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A Major Portion of the Latent Pseudorabies Virus Genome Is Transcribed in Trigeminal Ganglia of Pigs

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Pseudorabies virus (PRV) is a porcine herpesvirus that establishes latent infections in trigeminal ganglia. To determine whether PRV expresses any transcripts that could play a role in latency, the trigeminal ganglia of 14 pigs previously inoculated through the nose and latently infected with PRV(Ka) were assayed by in situ nucleic acid hybridization for the presence of PRV-specific RNA. Hybridizations employing probes encompassing the entire viral genome revealed that an area extending from 0.64 to 0.82 map units was transcriptionally active. The DNA probe that most consistently detected transcripts was *Bam*HI-8, a fragment which contains the gene for the immediate-early protein. With this probe, ganglia from 10 (71%) of 14 pigs scored positive for PRV RNA, although only 1 (8%) of 12 of the ganglia from the opposite side reactivated virus after explantation and culture of latently infected trigeminal ganglia. The RNA was transcribed from the strand opposite to that coding for the immediate-early protein; the signal was neuronally localized, with dense nuclear accumulation accompanied by variable numbers of grains over the cytoplasm. Northern RNA blot analysis showed that a discrete set of poly(A)$^-$ PRV transcripts were present in latently infected trigeminal ganglia. Additional in situ nucleic acid hybridization analysis revealed that the 3′ limit of the transcriptionally active area was located in a 1.2-kilobase fragment upstream and adjacent to the 5′ end of the immediate-early protein RNA, whereas the 5′ limit was as much as 4.9 kilobases downstream from the 3′ end of this RNA. PRV therefore expresses latent-phase transcripts that, although similar in many respects to latent-phase transcripts reported for other herpesviruses, have some unique properties.

One of the most important properties of neurotropic herpesviruses is their capacity to establish latent infections in the nervous system (22). After local or generalized stimuli these viruses can reactivate, produce disease in the host, and spread to additional individuals. To control such recurrences, an understanding of the phenomena involved with establishment, maintenance, and reactivation of the latent state is essential.

Recent studies in this laboratory and others have defined the areas of the herpes simplex virus type 1 (HSV-1) genome that are active during latency. In the nuclei of ganglionic neurons of mice (20, 24), rabbits (16), and humans (6, 10, 23) latently infected with HSV-1, RNA transcripts have been detected that are opposite in sense to the RNA for the HSV-1 immediate-early protein ICP0. These transcripts, termed latency-associated transcripts (LATs), are poly(A)$^-$ RNAs 2.1 and 1.5 kilobases (kb) in length (26, 27). LATs with some of the same properties have also been found in rabbits latently infected with bovine herpesvirus type 1 (BHV-1) (14). Varicella-zoster virus also expresses transcripts during the latent state, but there is controversy relative to the identification of cells harboring latent DNA. In supporting cells the transcripts are not restricted to the immediate-early regions of the viral genome, and some appear to be complementary to transcripts detected in acutely infected tissue culture cells (4). Taken together, the transcripts present in these tissues can be considered to be markers for latency; in the case of HSV-1, some portion of the transcriptional unit is involved in facilitating reactivation from the latent state (8, 11, 19, 21).

Early studies of pseudorabies virus (PRV) indicated that virus-specific RNA was not present in latently infected neurons (1), although PRV-specific DNA is abundantly present (7, 17). More recently, however, in studies in which in situ nucleic acid hybridization utilizing a whole genomic probe was employed, transcripts have been found (15), and RNA has also been detected by S1 nuclease analysis (2). This report confirms and extends these observations by further characterization of PRV gene expression during latency. Employing both in situ nucleic acid hybridization and Northern RNA blot technologies, we analyzed expression of the PRV genome latent in swine trigeminal ganglia, and we partially characterized the transcripts found. We report here that latent PRV expresses transcripts that, although they are similar in many respects to the HSV-1 LATs, are expressed from a much larger area of the genome.

MATERIALS AND METHODS

Virus and cells. PRV strain PRV(Ka) was kindly provided by Lawrence Feldman, Department of Microbiology and Immunology, University of California, Los Angeles, School of Medicine. Viral stocks were prepared and titrations were performed by standard methods on a line of rabbit skin (RS) cells grown in minimal essential medium plus 10% calf serum. The RK_{13} line of rabbit kidney (RK) cells was used for in vitro reactivation studies of explanted trigeminal ganglia tissue; cells were maintained in minimal essential medium plus 10% newborn calf serum and 5% fetal calf serum.

Clones. The *Bam*HI fragment 8 of PRV(Ka) DNA cloned

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into the Bluescript vector (Stratagene; no. 212205) was also provided by Lawrence Feldman. Bluescript contains promoters for both the T3 and T7 RNA polymerases and can be used to generate single-stranded RNA fragments. Other *Bam*HI fragments of PRV(Ka) were either cloned into pUC12 or kindly provided by Tamar Ben-Porat, Department of Microbiology, Vanderbilt University. In addition to *Bam*HI-8, PRV(Ka) DNA fragments *Kpn*I-E and *Sal*I-1 were initially cloned into pUC19. Subclones of these fragments and *Bam*HI-8 were prepared in Bluescript. Cloning was accomplished by standard techniques (13).

Infection of pigs and cocultivation of tissues. Three-week-old weanling pigs were pre-bled and tested by standard methods for serum-neutralizing PRV antibody by The Animal Disease Diagnostic Laboratory at Purdue University; all were negative. They were then infected intranasally with 1.0 ml of inoculum containing 10^6 PFU of PRV(Ka) for acutely infected ganglia or 10^4 PFU of PRV(Ka) for latent ganglia. To study the acute infections, one animal was killed on day 4 after infection. Thirty days or more after infection, pigs (14 total, two experiments) were bled once more to assess seroconversion and killed, and both trigeminal ganglia were removed. One ganglion was frozen and stored at −70°C for future use in in situ nucleic acid hybridization and Northern blot analysis; the second was used for an in vitro reactivation assay. Finally ganglia from two uninfected pigs were employed as negative controls in the nucleic acid hybridization experiments. Of the 22 pigs infected in two experiments, 1 was used as an acutely infected control, 14 survived for use as latent animals, and all developed neutralizing antibodies in serum. The titers of these antibodies (expressed as reciprocals of the dilution of serum needed to neutralize 50% of the input virus) ranged from 1:8 to 1:64.

In vitro recovery of virus. Two methods were used to reactivate virus from explanted ganglia. In the first experiment, tissue specimens obtained from six latently infected animals were cocultivated in 10% newborn calf serum–5% fetal calf serum on RK_{13} cells at 37°C. Every other day, one-half of the medium was removed and replaced with fresh medium. Cells were periodically checked for the appearance of plaques. Ganglia were removed and placed on new cell monolayers every week and cultivated for 40 days or more. In the second experiment, six ganglia were placed in 2 ml minimal essential medium plus 10% newborn calf serum and 200 mM dimethyl sulfoxide and placed at 37°C. Every other day a sample of the medium was plated on RS cells, and plaques were scored. This method has been reported to be a more sensitive assay than explant cocultivation on indicator cells in the absence of dimethyl sulfoxide (28). As a positive control for the assay systems, lumbosacral ganglia from mice latently infected via scarified rear footpads with HSV-1 strain KOS(M) (24) were tested concurrently with swine trigeminal ganglia in explant culture under identical conditions.

In situ nucleic acid hybridizations. In situ hybridizations were performed on frozen, cryostat-cut trigeminal ganglia tissue from latently infected, acutely infected, and uninfected pigs. The protocol was the same as previously reported (25). Tritiated double-stranded DNA probes were generated by nick translation (24), and tritiated single-stranded RNA (ssRNA) probes were made by transcribing PRV(Ka) fragments cloned into Bluescript with either T3 or T7 RNA polymerase according to the instructions of the manufacturer. Probes were hydrolyzed on ice and sized for hybridization as described by Cox et al. (3).

For slides that were hybridized with ssRNA probes, hybridization was at 56°C in 50% formamide. The slides were first rinsed three times in 4× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) for 5 min each time and treated with 20 μg of RNase A per ml for 30 min at 37°C in RNase buffer (0.5 M NaCl, 10 mM Tris [pH 8.0]), followed by two 15-min rinses in RNase buffer at 37°C. Slides were then rinsed numerous times in 2× SSC at room temperature, followed by a high-stringency rinse in 0.1× SSC for 10 min at 56°C and a 15-min rinse at room temperature in 0.1× SSC. Finally the slides were dehydrated in increasing concentrations of ethanol–0.3 M ammonium acetate, dipped in film emulsion, and placed at 4°C for 2 weeks (3).

Northern blot analysis. Northern blots were performed on total RNA or poly(A)$^+$ and poly(A)$^-$ RNA extracted from the trigeminal ganglia of one selected acutely or latently infected pig or uninfected pig as described previously (24). Blots were probed either with random hexamer-primed, ^{32}P-labeled DNA probes or ^{32}P-labeled riboprobes derived by transcribing Bluescript clones with T3 or T7 RNA polymerase. Blots hybridized with ssRNA probes were rinsed twice for 30 min each in 0.1× SSC–0.1% sodium dodecyl sulfate at 60°C, followed by a 1-h rinse at 68°C in the same solution.

RESULTS

In vitro recovery of virus. Of ganglia from 12 pigs cultivated in vitro to induce reactivation, only one produced virus; this was at 8 days after explanation. This ganglion was one of six tested in experiment 2 and was positive when cultivated in 200 mM dimethyl sulfoxide. None of the other ganglia was positive, although ganglia from mice latently infected with the HSV-1 strain KOS and cultivated at the same time and under identical conditions were all positive when tested with this assay system. This result indicates that in vitro-induced reactivation is not easily accomplished with this virus strain. Proving that the entire viral genome is present and biologically active is impossible at this juncture, but we have detected viral DNA with probes specific for areas outside the transcriptionally active area (data not shown). Potential reasons for our inability to detect infectious virus with in vitro cocultivation techniques are discussed in greater detail in the Discussion.

Genomic area transcribed. Sections from trigeminal ganglia presumed to be latently infected were treated with DNase to detect RNA and incubated with individual ^{3}H-labeled *Bam*HI fragments from the PRV(Ka) genome. Most of the fragments in the unique long region did not hybridize, and no part of the unique short region hybridized (Fig. 1). Several fragments in the repeat regions did hybridize, however, and the signal was present only over neurons. In terms of grains per neuron, fragment 8 gave the strongest positive signal. Fragments 8′ and 5, which flank fragment 8 in the internal repeat short region, also hybridized, although the signal was much weaker and was detected in fewer neurons. Unexpectedly, fragments 14 and 6 were also positive, and fragment 14, which spans the joint region between internal repeat short and unique long sequences, hybridized almost as strongly as did fragment 8. Similar results have recently been reported by Lokensgard et al. (12). Fragment 6, which is located in the unique long region near the internal repeat short sequence, was positive in only a few, rare neurons and gave a very weak signal. In sum, the signal was detected with probes that extend from 0.64 to 0.82 map units, an area of approximately 24.9 kb.

To determine the number of latently infected ganglia

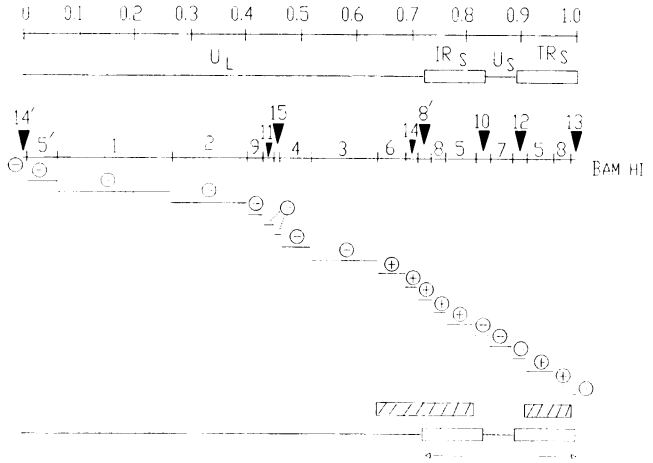

FIG. 1. Extent of latent PRV transcription in swine trigeminal ganglia established by in situ nucleic acid hybridization with double-stranded DNA probes. Tritiated double-stranded DNA clones corresponding to the *Bam*HI fragments of PRV(Ka) DNA were hybridized to sections of latently infected trigeminal ganglia. A diagram of the PRV genome is shown with the *Bam*HI restriction map of PRV(Ka) (9) at the top of the figure, and the probes tested are shown underneath the fragments they represent. Results of the hybridization (+ or −) are presented in the circles. Fragments were considered positive if, of approximately 30 tissue sections examined, 10 (30%) demonstrated hybridization signal. The same number of sections was scored before a fragment was considered negative. A summary drawing depicting the transcriptionally active area of the genome is presented in the hatched boxes at the bottom of the figure. The arrows represent the direction of IEP transcription. Abbreviations: U_L, unique long; IR_S, internal repeat short; U_S, unique short; TR_S, terminal repeat short.

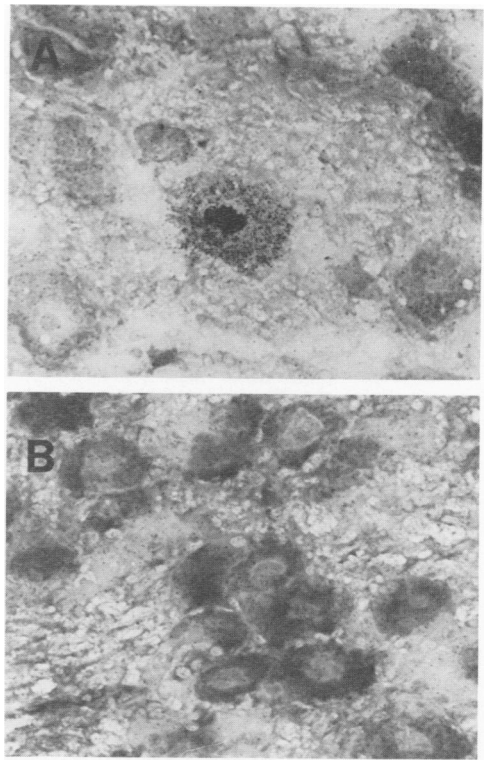

FIG. 2. PRV RNA in latently infected swine trigeminal ganglia. A ^{3}H-labeled PRV(Ka) *Bam*HI-8 fragment-derived DNA probe was employed to detect RNA in sections of pig trigeminal ganglia. (A) Latently infected trigeminal ganglion. (B) Uninfected trigeminal ganglion. Silver grains are present over one neuron in panel A. Magnification, ×500.

expressing transcripts, slides of individual latent trigeminal ganglia samples were treated with DNase and hybridized with a double-stranded DNA ^{3}H-labeled *Bam*HI-8 probe. The *Bam*HI-8 fragment was chosen not only because it contains the entire coding region of the only PRV immediate-early protein (IEP) (9) and it is the IE protein-coding regions that are active during HSV-1 (24) and BHV-1 (14) latency, but also because it was the fragment that had hybridized most strongly in the initial in situ hybridization studies. Of 14 ganglia tested, 10 (71%) were strongly positive for RNA from this IEP region. The signal was restricted to neurons and was mostly concentrated in the nucleus, although there was also substantial cytoplasmic signal (Fig. 2). These 10 positive ganglia were used in the in situ nucleic acid hybridization studies described below.

Direction of transcription. Since LATs in other systems localized to the immediate-early regions of the viral genome have been transcribed from the DNA strand opposite to that encoding the immediate-early genes (14, 24), we determined the direction of transcription of the RNA originating from this area of the genome in PRV(Ka). A *Bam*HI-8-derived clone (P7) in the Bluescript vector was transcribed with T3 or T7 RNA polymerase, generating ssRNA probes specific for IEP RNA and its complement. These probes were used in an in situ hybridization analysis of ganglia previously found to be expressing transcripts as detected by the *Bam*HI-8 fragment. Only the probe that detected transcripts originating from the strand opposite to that which transcribes IEP was positive (Fig. 3). Again, the signal was only found in neurons, and the most common pattern consisted of a dense nuclear signal with light to heavy scattered cytoplasmic grains, although a few neurons demonstrated signal which was limited to the nucleus. This pattern is different from that seen in latently HSV-1 infected ganglia, where the signal is confined to the neuronal nucleus (24). No signal was ever detected by the probe specific for the IEP transcript (Fig. 3), and uninfected samples were negative when either probe was used.

Northern blot analysis. To physically characterize the transcripts present, RNA extracted from acutely or latently infected or uninfected ganglia was hybridized with a ^{32}P-labeled double-stranded DNA *Bam*HI-8-derived probe. Although the Northern blots demonstrated a significantly lower level of resolution than those we have obtained with RNA extracted from mouse or rabbit tissue latently infected with HSV-1, PRV-specific transcripts were detected. RNA from two separate animals demonstrated the same pattern of hybridization: RNA migrating between approximately 4.5 and 5.5 kb and between approximately 1.0 and 2.0 kb (data not shown) in acutely and latently infected animals. When this RNA was further separated into poly(A)$^+$ and poly(A)$^-$ fractions (25) and probed with a ^{32}P-labeled riboprobe, P7-T3, which is specific for RNA complementary to IEP RNA, the same pattern was observed (Fig. 4). In addition, hybridization occurred only in the poly(A)$^-$ fraction (Fig. 4). Although these transcripts share sequences (they were detected with the same probe), we do not yet know the extent of the overlap.

Determination of the extent of transcription. With ssRNA probes, we next determined the limits of RNA expression relative to the genomic region encoding the IEP RNA.

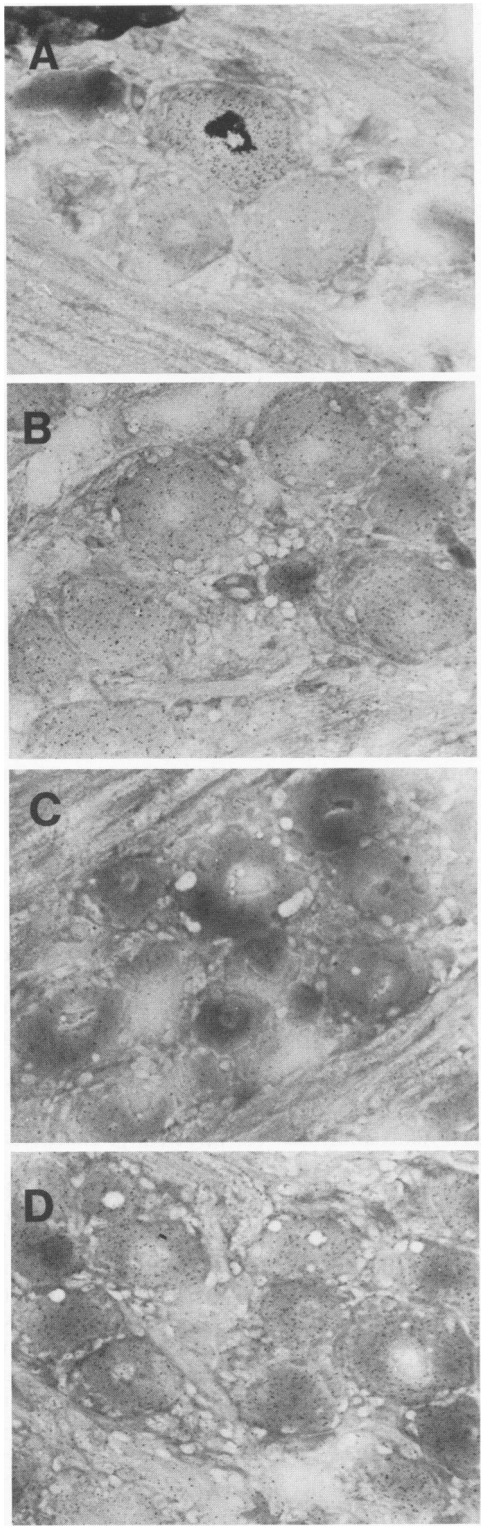

FIG. 3. Transcripts complementary to PRV IEP RNA present in latently infected swine trigeminal ganglia. Tritium-labeled riboprobes derived from the *Bam*HI-8 fragment of PRV(Ka) DNA were employed to detect PRV RNA in sections of latent pig trigeminal ganglia. (A) Latently infected ganglion hybridized with riboprobe P7-T3 (specific for an RNA complimentary to the IEP RNA). (B) Latently infected ganglion hybridized with riboprobe P7-T7 (specific for IEP RNA). (C) Uninfected pig trigeminal ganglion hybridized with riboprobe P7-T3. (D) Uninfected pig trigeminal ganglion hybridized with riboprobe P7-T7. Silver grains are present over one neuron in panel A. Magnification, ×500.

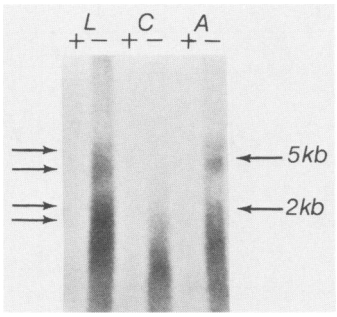

FIG. 4. Northern blot of poly(A)$^-$ and poly(A)$^+$ RNA extracted from latently infected swine trigeminal ganglia and hybridized with riboprobes derived from the *Bam*HI-8 fragment of PRV(Ka) DNA. Poly(A)$^+$ (+) and poly(A)$^-$ (−) RNA was extracted from the trigeminal ganglia of latently (L), and acutely (A) infected pigs as well as uninfected control (C) pigs and hybridized with a ^{3}H-labeled ssRNA P7-T3 probe specific for transcripts encoded by the DNA strand opposite to IEP. RNA bands at between approximately 4.5 and 5.5 kb are present in lanes containing poly(A)$^-$ RNA extracted from both acutely (A) and latently (L) infected ganglia. PRV specific RNA migrating at 1.0 to 2.0 kb is also detectable. None of these transcripts is seen in the uninfected control (C) lanes, and no signal was detected in any of the poly(A)$^+$ samples. Arrows on the left indicate the locations of these transcripts, and the arrows on the right show the positions of 5- and 2-kb eucaryotic rRNAs.

Regions outside of the *Bam*HI-8 fragment as well as within it were subcloned into Bluescript, and ssRNA probes generated from each of these clones were used in in situ nucleic acid hybridization analysis on latently infected ganglionic tissue. Only RNA complementary to IEP RNA could be detected (probes IIc through VIIc and P7-T3); no signal was ever detected with IEP-specific probes (Fig. 5).

The 3′ end of this RNA fraction is localized within the IIc clone; both strands of the probe immediately preceding it were negative. This area lies within a 1.2-kb region upstream of the 5′ end of IEP in the *Bam*HI-5 fragment. The 5′ end has been more difficult to localize. All subclones of *Bam*HI-8, *Kpn*I-E, and *Sal*I-1 derived from the area downstream of the 3′ end of IEP have given a positive signal. The 5′ end of these RNAs is at least 4.9 kb from the 3′ end of IEP but cannot extend beyond the *Bam*HI-6 fragment, since no hybridization was detected with *Bam*HI-3, which is immediately upstream of *Bam*HI-6. Taken together with the experiments summarized in Fig. 1, the data from these studies indicate that the area transcribed is at least 11 kb in length and suggest that splicing may be occurring, since no transcripts this long were detected on Northern blots. Alternatively, different transcriptional start sites may be used to produce the various transcripts detected here.

DISCUSSION

PRV, while latent in the trigeminal ganglia of infected pigs, expresses RNA from an unexpectedly large area of the genome extending from 0.64 to 0.82 map units, a region which includes the PRV IEP. A more detailed examination of the RNAs originating from this region by in situ nucleic acid hybridization analysis defined a transcript in the

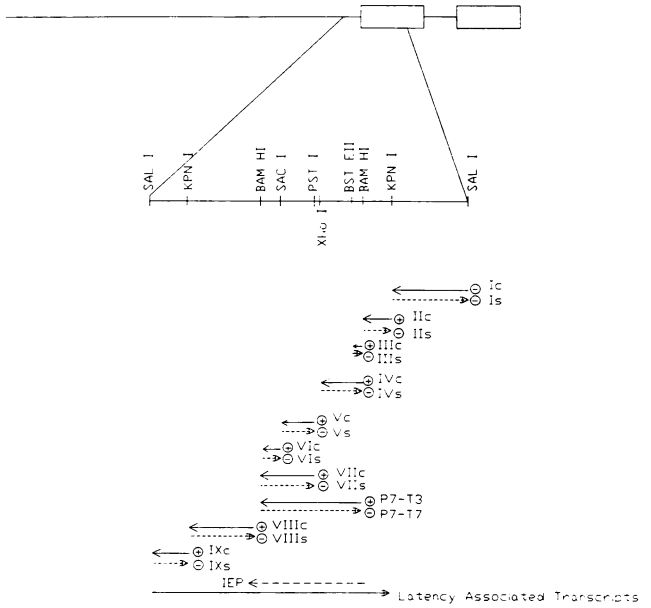

FIG. 5. Extent and direction of latent PRV transcription in swine trigeminal ganglia established by in situ nucleic acid hybridization with ssRNA probes. The upper part of the figure represents the PRV genome. The area magnified below this represents the restriction map of the *Sal*I-1 fragment of PRV(Ka) DNA. Each set of arrows represents a set of riboprobes derived from a single Bluescript clone. Broken arrows are probes specific for IEP RNA, and solid arrows are probes specific for the RNA complimentary to the IEP RNA, and solid arrows are probes specific for the RNA complimentary to the IEP RNA. Probes giving positive (+) and negative (−) signals are indicated. The criterion for scoring fragments as positive or negative is given in the legend to Fig. 1. Probes are labeled I through IX, starting from the right side of the fragment. c, IEP complementary specific probes; s, IEP-specific probe. Probes from the P7 *Bam*HI-8 clone are indicated as P7-T3 and P7-T7. The extent and direction of transcription from both the IEP RNA (←---) and its complement (→) are summarized at the bottom of the figure.

*Bam*HI-8 fragment, which is transcribed from the strand opposite to that coding for IEP. This transcript is found only in neurons and, although mainly restricted to the nucleus, is also present in the cytoplasm. The 3′ limit of this transcript is located within a 1.2-kb fragment upstream and adjacent to the 5′ end of IEP, whereas the 5′ end is located as much as 4.9 kb downstream of the 3′ end of IEP. Although this would indicate an RNA of roughly 11 kb in size, Northern blot analysis of latently infected pig ganglia indicates that the virus-specific RNA present is smaller than this.

These PRV RNAs have several characteristics in common with the LATs described for HSV-1 (22, 24). The HSV-1 LATs consist of 2.1- and 1.5-kb poly(A)$^-$ RNA species that are derived from a larger transcript (5). The smaller class of PRV transcripts detected in pig trigeminal ganglia, which are of a similar size, are poly(A)$^-$, and the in situ nucleic acid hybridization mapping data presented here indicate that they, too, may be part of a larger transcriptional unit. The PRV LAT is an RNA complementary to the IEP RNA of PRV, just as the HSV-I LAT and BHV-1 LAT (14, 24) are complementary RNAs to HSV-1 and BHV-1 immediate-early genes. The RNAs expressed during PRV latency are therefore similar to the LATs of two other herpesviruses.

There are, however, also some striking differences between the PRV RNAs and their HSV-1 and BHV-1 counterparts. In PRV, a substantial proportion of the transcripts are present in the cytoplasm of latently infected neurons. This is in contrast to the HSV-I LATs, which are restricted to the nucleus with little or no cytoplasmic signal (24), and may indicate that a certain proportion of the transcripts are translated. PRV also expresses other poly(A)$^-$ RNAs (between 4.5 and 5.5 kb) during latency which have not been found on Northern blots prepared from ganglia latently infected with either HSV-1 or BHV-1. At the moment, the relationship between the larger and smaller PRV transcripts is not clear.

Our conclusion that latent phase gene expression by PRV is similar in some aspects to that of two other alphaherpesviruses is in contrast to the results reported in a recent paper by Cheung (2). Using the Becker strain of PRV, he identified RNAs of 2.0 and 0.95 kb in the trigeminal ganglia of latently infected pigs. Both of these transcripts were poly(A)$^+$ and located just downstream of the 3′ end of IEP in a region that, as we have shown here, is also positive by in situ hybridization analysis of PRV(Ka)-infected swine trigeminal ganglia tissue. The primary means of detection of these RNAs was S1 nuclease analysis. This is in contrast to our data, which indicate that PRV expresses several RNAs during latency, all of which are poly(A)$^-$, although our Northern blots may not have been sensitive enough to detect the poly(A)$^+$ transcripts described by Cheung. Several of the probes (e.g., P7T3) that were strongly positive in our in situ nucleic acid hybridization experiments were negative when tested by Cheung, and it is possible that any larger RNA fragments protected by probes, which fragments should hybridize to the 5-kb RNAs, were missed due to the size restrictions of the polyacrylamide gels used in S1 nuclease analysis.

Although the differences between our results and those of Cheung may be due to differences in the sensitivities of the techniques used, an alternative explanation for this discrepancy would be that the two strains differ in the processing of these RNA transcripts. In the PRV strains, for which data are available on both reactivation and the presence of transcripts in latently infected pig trigeminal ganglia, each case is different. Both the Sullivan and Becker strains reactivate at a high level in vitro, but only the Becker strain expresses a detectable LAT (1, 15). PRV(Ka), by contrast, does not reactivate well but is transcriptionally active during latency. Other PRV isolates that do not reactivate well have also been identified (18), but no data on the expression of an LAT from these viruses are available. It is possible that strain differences in either RNA expression or processing during latency do exist, and that the availability of certain poly(A)$^-$ or poly(A)$^+$ species of RNA may influence the ability of the virus to reactivate in vitro at a high frequency from latently infected tissue. Alternatively, certain isolates of PRV may demonstrate varied levels of spontaneous reactivation, which may contribute to the differences seen here, especially since we have found that during an acute infection in tissue culture, PRV expresses transcripts that are poly(A)$^+$ from the region active during latency (unpublished results). Thus we cannot exclude the possibility, acknowledged by Cheung, that the poly(A)$^+$ RNA species he detected are from reactivating ganglia.

We conclude that PRV expresses RNAs during latency and that these RNAs, although different in many ways, display some of the same characteristics of the LATs found in two other herpesviruses, HSV-1 and BHV-1. PRV LATs, if they vary among different PRV strains, might affect the capacity of the virus to reactivate, a phenomenon seen in

HSV-1 latent infections when an LAT is absent (8, 11, 19, 21). Such differences could facilitate the eventual delineation of the function of these transcripts in the pathogenesis of the latent infection of pseudorabies virus in infected pigs.

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LITERATURE CITED

1. **Ben-Porat, T., A. M. Deatly, B. C. Easterday, D. Galloway, A. S. Kaplan, and S. McGregor.** 1984. Latency of pseudorabies virus. Curr. Top. Vet. Med. Anim. Sci. **27**:365–383.
2. **Cheung, A. K.** 1989. Detection of pseudorabies virus transcripts in trigeminal ganglia of latently infected swine. J. Virol. **63**: 2908–2913.
3. **Cox, K. H., D. V. Deleon, L. M. Angerer, and R. C. Angerer.** 1984. Detection of mRNAs in sea urchin embryos by in situ hybridization using asymmetric RNA probes. Dev. Biol. **101**: 484–502.
4. **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.
5. **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.
6. **Gordon, Y. J., B. Johnson, E. Romanowski, and T. Aravllo-Cruz.** 1988. RNA complimentary to herpes simplex virus type 1 ICP0 gene demonstrated in neurons of human trigeminal ganglia. J. Virol. **62**:1832–1835.
7. **Gutenkust, D. E.** 1979. Latent pseudorabies virus infection in swine detected by RNA-DNA hybridization. Am. J. Vet. Res. **40**:1568–1572.
8. **Hill, J., F. Sederati, R. T. Javier, E. K. Wagner, and J. G. Stevens.** 1990. Herpes simplex virus latent phase transcription facilitates in vivo reactivation. Virology **174**:117–125.
9. **Ihara, S., L. Feldman, S. Watanabe, and T. Ben-Porat.** 1983. Characterization of the immediate-early functions of pseudorabies virus. Virology **131**:437–454.
10. **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.
11. **Leib, D. A., C. L. Bogard, M. Kosz-Vnenchak, K. A. Hicks, D. M. Coen, D. M. Knipe, and P. A. Schaffer.** 1989. A deletion mutant of the latency-associated transcript of herpes simplex virus type 1 reactivates from the latent state with reduced frequency. J. Virol. **63**:2893–2900.
12. **Lokensgard, J. R., D. G. Thawley, and T. W. Molitor.** 1990. Pseudorabies virus latency: restricted transcription. Arch. Virol. **110**:129–136.
13. **Maniatis, T. E., F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **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.
15. **Rock, D. L., W. A. Hagemoser, F. A. Osorio, and H. A. McAllister.** 1988. Transcription from the pseudorabies virus genome during latent infection. Arch. Virol. **98**:99–106.
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. **Rziha, H. J., T. C. Mettenleiter, V. Ohlinger, and G. Wittmann.** 1986. Herpesvirus (pseudorabies virus) latency in swine: occurrence and physical state of viral DNA in neural tissues. Virology **155**:600–613.
18. **Sabo, A., and J. Rajcani.** 1976. Latent pseudorabies virus infection in pigs. Acta Virol. **20**:208–214.
19. **Sederati, F., K. M. Izumi, E. K. Wagner, and J. G. Stevens.** 1989. Herpes simplex virus type 1 latency-associated transcription plays no role in establishment or maintenance of a latent infection in murine sensory neurons. J. Virol. **63**:4455–4458.
20. **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.
21. **Steiner, I., J. G. Spicack, R. P. Linette, S. Moira-Brown, A. R. Maclean, J. H. Subak-Sharpe, and N. W. Fraser.** 1989. Herpes simplex virus type 1 latency-associated transcripts are evidently not essential for latent infection. EMBO J. **8**:505–511.
22. **Stevens, J. G.** 1989. Human herpesviruses: a consideration of the latent state. Microbiol. Rev. **53**:318–332.
23. **Stevens, J. G., L. Haarr, D. D. Porter, M. L. Cook, and E. K. Wagner.** 1988. Prominence of the herpes simplex virus latency-associated transcripts in trigeminal ganglia from seropositive humans. J. Infect. Dis. **158**:117–122.
24. **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 prominent in latently infected neurons. Science **235**;1056–1059.
25. **Wagner, E. K., G. Devi-Rao, L. T. Feldman, A. T. Dobson, Y. Zhang, W. M. Flanagan, and J. G. Stevens.** 1988. Physical characterization of the herpes simplex virus latency-associated transcripts in neurons. J. Virol. **62**:1194–1202.
26. **Wagner, E. K., W. M. Flanagan, G. Devi-Rao, Y. Zhang, J. M. Hill, K. P. Anderson, and J. G. Stevens.** 1988. The herpes simplex virus latency-associated transcript is spliced during the latent phase of infection. J. Virol. **62**:4577–4585.
27. **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**:1052–4058.
28. **Whitby, A. J., W. A. Blyth, and T. J. Hill.** 1987. The effect of DNA hypomethylating agents on the reactivation of herpes simplex virus from latently infected mouse ganglia *in vitro*. Arch. Virol. **97**:137–144.