLT are cross-hatched.

three classes. (i) Immediate-early genes are expressed without de novo protein synthesis, and protein synthesis can be blocked by the addition of an inhibitor such as cycloheximide; (ii) early genes are expressed before viral DNA synthesis, and DNA synthesis can be inhibited by the addition of DNA polymerase inhibitor such as phosphonoacetic acid (PAA); and (iii) late genes are expressed after viral protein synthesis and DNA replication. To determine the time of EP0 mRNA expression during the replicative cycle, oligo(dT)-selected RNAs isolated from cycloheximide-treated, PAA-treated, or nontreated cultures were probed with nick-translated *Bam*HI-P fragments in Northern blot experiments (Fig. 9). A 1.75-kb mRNA was detected starting at 2 h postinfection (Fig. 9B). This RNA was not synthesized in the presence of cycloheximide or inhibited by PAA at 4 h postinfection. The results indicated that EP0 is an early gene. Identical results have been obtained with PRV-Becker-infected cultures probed with the *Bam*HI-P fragment or an 18-mer oligonucleotide (5′-GGTGCTCGTCAGGGTC CA-3′) complementary to the mRNA at nucleotides 1399 to 1416 (data not shown).

Comparative analysis of the PRV and HSV-1 LLTs. Figure 10 is a schematic diagram illustrating the transcription activities of PRV and HSV-1 during a latent or productive infection. The spatial arrangements of PRV LLT, EP0, and IE180 are very similar to those of HSV-1 LLT, ICP0, and ICP4. Since conserved protein domains are present in respective homologous proteins (ICP0 versus EP0 and ICP4 versus IE180), it is expected that they exhibit similar biochemical functions. One would also predict that the protein products of PRV LLT and HSV-1 LLT share some common protein domains. To examine this possibility, all three frames of the HSV-1 DNA sequence from nucleotides 118000 to 132000 (24, 25) were translated. The sequence of each of these translation products was compared with the deduced amino acid sequence of PRV LLT ORF-2. Striking homologous sequences, 39% amino acid residue identity, were detected over a stretch of 400 amino acid residues between the deduced PRV LLT polypeptide and one of the deduced translation products of HSV-1 LLT (Fig. 11). The conserved protein domains are similarly located in their respective LLTs, which are complementary to the conserved region 4 of ICP4 and IE180. Homologous amino acid sequences were not observed in the vicinity complementary to the zinc finger region of EP0 and ICP0 or the conserved region 2 of IE180 and ICP4. Thus, the homologous protein domain presented in Fig. 11 appeared to be specific. The data suggest that HSV-1 LLT extends from the currently proposed 3′ end (13, 55) and overlaps ICP4 in the antiparallel orientation indicated in Fig. 10.

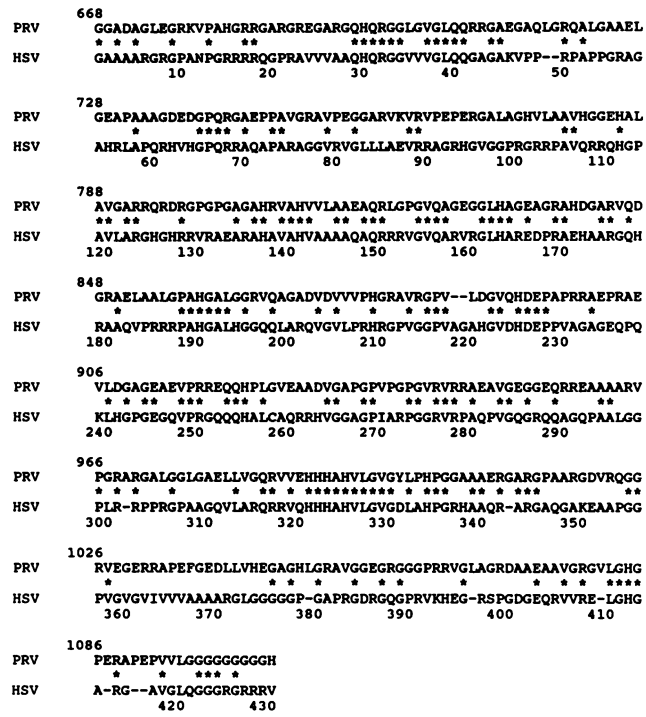

FIG. 11. Comparison of the deduced amino acid sequences of PRV LLT ORF-2 and an ORF of HSV-1 LLT. The coordinates for the PRV sequence correspond to the amino acid residue of the deduced amino acid sequence of PRV LLT ORF-2; the coordinates for the HSV sequence are arbitrary. Gaps (dashes) were introduced into the sequence for maximum homology. Identical residues between the two sequences are indicated by asterisks.

DISCUSSION

The results presented above show that PRV LLT is present in the cDNA library generated from RNAs of a latently infected animal and not detectable in productively infected cultures. The latency gene conforms to the well-established structure of a eukaryotic gene. LLT is transcribed 34 nucleotides downstream of a TATA promoter sequence. At the splice junction, consensus dinucleotides are present at the intron boundaries. At the 3′ terminus, a polyadenylation signal is present 27 nucleotides prior to the poly(A) addition site. These features together with the large ORF-2 suggest that LLT codes for a polypeptide, possibly by ORF-2, although there is no evidence to exclude potential translation from the other two ORFs. The presence of another TATA sequence in the 5′ noncoding region of the latency gene poses some intriguing possibilities about the regulation of this latency gene in different tissues or various stages of viral latency. As to the conflicting reports (6, 33) concerning the poly(A) nature of the RNAs expressed during latency, a possible explanation is that Northern blot analysis detected the abundant nonpoly(A) LATs (0.95, 2.0, and 5.0 kb) and S1 analysis detected the poly(A) LLT. Alternatively, there may be multiple processed forms of the RNAs not identified yet.

This work also showed that the PRV EP0, HSV-1 ICP0, and varicella-zoster virus gene 61 proteins contain a highly conserved region. Among the amino acid residues in this homologous region, there exists a cysteine-rich zinc finger similar to that of many DNA-binding proteins (5). Since this domain is conserved among three different viruses of the same family, it is likely an important element for each virus. Interestingly, by conventional classification, EP0 is an early protein whereas ICP0 is an immediate-early polypeptide. Although antibodies to EP0 have been detected in PRV-infected pig serum (unpublished data), the function(s) of EP0 has not been elucidated. However, by analogy, EP0 may possess functions similar to those associated with ICP0. ICP0 is nonessential for virus replication, but mutants defective in this gene exhibit impaired growth in tissue culture (40, 46). In vitro experiments have shown that ICP0 is a general transactivator of all three classes of HSV genes and functions synergistically with ICP4 (14–16, 30, 31, 34). The ICP0 zinc finger domain is essential for the transactivation activities (54). ICP4 is a transactivator of HSV early and late genes (38) and an inhibitor of HSV immediate-early gene promoters (29). Recent reports demonstrated that ICP0 may play a role in the reactivation of latent HSV (19, 54).

With HSV-1, other than the smaller LATs, recent reports suggest that there may be an 8.5-kb latency transcript that contains the entire ICP0 sequence in the antiparallel orientation but does not overlap ICP4 (13, 55). On the basis of the similarities of the biological functions and genome organization of HSV-1 and PRV, particularly within the area of latency transcription, it is expected that any functionally important domain(s) of the latency protein would be conserved between the two viruses. None of the amino acid sequences deduced from all three frames of the HSV-1 DNA sequences encoded by the previously proposed 8.5-kb latency RNA has any obvious homology with the deduced amino acid sequence of PRV LLT ORF-2. Since PRV LLT traverses the entire length of IE180, a homolog of HSV-1 ICP4, it is possible that the same situation occurs in HSV-1. If that is the case, the AATAAA sequence at the 3′ end of ICP4 would not function as a polyadenylation signal in HSV-1 LLT synthesis, a situation similar to that in PRV, in which the AATAAA sequence 3′ of IE180 does not function in PRV LLT synthesis. When the antiparallel DNA sequence of ICP4 is incorporated into the hypothetical HSV-1 latency gene, one of the ORFs shows significant amino acid sequence homology with the ORF-2 of PRV LLT. Whether this speculative situation occurs in HSV-1 remains to be seen. However, recent evidence from in situ hybridization experiments with both HSV-1 and HSV-2 showed that some HSV latency transcripts may extend halfway through their respective ICP4 genes (27, 28), a region that includes the coding sequence for the protein domain homologous to PRV LLT noted in my computer analysis.

Since PRV LLT is the only gene transcribed during latency, it is expected to play an important role in the establishment, maintenance, or reactivation of the latent virus. The spatial arrangements of the genes discussed and the conserved protein domains observed suggest that PRV and HSV homologous polypeptides, i.e., PRV LLT versus HSV-1 LLT, IE180 versus ICP4, and EP0 versus ICP0, are involved in a common pathway of herpesvirus latency. It is possible that the balance between a productive and latent infection depends on the molecular interactions of these viral genes and certain cellular factors.

ACKNOWLEDGMENTS

I thank L. Patrilla and B. Swearingen for technical assistance and L. Hornung for typing the manuscript.

REFERENCES

1. **Aviv, H., and P. Leder.** 1972. Purification of biologically active globin messenger RNA by chromatography on oligoothymidylic acid-cellulose. Proc. Natl. Acad. Sci. USA **69**:1408–1412.
2. **Beale, E. G., N. B. Chrapkiewicz, H. A. Scoble, R. J. Metz, D. P. Quick, R. L. Nobel, J. E. Donelson, K. Biemann, and D. K. Granner.** 1985. Rat hepatic cytosolic phosphoenolpyruvate carboxykinase (GTP). Structures of the protein, messenger RNA and gene. J. Biol. Chem. **260**:10748–10760.
3. **Ben-Porat, T., and A. S. Kaplan.** 1985. Molecular biology of pseudorabies virus, p. 105–173. *In* B. Roizman (ed.), The herpesvirus, vol. 3. Plenum Publishing Corp., New York.
4. **Beran, G. W., E. B. Davies, P. V. Arambulo, C. A. Will, H. T. Hill, and D. L. Rock.** 1980. Persistence of pseudorabies virus in infected swine. J. Am. Vet. Med. Assoc. **176**:998–1000.
5. **Berg, J. M.** 1986. Potential metal-binding domains in nucleic acid binding proteins. Science **232**:485–487.
6. **Cheung, A. K.** 1989. Detection of pseudorabies virus transcripts in trigeminal ganglia of latently infected swine. J. Virol. **63**: 2908–2913.
7. **Cheung, A. K.** 1989. DNA nucleotide sequence analysis of the immediate-early gene of pseudorabies virus. Nucleic Acids Res. **17**:4637–4646.
8. **Cheung, A. K., C. Vlcek, V. Paces, and M. Schwyzer.** 1990. Update and comparison of the immediate-early gene DNA sequences of the two pseudorabies virus isolates. Virus Genes **4**:261–265.
9. **Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18**:5294–5299.
10. **Croen, K. D., J. M. Ostrove, L. J. Pragovic, and S. E. Straus.** 1988. Patterns of gene expression and sites of latency in human nerve ganglia are different for varicella-zoster and herpes simplex viruses. Proc. Natl. Acad. Sci. USA **85**:9773–9777.
11. **Davies, E. B., and G. W. Beran.** 1980. Spontaneous shedding of pseudorabies virus from clinically recovered postparturient sow. J. Am. Vet. Med. Assoc. **176**:1345–1347.
12. **Deatly, A. M., J. G. Spivak, 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.
13. **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.
14. **Everett, R. D.** 1984. Transactivation of transcription by herpes virus products: requirement for two HSV-1 immediate-early polypeptides for maximum activity. EMBO J. **3**:3135–3141.
15. **Gelman, I. H., and S. Silverstein.** 1985. Identification of immediate-early genes from herpes simplex virus that transactivate the virus thymidine kinase gene. Proc. Natl. Acad. Sci. USA **82**:5265–5269.
16. **Gelman, I. H., and S. Silverstein.** 1986. Coordinate regulation of herpes simplex virus gene-expression is mediated by the functional interaction of 2 immediate-early gene products. J. Mol. Biol. **191**:395–409.
17. **Gordon, Y. J., B. Johnson, E. Romanowski, and T. Aravllo-Cruz.** 1988. RNA complementary to herpes simplex virus type 1 ICP0 gene demonstrated in neurons of human trigeminal ganglia. J. Virol. **62**:1832–1835.
18. **Gubler, U., and B. J. Hoffman.** 1983. A simple and very efficient method for generating cDNA libraries. Gene **25**:263–269.
19. **Harris, R. A., R. D. Everett, X. Zhu, S. Silverstein, and C. M. Preston.** 1989. Herpes simplex virus type 1 immediate-early protein vMW110 reactivates latent herpes simplex virus type 2 in an in vitro latency system. J. Virol. **63**:3513–3515.
20. **Huyah, T., R. A. Young, and R. W. Davis.** 1985. Constructing and screening cDNA libraries in λgt10 and λgt11, p. 49–78. *In* D. M. Glover (ed.), DNA cloning techniques, a practical approach, vol. I. IRL Press, Oxford.
21. **Krause, P. R., K. D. Croen, S. E. Straus, and J. M. Ostrove.** 1988. Detection and preliminary characterization of herpes simplex virus type 1 transcripts in latently infected human trigeminal ganglia. J. Virol. **62**:4819–4823.
22. **Lokensgard, J. R., D. G. Thawley, and T. W. Molitor.** 1990. Pseudorabies virus latency: restricted transcription. Arch. Virol. **110**:129–136.
23. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **68**:499–560.
24. **McGeoch, D. J., M. A. Dalrymple, A. J. Davison, A. Dolan, M. C. Frame, D. McNab, L. J. Perry, J. E. Scott, and P. Taylor.** 1988. The complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1. J. Gen. Virol. **69**:1531–1574.
25. **McGeoch, D. J., A. Dolan, S. Donald, and D. H. K. Brauer.** 1986. Complete DNA sequence of the short repeat region in the genome of herpes simplex virus type 1. Nucleic Acids Res. **14**:1727–1745.
26. **McMaster, G. K., and G. G. Carmichael.** 1977. Analysis of single and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. Proc. Natl. Acad. Sci. USA **74**:4835–4838.
27. **Mitchell, W. J., S. L. Deshmane, A. Dolan, D. J. McGeoch, and N. W. Fraser.** 1990. Characterization of herpes simplex virus type 2 transcription during latent infection of mouse trigeminal ganglia. J. Virol. **64**:5342–5348.
28. **Mitchell, W. J., R. P. Lirette, and N. W. Fraser.** 1990. Mapping of low abundance latency-associated RNA in the trigeminal ganglia of mice latently infected with herpes simplex virus type 1. J. Gen. Virol. **71**:125–132.
29. **O'Hare, P., and G. S. Hayward.** 1984. Expression of recombinant genes containing herpes simplex virus delayed-early and immediate-early regulatory regions and transactivation by herpesvirus infection. J. Virol. **52**:722–731.
30. **O'Hare, P., and G. S. Hayward.** 1985. Evidence for a direct role for both the 175,000- and 110,000-molecular-weight immediate-early proteins of herpes simplex virus in the transactivation of delayed-early promoters. J. Virol. **53**:751–760.
31. **Perry, L. J., F. J. Rixon, R. D. Everett, M. C. Frame, and D. J. McGeoch.** 1986. Characterization of th IE110 gene of herpes simplex virus type 1. J. Gen. Virol. **67**:2365–2380.
32. **Pirtle, E. C., M. W. Wathen, P. S. Paul, W. L. Mengeling, and J. M. Sacks.** 1984. Evaluation of field isolates of pseudorabies (Aujeszky's disease) virus as determined by restriction endonuclease analysis and hybridization. Am. J. Vet. Res. **45**:1906–1912.
33. **Priola, S. A., D. P. Gustafson, E. K. Wagner, and J. G. Stevens.** 1990. A major portion of the latent pseudorabies virus genome is transcribed in trigeminal ganglia of pigs. J. Virol. **64**:4755–4760.
34. **Quinlan, M. P., and D. M. Knipe.** 1985. Stimulation of expression of a herpes simplex virus DNA-binding protein by two viral functions. Mol. Cell Biol. **5**:957–963.
35. **Rigby, P. W. J., M. Dieckmann, C. Rhoades, and P. J. Berg.** 1977. Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase. J. Mol. Biol. **113**:237–251.
36. **Rock, D. L., S. L. Beam, and J. E. Mayfield.** 1987. Mapping bovine herpesvirus type 1 latency-related RNA in trigeminal ganglia of latently infected rabbits. J. Virol. **61**:3827–3831.
37. **Rock, D. L., A. B. Nesburn, H. Ghiasi, J. Ong, T. L. Lewis, J. R. Lokensgard, and S. 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.
38. **Roizman, B., and A. E. Sears.** 1990. Herpes simplex viruses and their replication, p. 1795–1841. *In* B. N. Fields and D. M. Knipe (ed.), Virology, 2nd ed., vol. 2. Raven Press, New York.
39. **Sabo, A., and S. Grunert.** 1971. Persistence of virulent pseudorabies virus in herds of vaccinated and nonvaccinated pigs. Acta Virol. **15**:87–94.

40. **Sacks, W. R., and P. A. Shaffer.** 1987. Deletion mutants in the gene encoding the herpes simplex virus type 1 immediate-early protein ICP0 exhibit impaired growth in cell culture. J. Virol. **61**:829–839.
41. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
42. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–518.
43. **Spivack, J. G., and N. W. Fraser.** 1987. Detection of herpes simplex virus type 1 transcripts during a latent infection in mice. J. Virol. **61**:3841–3847.
44. **Stevens, J. G., L. Haarr, D. D. Porter, M. L. Cook, and E. K. Wagner.** 1988. Prominence of the herpes simplex virus latency-associated transcript in trigeminal ganglia from seropositive humans. J. Infect. Dis. **158**:117–122.
45. **Stevens, J. G., E. K. Wagner, G. B. Devi-Rao, M. L. Cook, and L. T. Feldman.** 1987. RNA complementary to a herpesvirus alpha gene mRNA is predominant in latently infected neurons. Science **235**:1056–1059.
46. **Stow, N. D., and E. C. Stow.** 1986. Isolation and characterization of a herpes simplex virus type 1 mutant containing a deletion within the gene encoding the immediate-early polypeptide VMW110. J. Gen. Virol. **67**:2571–2585.
47. **Vafai, A., R. S. Murray, M. Wellish, M. Delvin, and D. H. Gilden.** 1988. Expression of varicella-zoster virus and herpes simplex virus in normal human trigeminal ganglia. Proc. Natl. Acad. Sci. USA **85**:2362–2366.
48. **Van Oirschat, J. R., and A. L. J. Gielkens.** 1984. *In vivo* and *in vitro* reactivation of latent pseudorabies virus in pigs born to vaccinated sows. Am. J. Vet. Res. **45**:567–571.
49. **Vlcek, C., Z. Kozmik, V. Paces, S. Schirm, and M. Schwyzer.** 1990. Pseudorabies virus immediate-early gene overlaps with an oppositely oriented open reading frame—characterization of their promoter and enhancer regions. Virology **179**:365–377.
50. **Vlcek, C., V. Paces, and M. Schwyzer.** 1989. Nucleotide sequence of the pseudorabies virus immediate early gene encoding a strong transactivator protein. Virus Genes **2**:335–346.
51. **Wagner, E. K., G. B. Devi-Rao, L. T. Feldman, A. T. Dobson, Y. F. Zhang, W. M. Flanagan, and J. Stevens.** 1988. Physical characterization of the herpes simplex virus latency-associated transcripts in neurons. J. Virol. **62**:1194–1202.
52. **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.
53. **Whittman, G., V. Ohlinger, and H. J. Rziha.** 1983. Occurrence and reactivation of latent Aujeszky's disease virus following challenge in previously vaccinated pigs. Arch. Virol. **75**:29–41.
54. **Zhu, X., J. Chen, C. H. S. Young, and S. Silverstein.** 1990. Reactivation of latent herpes simplex virus by adenovirus recombinants encoding mutant IE-0 gene products. J. Virol. **64**:4489–4498.
55. **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.